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Contents

Contents

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Contents

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EDITORIAL

Prediction of severe acute pancreatitis: Current knowledge and novel insights

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Abstract

Acute pancreatitis (AP) is a common and potentially lethal acute inflammatory process with a highly variable clinical course. It is still unclear why some patients progress to organ failure and others do not. Ability to predict which patients will develop severe disease is limited. Routine clinical and laboratory data and multi-factorial clinical scores measured on admission and during the first 48 h of hospitalization are currently the standards of care used to estimate the magnitude of the inflammatory response to injury. Current literature highlights several common environmental, metabolic and genetic factors that increase the risk of AP development and subsequent adverse sequelae. Several cytokines have been found to play a critical role in the pathogenesis of AP by driving the subsequent inflammatory response, to include tumor necrosis factor-α (TNF-α), Interleukin-1 (IL-1), IL-6 and monocyte chemotactic protein-1 (MCP-1). Large, prospective studies are still needed to address these questions by identifying AP risk factors and serum biomarkers of severe disease.

Key words: Acute pancreatitis; Prediction; Severity; Monocyte chemotactic protein-1

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INTRODUCTION

Acute pancreatitis (AP) is a common and potentially lethal acute inflammatory process with a highly variable clinical course. It accounts for greater than 300 000 emergency room visits annually in the US, which is steadily increasing, with a mean length of hospital stay of 7 d[1].

Approximately 20% of affected individuals will develop a severe clinical course in association with the development of a systemic inflammatory response syndrome (SIRS), multiple organ failure (MOF), and on occasion death. Despite substantial animal model research[2], it is still unclear as to why some patients progress to organ failure and others do not, or at what step in the inflammatory cascade will an intervention have an impact upon disease progression. Predictive disease severity scoring systems are widely used in clinical practice; but in reality they reflect the inflammatory response rather than the severity of the insult experienced by the pancreatic parenchyma.

Several clinical and molecular pre-AP susceptibility and severity factors have been identified which may modify an individual's predisposition to AP, and the associated risk of severity. Obesity is one such important factor. An elevated BMI ($\geq$ 30 kg/m^2) significantly increases the extent of AP severity (OR, 2.6; 95% CI, 1.5-4.6) and is implicated in both local and systemic complications[3]. The severity risk increases at an OR of 1.2 per 5 units of BMI. Severe AP is associated with android fat distribution, increased waist-hip ratio (> 1.0) and appears to correlate with an "overactive" immune response.

Alcohol consumption is another risk factor associated with severe AP as it lowers the threshold for intrapancreatic trypsin activation and shifts pancreatic acinar cell death from apoptosis to necrosis as demonstrated in alcohol-fed animals[4]. Our group reaffirmed this finding in human subjects consuming two or more alcoholic drinks per day[5]. Furthermore, active tobacco smoking has been suggested as a susceptibility

factor for AP (RR, 2.14; 95% CI, 1.48-3.09)[6].

In preliminary genetic susceptibility factor studies, the presence of a single nucleotide polymorphism in the gene of a potent chemokine, named monocyte chemotactic protein-1 (MCP-1), at position -2518 A/G predicted that the inflammatory response to AP would be systemic and associated with death[7]. The G allele was present in 86% of severe pancreatitis cases, 46% of mild pancreatitis cases and 43% of controls. The presence of the G allele increased the risk of developing severe AP seven fold (OR, 7.7; 95% CI, 1.6-100).

Routine clinical and laboratory data and multifactorial clinical scores measured on admission and during the first 48 h of hospitalization are currently the standards of care used to estimate the magnitude of the inflammatory response to injury, and to predict whether or not intensive care support is needed to address inflammation-associated complications. Admission hematocrit, C-reactive protein (CRP) at 48 h, Ranson's criteria and the Acute Physiology and Chronic Health Evaluation (APACHE-Ⅱ) scores are the most popular. In addition, a variety of cytokines, chemokines, and other markers of the inflammatory response have been evaluated as predictors of severe AP, as well as markers of development of specific organ-system failure.

Collectively, the literature highlights several common environmental, metabolic and genetic factors that are predisposing factors increasing the risk of AP development and subsequent adverse sequelae. The mechanisms by which such factors increase the risk of severe disease, and whether or not they directly interact with or potentiate one another remains speculative. Knowledge of the inflammatory cascade is important in recognizing when the peak response occurs for various cytokines and inflammatory mediators.

Several reports have evaluated patients with endoscopic retrograde cholangiopancreatography (ERCP) induced pancreatitis, and studied post-ERCP cytokine profiles. Cytokines play a critical role in the pathogenesis of AP by driving the subsequent inflammatory response. Patients with post-ERCP AP have an amylase and lipase increase within the first hour reaching a maximum value between 4 h and 12 h following ERCP[8]. Interleukin-6 (IL-6) increases to a maximal concentration at 24-48 h, and the highest CRP concentrations are established 72 h following an ERCP. In another study, in patients who developed post-ERCP pancreatitis, the serum levels of these cytokines including tumor necrosis factor-α (TNF-α), IL-1, IL-6, IL-8, and IL-10 rose significantly at 8 and 24 h but not at 1 h and 4 h when compared to patients without pancreatitis[9]. These data suggest that serum markers (amylase/lipase) are detected early, but that the acute inflammatory response does not fully develop until at least 8-12 h after the initial pancreatic insult. These data may be useful for determining the extent of pancreatic injury, the timing of the acute inflammatory response and for assessing such inflammatory markers in equation-based models.

TNF-α

TNF-α is a pleiotropic cytokine expressed in acinar cells, and is a key regulator of other pro-inflammatory cytokines and leukocyte adhesion molecules which acts as a priming activator of immune cells[10]. It is also a cell death signal through the TNF-α-related apoptosis induced ligand (TRAIL) receptor pathway, with the potential to cause severe tissue damage. TNF-α plays a pivotal role in severe AP, acting early in the disease course, and is quickly cleared. As a result of its rapid clearance, TNF-α serum levels are less useful as biomarkers of early events than downstream cytokines (e.g. IL-6). To limit the systemic effect of TNF-α, the body releases TNF-α inhibitors. The soluble TNF receptor (sTNFR) attenuates the effects of TNF-α by binding to TNF-α in the serum and thus acts as an anti-inflammatory molecule. sTNFR levels have been found to predict severity in AP with an accuracy of 96%, and also to have a high sensitivity for mortality[11].

IL-1

IL-1 is another major pro-inflammatory cytokine that can drive the SIRS response. It has recently been shown to be the major cytokine mediating inflammation in sterile necrosis[12], which is often problematic in severe AP. In contrast to TNF-α, IL-6 does not directly cause pancreatic damage[13]. It has been used as a biomarker of disease severity and has similar accuracy to IL-6 in predicting severe AP on admission (82% *vs* 88%)[11]. IL-1 receptor antagonist (IL1-RA) levels also correlate with the inflammatory response and severity in AP and may in fact be superior to IL-6 or CRP within the first 48 h.

IL-6

IL-6 is a multifunctional cytokine released by macrophages in response to tissue injury and constitutes the principal mediator in the synthesis of acute-phase proteins, in addition to transitioning the acute inflammatory response to a chronic response. It is an accurate early predictor of severity in AP, with a sensitivity range of 89% to 100% and 90% accuracy within the initial 24 h[11]. It has also been shown to be superior to CRP and the APACHE-Ⅱ score at 24 h following admission.

MCP-1

MCP-1 is a potent chemokine which is released early in the inflammatory process. MCP-1 serum concentrations have been shown to display a dramatic increase in patients with AP who develop local complications or remote organ failure. A close correlation has also been found between the incidence of remote organ failure and the degree of MCP-1 level elevation[7,14]. As highlighted earlier, a common single nucleotide polymorphism on the MCP-1 gene is shown to predispose to severe AP. Macrophage migration inhibitory factor (MIF) is a unique chemokine; that participates in inflammation, immune response and cell growth. Serum MIF levels

have been found to be higher in patients with severe AP than patients with mild disease[11].

Although altering the inflammatory response in animals translates into a possible benefit, the potential translational benefit to humans has not been confirmed to date. For example, the platelet activating factor (PAF) inhibitor, Lexipafant displayed early promise. However, it was not deemed to be an effective treatment in a large, multi-national study of 1500 patients[15]. Although IL-10 decreases the severity of AP in mouse models, and could be of potential benefit in humans, sufficiently powered human studies have yet to be reported in the literature.

The discriminatory power of general prediction schemes improved considerably in the early 1990's. Indeed, Ranson's criteria and APACHE Ⅱ score achieved reasonable discrimination with receiver-operating characteristic curve (ROC) area under the curve (AUC) values approaching 0.8 in most validation studies. Yet, these classification tools are designed to predict ICU mortality and not potentially preventable complications; they are, therefore, least useful in the middle prediction range where the clinician needs most support and information to direct management. Although these tools are of assistance in medical decision making at the extreme end of the prediction range, their use has been confined to a global ICU performance assessment and criteria for clinical trial enrolment.

Successful prediction of individual outcomes is undoubtedly one of the holy grails in the care of the critically ill. Remarkably, although progress has been made along all those fronts in risks and markers for severe AP, little has been achieved in translating data and quantitative tools into clinically useful and appealing predictive knowledge for physicians managing patients with AP.

Large, prospective studies are needed to address these questions by identifying AP risk factors and serum biomarkers of severe disease. Such data could be potentially used to develop patient-specific predictive algorithms of AP risk and to guide the treatment decision-making process early in the disease course. Such studies could aim to: firstly, determine the role of demographic, environmental, genetic and physiological variables on the initiation, progression, severity and clinical outcomes of AP; secondly to identify biomarkers that reflect the extent of pancreatic injury and the acute inflammatory response which are critical in the assessment of the activity of potentially pathologic cascades; thirdly to build advanced statistical models based on pre-injury risk factors and biomarkers of pancreatic injury and inflammation to accurately predict primary and secondary outcomes of AP, including organ failure, complications and death; and finally to guide the research on inflammatory cascade blocking agents administered early in the disease course based on patient-specific predictive algorithms.

REFERENCES

1 **Whitcomb DC**. Clinical practice. Acute pancreatitis. *N Engl J Med* 2006; **354**: 2142-2150
2 **Steinberg WM**, Schlesselman SE. Treatment of acute pancreatitis. Comparison of animal and human studies. *Gastroenterology* 1987; **93**: 1420-1427
3 **Martinez J**, Sanchez-Paya J, Palazon JM, Suazo-Barahona J, Robles-Diaz G, Perez-Mateo M. Is obesity a risk factor in acute pancreatitis? A meta-analysis. *Pancreatology* 2004; **4**: 42-48
4 **Wang YL**, Hu R, Lugea A, Gukovsky I, Smoot D, Gukovskaya AS, Pandol SJ. Ethanol feeding alters death signaling in the pancreas. *Pancreas* 2006; **32**: 351-359
5 **Papachristou GI**, Papachristou DJ, Morinville VD, Slivka A, Whitcomb DC. Chronic alcohol consumption is a major risk factor for pancreatic necrosis in acute pancreatitis. *Am J Gastroenterol* 2006; **101**: 2605-2610
6 **Lindkvist B**, Appelros S, Manjer J, Berglund G, Borgstrom A. A prospective cohort study of smoking in acute pancreatitis. *Pancreatology* 2008; **8**: 63-70
7 **Papachristou GI**, Sass DA, Avula H, Lamb J, Lokshin A, Barmada MM, Slivka A, Whitcomb DC. Is the monocyte chemotactic protein-1 -2518 G allele a risk factor for severe acute pancreatitis? *Clin Gastroenterol Hepatol* 2005; **3**: 475-481
8 **Messmann H**, Vogt W, Holstege A, Lock G, Heinisch A, von Furstenberg A, Leser HG, Zirngibl H, Scholmerich J. Post-ERP pancreatitis as a model for cytokine induced acute phase response in acute pancreatitis. *Gut* 1997; **40**: 80-85
9 **Chen CC**, Wang SS, Lu RH, Lu CC, Chang FY, Lee SD. Early changes of serum proinflammatory and anti-inflammatory cytokines after endoscopic retrograde cholangiopancreatogr aphy. *Pancreas* 2003; **26**: 375-380
10 **Papachristou GI**, Clermont G, Sharma A, Yadav D, Whitcomb DC. Risk and markers of severe acute pancreatitis. *Gastroenterol Clin North Am* 2007; **36**: 277-296, viii
11 **Malleo G**, Mazzon E, Siriwardena AK, Cuzzocrea S. Role of tumor necrosis factor-alpha in acute pancreatitis: from biological basis to clinical evidence. *Shock* 2007; **28**: 130-140
12 **Chen CJ**, Kono H, Golenbock D, Reed G, Akira S, Rock KL. Identification of a key pathway required for the sterile inflammatory response triggered by dying cells. *Nat Med* 2007; **13**: 851-856
13 **Denham W**, Yang J, Fink G, Denham D, Carter G, Bowers V, Norman J. TNF but not IL-1 decreases pancreatic acinar cell survival without affecting exocrine function: a study in the perfused human pancreas. *J Surg Res* 1998; **74**: 3-7
14 **Rau B**, Baumgart K, Kruger CM, Schilling M, Beger HG. CC-chemokine activation in acute pancreatitis: enhanced release of monocyte chemoattractant protein-1 in patients with local and systemic complications. *Intensive Care Med* 2003; **29**: 622-629
15 **Johnson CD**, Kingsnorth AN, Imrie CW, McMahon MJ, Neoptolemos JP, McKay C, Toh SK, Skaife P, Leeder PC, Wilson P, Larvin M, Curtis LD. Double blind, randomised, placebo controlled study of a platelet activating factor antagonist, lexipafant, in the treatment and prevention of organ failure in predicted severe acute pancreatitis. *Gut* 2001; **48**: 62-69

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EDITORIAL

Hypnosis and upper digestive function and disease

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Abstract

Hypnosis is a therapeutic technique that primarily involves attentive receptive concentration. Even though a small number of health professionals are trained in hypnosis and lingering myths and misconceptions associated with this method have hampered its widespread use to treat medical conditions, hypnotherapy has gained relevance as an effective treatment for irritable bowel syndrome not responsive to standard care. More recently, a few studies have addressed the potential influence of hypnosis on upper digestive function and disease. This paper reviews the efficacy of hypnosis in the modulation of upper digestive motor and secretory function. The present evidence of the effectiveness of hypnotherapy as a treatment for functional and organic diseases of the upper bowel is also summarized, coupled with a discussion of potential mechanisms of its therapeutic action.

Key words: Hypnosis; Hypnotherapy; Gastric emptying; Small bowel transit; Functional dyspepsia; Functional esophageal disorders; Functional bowel disorders

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INTRODUCTION

Hypnosis can be defined as an altered state of consciousness, different from both sleep and normal wakefulness, characterized by highly focused attention and heightened compliance with suggestion[1]. As a rule, the onset of this state is facilitated by eye closure. A number of other phenomena are often described as associated with hypnosis, including altered perception of passage of time, partial or complete amnesia for the events experienced, and attenuation of stress experiences[1-3]. In addition, subjects may show enhanced compliance to suggestion given during hypnosis meant to influence favorably their behavior after the trance state has been terminated (post-hypnotic suggestion)[1]. Furthermore, a more contentious property of hypnosis is either increased access to memories, feelings, and perceptions which are normally kept below the level of conscious awareness, or *vice versa* enhanced suppression of these from the conscious mind[4-6].

Clinical hypnosis is the method of deliberately inducing the state of hypnosis in a patient through verbal guidance, and making use of its characteristic properties for targeted therapeutic purposes. The possibilities of hypnosis as a healing method stem principally from the heightened responsiveness to suggestion in this altered mental state. Hypnotic and post-hypnotic suggestions can be used to facilitate desired therapeutic changes in feelings, behavior and physiology, and this can be useful not only for mental health purposes, but also in medicine[1]. Although a single hypnosis session targeting a simple symptom or bodily function can sometimes yield useful results, treatment of complex psychological and somatic conditions with hypnosis typically requires a structured form of therapeutic intervention, hypnotherapy, administered in a series of several therapy sessions[1].

Hypnosis has a long history of application as a clinical tool in medicine, dating back to the early 18th

Century, when it was used with considerable success for the purpose of inducing anesthesia during surgery in thousands of cases, predominantly by British physicians. Only the availability of chemical anesthesia with ether and chloroform in 1846 and 1847 made this application obsolete[7].

In the latter half of the 19th century, hypnosis became prominently utilized in the treatment of psychiatric conditions like hysteria by some of Europe's foremost authorities in neurology and psychiatry of that time, such as Sigmund Freud in Austria and Jean-Martin Charcot in France[8]. Ever since then, hypnosis has been more widely recognized as a treatment aid for mental health problems than for physical ailments. However, medical uses of hypnosis continued, and sufficient experience with various advantageous medical applications gradually accumulated for the technique of clinical hypnosis to earn formal acceptance in mainstream medicine[7,8]. Hypnosis gained official approval as a medical treatment, first by the British Medical Association in 1955 and then by the American Medical Association in 1958, in a report that stated that hypnosis had "definite and proper applications in medicine and dentistry", and recommended that physicians should receive training in the technique[7,8]. However, even today, most medical school curricula in the U.S. and elsewhere provide no training or education in hypnosis. Although clinical hypnosis is currently practiced by thousands of health professionals in many Western countries, it is practiced by a variety of professional disciplines, including psychologists, counselors, clinical social workers, dentists, nurses and nurse practitioners, but relatively few physicians[7,8]. In many places, the great majority of practitioners providing hypnosis are mental health professionals who rarely use it to treat physical conditions. Additionally, hypnosis services are commonly offered also by large numbers of lay hypnotherapists without any qualifications or formal education in treating medical problems[8]. These limitations, as well as myths, misconceptions and apprehensions that still linger in the public's mind from the exploitation and inaccurate portrayal of hypnosis in stage shows, movies and other popular media, has continued to hamper a widespread proper medical use of hypnosis.

Nonetheless, several medical applications of clinical hypnosis have been sufficiently investigated and considered effective in multiple formal studies. A review by a 1995 National Institutes of Health panel in the U.S. concluded that there is "strong evidence for the use of hypnosis in alleviating pain associated with cancer"[9]. Published systematic reviews of randomized clinical trials have also deemed hypnosis to be effective for treating nausea and vomiting associated with cancer chemotherapy[10] as well as the most promising psychological treatment for controlling procedure-related pain and distress in children and adolescents[11]. Furthermore, three separate systematic reviews published in the past three years[12-14] have concluded that hypnotherapy is an effective treatment for irritable bowel syndrome.

Research on the use of hypnosis for gastrointestinal disorders began with a randomized placebo-controlled study of hypnotherapy for treatment-refractory irritable bowel syndrome (IBS) in England, published in the Lancet in 1984[15]. In this study, by Peter Whorwell and colleagues in Manchester, England, the investigators randomly allocated 30 patients with IBS which was refractory to standard medical care, to either seven sessions of hypnotherapy or to the same amount of supportive psychotherapy plus placebo pills. The hypnosis approach used was a structured intervention developed by this Manchester team called gut-focused hypnotherapy. This technique aims primarily to normalize disordered bowel function, but additionally provides relaxation, coping skills, and ego-strengthening suggestion[16]. After the treatment, the patients in the hypnosis group showed substantial improvement in all cardinal IBS symptoms, and were significantly more improved on all outcome variables than the supportive psychotherapy group[15]. In a later paper, the investigators reported that the benefits of hypnotherapy in the same group of patients persisted up to 18 mo[17].

This study, albeit small, was a landmark trial, demonstrating for the first time the substantial possibilities that hypnosis offers for ameliorating gastrointestinal symptoms. Since then, positive results on the efficacy of hypnotherapy as a treatment for IBS have been reported by independent investigators both in uncontrolled and controlled trials (Table 1)[18-22]. The Manchester group has created a Hypnotherapy Unit, where this mode of therapy is routinely offered to functional GI patients who do not gain satisfactory benefit from more conventional medical treatment[16]. This group recently reported the long-term outcomes of the first 250 IBS patients treated in their clinic[22,23]. The results show an impressive 71% overall response rate to treatment, more than 50% average reduction in bowel symptom severity, and with four out of five treatment responders maintaining the full therapeutic benefit for one to five years after treatment termination[22,23].

The Manchester group has also expanded their experience from IBS therapy to other functional bowel disorders[16]. They demonstrated that functional esophageal disorders and functional gastroduodenal disorders are also suitable targets for hypnotherapy, with equally satisfactory results (Table 1)[24,25]. A small, but significant group of papers now provides evidence that hypnosis and hypnotherapy may effectively influence upper digestive function and disease. The aim of this review is to focus on this literature and to highlight the potential of hypnotherapy as a treatment option for upper digestive functional disorders.

HYPNOSIS AND UPPER DIGESTIVE FUNCTION

Gastric acid production is the bowel function where the influence of hypnosis was first investigated[26,27]. In the past, gastric acid secretion was an important research domain for gastroenterologists and its responsiveness to

Table 1 Randomized controlled trials of hypnosis treatment for severe functional bowel disorders

Authors & yr	No. of patients	Control treatment	Positive outcome	Follow-up (mo)
A: Irritable bowel syndrome				
Whorwell *et al* (1984)	30	Psychotherapy plus Placebo Pill	Hypnosis 100% $P < 0.0001$	12
Galovski & Blanchard (1998)	11	Waiting List	Hypnosis 82% $P = 0.016$	2
Palsson *et al* (2002)	24	Waiting List	Hypnosis 87% $P = 0.002$	10
B: Functional Dyspepsia				
Calvert *et al* (2002)	126	Psychotherapy plus Placebo Pill or Ranitidine 300 mg daily	Hypnosis 73% *vs* Placebo $P < 0.02$ *vs* Ranitidine $P < 0.01$	14
C: Non-cardiac chest pain				
Jones *et al* (2006)	28	Psychotherapy plus Placebo Pill	Hypnosis 80% $P = 0.008$	4

Note: Randomized controlled studies run in primary care are not reported for different patient population.

emotions and psychological stress were documented[28,29]. This interest was driven by the belief that peptic ulcer disease was a psychosomatic disease, and excess gastric acid secretion the pathophysiological mechanism linking emotion to the disease[30,31]. As a consequence, acid secretion was an attractive parameter to attempt to influence by hypnosis. A few studies were published in the nineteen sixties and early seventies examining the gastric secretory responses to hypnotic conditions, where either food-related (hunger-eating) or emotion-related (sleep-relaxation) suggestions were provided[27-29]. These early studies were flawed by small samples and questionable research methodology, and produced contradictory results. In 1989, however, Klein and Spiegel published a well-designed trial investigating the ability of hypnosis to modulate gastric acid secretion in highly hypnotizable healthy volunteers, as defined by accepted scales of trance depth[32]. The study was conducted in two centers, by two experienced hypnotherapists using two different hypnosis induction techniques. After naso-gastric intubation, gastric secretion was measured both basally and after pentagastrin stimulation in two separate studies. In the first study (acid stimulation test), acid secretion was collected in 28 subjects (13 females, age range 18-60 years) after hypnotic instructions to visualize and eat the most delicious meal possible. All the sensory aspects of the eating process, including food appearance, aroma, texture and taste, were explored and reinforced by hypnotic suggestions from the therapist. The second study consisted of two separate sessions that were held in random order. In the no-hypnosis session, the peak acid output (PAO) was obtained after maximal pentagastrin stimulation in 17 subjects (7 females, age range 18-60 years), but hypnosis was not provided. The procedure for the hypnosis sessions was the same, but deep muscle relaxation and intense imagery to divert one's attention from eating were provided. Imagery involved either lying on a beach, watching a sunset, or meeting a friend somewhere else. In both studies, none of the subjects reported difficulty in following the hypnotic suggestion or adverse side effects. Hypnotic suggestion of eating significantly increased gastric acid output compared to basal conditions[32]. In addition, the pentagastrin-stimulated PAO was significantly lowered in the averting-food hypnosis condition compared to the no-hypnosis session[32]. The authors concluded that gastric acid secretion may be modulated by hypnosis in highly hypnotizable subjects. Treatment mechanisms of action were left unexplored. But, the authors postulated that hypnosis influenced cognitive processing within the central nervous system[32]. Since the relevance of gastric secretion in peptic ulcer disease has diminished, no other centers have tried to replicate these positive results.

Two additional studies have evaluated the influence of hypnosis on upper digestive transit. In 1991 Beugerie *et al* studied the ability of hypnosis to modulate the orocaecal transit time of 10 g lactulose in six healthy volunteers[33]. Orocaecal transit time was measured by the hydrogen breath test. Oral ingestion of a poorly absorbable carbohydrate (lactulose) results in a sustained rise in breath hydrogen, which occurs within minutes of the substrate entering the cecum[34]. The orocaecal transit time is the interval elapsing from the ingestion of the substrate to the evidence of a persistent increment in breath hydrogen concentration[34]. It is commonly considered a non-invasive, reliable index of small bowel transit, particularly when lactulose is included in a caloric meal to securely interrupt the fasting motility pattern of the small bowel[35]. The subjects in this trial were recruited irrespectively to their hypnotizability, but two of them had previously been hypnotized. Orocaecal transit was evaluated on three occasions in random order: (A) control session without hypnosis; (B) hypnotic session with suggestion of deep relaxation; (C) hypnotic session with visualization of a cascading waterfall to promote transit acceleration[33]. All hypnosis sessions were started just before orocaecal transit and maintained till the transit time elapsed. The mean orocaecal transit time was significantly longer during the hypnotic relaxation session compared to the control session[33]. On the contrary, the hypnotic acceleration session did not result in significant modification of small bowel transit time[33]. The small sample size and the limited breath technique used (lactulose not administered together with a caloric test meal) did flaw the results of this study. However, it was the first study showing an influence of hypnosis on upper digestive function in individuals not selected for high hypnotizability.

The potential influence of hypnosis on gastric emptying rates has been evaluated only recently, by Chiarioni and coworkers in Italy[36]. In this study, the gastric emptying rate of a typical Mediterranean meal

(pasta with meat sauce, cheese, bread) was tested by a non-invasive ultrasonography technique. Real-time ultrasonography was used to measure the diameters of the gastric antrum in the sagittal plane passing through the aorta. Serial measurements were taken before the meal, immediately after eating and at 30 min intervals thereafter to obtain total emptying time of the meal. The total emptying time of the meal has been validated as reliable index of gastric motor function both in health and in disease when compared with total emptying time measured by gastric scintigraphy[37]. Gastric emptying rates and epigastric sensations were evaluated in 11 healthy volunteers from the hospital staff and in 15 patients with severe functional dyspepsia unresponsive to standard care under three conditions according to a fixed schedule to avoid a carry-over effect: (A) basal session, (B) prokinetic drug session (cisapride 10 mg po 30 min before meal), and (C) hypnosis session (90 min hypnosis session 30 min after finishing meal). An additional session was run in eight healthy volunteers while listening to relaxing music, to address the potential influence of both repeated testing and posture. Cisapride is a prokinetic agent that has been shown to significantly improve both gastric emptying and symptoms in functional dyspepsia compared to placebo, before being withdrawn from the market for its cardiovascular side-effects[38,39]. The method of progressive relaxation by verbal suggestion was used for hypnosis induction. Techniques to deepen the hypnosis included induction of limb heaviness and warmth. The hypnotically warmed hand was then placed over the epigastrium to associate suggestion of improved well being and gastric function mediated by the warmth of the hand. Imagery was provided of water flowing in a river and in a waterfall. This was related to suggestions for improved well-being and gastric function, derived from the gut-oriented suggestions developed by the Manchester group to treat irritable bowel syndrome[16]. The hypnosis session was completed by the classic Hartland's ego-strengthening technique, providing direct and broad hypnotic suggestions to increase the patient's confidence[40]. In patients with functional dyspepsia, gastric emptying was significantly shortened by cisapride and even more by hypnosis compared to the basal session[36]. In healthy volunteers, gastric emptying was significantly accelerated by hypnosis, but not by cisapride, compared to the basal session[36]. The relaxing music session did not influence gastric emptying rates. Epigastric sensations (i.e. fullness and discomfort) were significantly improved by hypnosis in the dyspeptic patients, but not by cisapride[36]. Interestingly, symptomatic improvement did not correlate with improved gastric motor function, leaving the mechanism/s of action of hypnosis unexplained[36]. Limitations of the study were lack of randomization and the highly selected study population.

HYPNOTHERAPY TO TREAT UPPER DIGESTIVE DISEASES

Hypnotherapy delivered as a structured, multi-session focused intervention has been most extensively used to treat IBS according to the protocols of the Manchester group or the North Carolina group[13,16]. However, the Manchester group has also provided experimental evidence to support the use of hypnotherapy in some upper digestive diseases. The first of these was a controlled study to prevent relapse of peptic ulcer[41]. The investigation was published in 1988 when peptic ulcer was considered to be a psychosomatic disorder caused by increased gastric secretion[30,31]. Thirty patients with frequently relapsing duodenal ulcer were randomized to receive either seven sessions of gut-focused hypnotherapy plus ranitidine 150 mg twice daily or seven routine consultations at a GI clinic without hypnosis plus the same ranitidine dosage over a 10-wk interval[41]. Hypnosis was induced with an arm-levitation technique followed by a combination of standard deepening procedures. The subject was then asked to place her/his hand over the abdomen, feel a sense of warm beneath the hand, and relate this to the control of gastric secretions. Reinforcement by visualization was used depending on the patient's ability. Patients were also given an audio tape for daily autohypnosis. At one year follow-up, all the subjects in the no-hypnosis group had relapsed while only 53% in the hypnotherapy group showed endoscopic evidence of relapsing duodenal ulcer[41]. The authors concluded that hypnotherapy is helpful in maintaining remission in those patients with peptic ulcer who are prone to relapse[41]. Shortly after the study, consensus developed that Helicobacter pylori infection of the stomach is the primary cause of peptic ulcer disease, and hypnotherapy was, therefore, not pursued further as potential treatment for peptic disease[42]. Nonetheless, this remains the first study to investigate the efficacy of hypnotherapy to treat upper digestive diseases.

Recently, the Manchester group assessed the efficacy of hypnotherapy for upper digestive functional diseases in two controlled trials; one on functional dyspepsia (FD) and the other for non-cardiac chest pain (NCCP)[24,25]. Functional dyspepsia refers to symptoms thought to originate in the gastroduodenal region in the absence of any organic or metabolic disease that is likely to explain the symptoms[43]. Postprandial fullness, early satiety, epigastric pain and/or burning may be reported as symptoms in FD[43]. Delayed gastric emptying, abnormal gastric tone, altered visceral perception, and autonomic imbalance have all been considered as potential etiologic factors[43,44]. In addition, comorbidity with psychiatric disorders, especially anxiety disorders, is reported to be high in FD[45]. Up to 30% of people in the community report having dyspeptic symptoms each year[43,45]. Symptomatic drug treatment, especially proton pump inhibitor medications, are often used for FD symptoms. But, the results are unsatisfactory[43,44,46]. To investigate the efficacy of hypnotherapy in FD, Calvert and coworkers randomly assigned 126 FD patients to receive either 12 hypnotherapy sessions, supportive therapy plus placebo tablets, or medical treatment with ranitidine 150 mg twice daily[24]. Patients underwent a 16-wk

treatment phase followed by a 40-wk follow-up phase where no further study interventions were undertaken. Hypnosis was induced using eye fixation followed by progressive muscular relaxation and deepened by standard procedures. The patients were then asked to place their hands on their abdomens and imagine a reduction of all symptoms. Suggestions of improvement in gastric motor function, sensitivity and gut secretion activity were also given. Reinforcement by appropriate visualization processes were administered as well. At the short term follow-up (16 wk), hypnotherapy significantly ameliorated symptoms compared to both the supportive therapy and the medical treatment groups[25]. Analogous improvements were observed when quality of life scores (QOL) were considered. Anxiety scores were lower after hypnotherapy; but there was no correlation between improvement in anxiety and FD symptom improvement[24]. No differences were evident between groups in terms of depression scores. Improvement in FD symptoms and QOL were well-maintained at long term follow-up (56 wk)[24]. In addition, patients in the hypnotherapy group were significantly less likely to consult the referring physician and to establish additional drug treatments than were the subjects in the other two groups[24]. The authors concluded that hypnotherapy is an effective treatment for functional dyspepsia both in the short and long term, but the mechanism/s of action remained speculative[24]. Hypnotherapy seems also to be cost-effective for the observed reduction in medication use and consultation rate at long term follow-up. This study was methodologically sound by most standards: (A) study design and sample size were both adequate, and (B) the double placebo control condition plus the standard care arm were likely to have produced a high expectation of therapeutic effect. Replication of these positive results by independent investigators is eagerly awaited.

Recently, the Manchester group has extended the application of hypnotherapy to non-cardiac chest pain, a condition later redefined as functional chest pain of presumed esophageal origin by the Rome Ⅲ Committee[25,47]. This functional disorder refers to relapsing episodes of unexplained chest pain that is usually located in the midline of the chest and of visceral quality[47]. The pain involved may be similar in nature to the one reported by angina patients, and by those affected by other esophageal disorders including achalasia and gastro-esophageal reflux disease (GERD)[47]. To diagnose functional chest pain, heart disease needs to be excluded as well as structural esophageal diseases, GERD, and esophageal motility disorders with defined histopathologic bases (i.e. achalasia, scleroderma of the esophagus)[47]. Epidemiology of functional chest pain is ill defined; but one should consider that 15%-30% of coronary angiograms performed for chest pain are negative for ischemic heart disease[47,48]. Disordered esophageal motility, altered visceral perception, and abnormal central signal processing with secondary errors in autonomic response have all been reported, alone or in combination, as potential causative factors[47]. In addition, overrepresentation of psychiatric disorders, particularly depression, anxiety and somatization disorders, have been described in functional chest pain of presumed esophageal origin[49]. Quality of life is impaired in continued pain and spontaneous recovery is rare[47]. In these patients, a therapeutic trial with proton pump inhibitors is mandatory to exclude symptomatic reflux disease[50]. Antidepressants may be of help, but their continuous use is associated with a high rate of side effects[47,51].

To address the effect of hypnotherapy in NCPP, the Manchester group randomized 28 patients with functional chest pain to receive either 12 sessions of individualized hypnotherapy or 12 sessions of supportive listening plus placebo tablets to control for expectancy and equalize the amount of time spent with a clinician[25]. All the patients were referred by the local cardiothoracic center after negative coronary angiography for angina-like chest pain. Reflux disease as a potential causative factor of chest pain was excluded in all subjects either by normal 24 h pH monitoring or by non-responsiveness to a proton pump inhibitor trial. Hypnosis was induced by eye closure, followed by progressive muscle relaxation and deepened by standard techniques. Suggestions focused on improved esophageal functioning and sensitivity were then introduced by using both imagery and conditioning techniques. In addition, direct suggestions of reduced pain and improved general health were given on a repetitive basis at each session. After treatment, 80% of patients in the hypnotherapy group described their chest pain as completely better or moderately better, compared to only 23% of patients in the control group[25]. This benefit persisted long-term (2 years), as reported by the authors in a follow-up paper[52]. Hypnotherapy also resulted in a significantly greater reduction in pain intensity scores, greater improvements in quality of life, and a greater reduction in medication usage when compared to the control treatment[25]. There were no significant differences between treatment groups in terms of improvement of either anxiety or depression scores as assessed by the Hospital anxiety and depression scale[25].

Limitations of this trial include the small sample size and the high patient selection. As in previous studies, the mechanism of action of hypnosis was left unexplored in this study. However, it remains the only randomized trial to show that hypnotherapy is effective treatment for functional chest pain, a disabling disorder that responds poorly to conventional care. Therefore, additional larger studies evaluating the effect of hypnotherapy on functional chest pain of presumed esophageal origin should be pursued.

MECHANISM OF ACTION OF HYPNOSIS AND HYPNOTHERAPY

The mechanism of action of hypnosis is ill defined; but we may speculate that many factors possibly contribute to its influence on physiological function and symptoms in the upper digestive tract. Abnormal motor activity and

altered autonomic function have both been reported in functional gastroduodenal and esophageal diseases[43,47]. In functional dyspepsia, delayed gastric emptying seems particularly common in patients complaining of nausea, fullness and vomiting; but this is controversial[43,53]. Other disturbances of gastroduodenal motility have been described in functional dyspepsia (e.g. antral hypomotility, gastric dysrhythmia, reduced frequency of interdigestive migrating motor complexes); but their relationship to the symptoms is less documented[43]. On the contrary, evidence of increased gastric visceral perception (so-called hypersensitivity) in a subset of functional dyspepsia patients is well documented in the literature[43,54]. This altered perception may be mediated by the autonomic imbalance both on a cortical and a peripheral level often described in functional bowel disorders[55]. Hypnosis induces a state of profound relaxation consistent with a generalized decrement in sympathetic nervous system activity[1,3]. This relaxation response is not specific to hypnosis, but may be induced by different techniques such as autogenic training, yoga, and meditation[3]. The physiological changes of the relaxation response include simultaneous lowering of blood pressure, heart and respiratory rates, which are opposite to those induced by stressful events[3]. These changes are actually distinct from those observed during sleep and characterize a wakeful hypometabolic state[56]. In addition, the relaxation response seems to last longer than the actual hypnosis interval[3]. A distinct feature of the relaxation response is that its action seems to be mediated through a reduction in epinephrine end-organ responsivity[3]. Stress has been shown to increase gastric acid secretion, and it used to be considered a risk factor to developing peptic ulcer disease[30,57]. We may speculate a potential influence of hypnosis on gastric secretion through modulation of the sympathetic tone. In addition, experimental stress delays gastric emptying and increases plasma levels of noradrenaline plus accelerating small bowel transit[58-60]. Therefore, the capability of a single session of hypnosis either to accelerate gastric emptying or to slow small bowel transit may be secondary to the relaxation response. However, a recent study investigating hypnosis mechanisms of action showed that hypnotherapy did not change cardiovascular responses in IBS[23]. The only parameter of sympathetic tone that was significantly decreased by hypnotherapy was skin conductance, a measure reflecting sweat gland responses to stress[23].

The effect of hypnosis in gastric visceral sensitivity has not been investigated. However, in IBS the influence of hypnotherapy on rectal perception has been evaluated with controversial results. The Manchester group provided experimental evidence that hypnosis improved rectal sensitivity; but this was not confirmed by a recent study by the North Carolina group[23,61]. In addition, one should consider that significant symptom improvement has been reported in functional bowel diseases without correlating with gut sensorimotor functioning modifications[23,36,12]. Therefore, the symptomatic improvement observed after hypnosis should also be related to some modulation of perception at a cortical level. Brain imaging studies have shown a variety of alterations in cortical activation pattern to visceral sensitive stimulation (rectal distension, esophageal distension and acid perfusion) in patients with functional bowel disorders compared with controls[62]. However, a consistent finding has been the reported excessive activation of the anterior cingulated cortex where the affective response to pain is elaborated[62]. It has also been shown that non-painful esophageal distension activated the somatosensory and anterior cingulated cortex while visual stimulation activated a different central area (visual cortex), thus postulating a more specific response to visceral stimulation[63]. Studies on somatic pain have shown that hypnosis is capable of decreasing reported pain sensation in response to pain-inducing stimuli, while the neurophysiological reactions of spontaneous and evoked EEG were unaffected (i.e. cerebral potentials were modified as the subject was actually feeling pain)[64]. Further supporting evidence has been given by studies on somatic pain analgesia where hypnosis reduced activity of the anterior cingulate cortex, but not that of the somatosensory cortex[65]. This dissociation of sensory and affective components of pain under hypnosis would also be consistent with the new "dissociation theory" to explain the effectiveness of hypnotherapy in psychopathology[1,8,66]. There is growing evidence that patients with IBS, functional chest pain and probably functional dyspepsia show increased levels of vigilance toward gut pain related sensations, easily interpreting them as symptoms of disease as a consequence[62,67,68]. Modulating the affective component of pain ratings may be one of the therapeutic mechanisms of hypnotherapy in functional bowel disease.

An additional reason for the effectiveness of hypnotherapy in functional bowel diseases could be related to the focus of many protocols on reducing the catastrophising cognitions commonly present in these patients[67,68]. Gonsalkorale and coworkers reported that hypnotherapy improved symptom-related cognitions in IBS by using a dedicated cognitive scale[69]. In this study, improved cognitive scores correlated with symptomatic improvement[69]. Finally, the role of the placebo effect of hypnotherapy needs to be considered in producing the beneficial hypnotherapy outcomes observed. In many hypnotherapy trials patients affected by severe, unremitting symptoms of functional bowel disorder have been included[13,24,25,36]. The motivation to undergo a new treatment and therapy expectancy in these patients are predicted to be high[70]. In addition, the most powerful placebo effect is to be expected in patients suffering from chronic pain syndromes[70]. In this context, the placebo effect is stronger when complex interventions such as hypnotherapy are provided[70]. Unfortunately, to undertake a double blind controlled trial of treatments such as hypnotherapy is almost impossible because the recipient will know what treatment is provided and establishing a sham hypnosis therapy is not doable[16]. Therefore, appropriate control treatments (e.g. supportive

listening and placebo pills) are desirable options when designing a meaningful trial of hypnotherapy[13,16]. However, the results of such placebo-controlled studies conducted so far on hypnosis for IBS[15,24] and FD, using a powerful double placebo of inert pills combined with supportive listening, suggest that the placebo effect only plays a small role in the therapeutic impact of hypnotherapy on these conditions.

LIMITATIONS OF HYPNOTHERAPY IN UPPER DIGESTIVE DISEASES

Only two studies, coming from the same center, have tested the efficacy of hypnotherapy in the treatment of upper digestive functional diseases[24,25]. These studies provide encouraging evidence that hypnotherapy is effective treatment for functional dyspepsia and functional chest pain of presumed esophageal origin[19,20]. However, these results need to be replicated in less selected populations, and by independent investigators before a more widespread use of hypnotherapy to treat upper digestive dysfunction can be recommended. In addition, hypnotherapy is a time consuming, labor intensive and costly treatment, and the number of health care providers trained in hypnosis is limited. Non-medical qualified hypnotherapists and hypnosis audiotapes may reduce costs, but the effectiveness of these alternative delivery methods on outcomes have not been thoroughly investigated[13,16]. Specific knowledge in gut-directed hypnosis is required to obtain successful outcomes in treating gastrointestinal disorders, and such training has not been widely available[13,16]. In an effort to overcome this problem, some centers are providing gut-focused hypnosis scripts to treat IBS[13]. Finally, skepticism by some patients and physicians about the use of a psychological intervention for a gut disease may deter them from trying this treatment option. This may be particularly true for hypnosis because of the aura of magic and mystery associated with it.

CONCLUSION

Hypnosis is an altered state of consciousness characterized by highly focused attention and heightened compliance with suggestion[1]. Clinical hypnosis can be used to treat a range of complex psychological or somatic diseases, but this generally requires a structured form of hypnotherapy intervention consisting of several sessions[1,8]. Hypnosis has a long history of applications in medicine, and is now formally recognized as a valuable aid for various medical problems. However, a limited number of health professionals offer hypnotherapy for medical problems, and it has traditionally been hampered by misconceptions shrouding this psychological intervention[8,16]. Yet, sufficient evidence has amassed over the years to firmly support the effectiveness of hypnotherapy for various pain problems, as well as to treat IBS, a complex and prevalent functional disorder of the lower bowel[12,13,16]. Recently, a few studies have addressed the potential influence of both single-session hypnosis and a course of hypnotherapy on upper digestive function and diseases with encouraging results.

Hypnosis delivered on a single session by an expert therapist has been shown capable of modulating gastric secretion and accelerating gastric emptying in healthy volunteers[32,36]. In addition, hypnosis has improved gastric emptying and epigastric sensations in severe functional dyspepsia[36]. Small bowel transit may also be influenced by hypnosis[33].

In the past, hypnotherapy has been used with a successful outcome to decrease the relapsing rate of peptic ulcer disease[41]. More recently, two randomized controlled trials have shown hypnotherapy to be a highly effective treatment for functional dyspepsia and functional chest pain of presumed esophageal origin unresponsive to standard care[24,25]. In both of these upper gastrointestinal diseases, clinical benefits were well maintained at long-term follow-ups[24,52]. However, both of these studies were carried out by the same research team -- the Manchester group in England[16]. Additional well designed studies from independent investigators are eagerly awaited to substantiate the efficacy of hypnotherapy in this domain.

REFERENCES

1 **Heap M**. The nature of hypnosis. *Eur J Gastroenterol Hepatol* 1996; **8**: 515-519
2 **von Kirchenheim C**, Persinger MA. Time distortion--a comparison of hypnotic induction and progressive relaxation procedures: a brief communication. *Int J Clin Exp Hypn* 1991; **39**: 63-66
3 **Benson H**. Hypnosis and the relaxation response. *Gastroenterology* 1989; **96**: 1609-1611
4 **Wickramasekera I**. How does biofeedback reduce clinical symptoms and do memories and beliefs have biological consequences? Toward a model of mind-body healing. *Appl Psychophysiol Biofeedback* 1999; **24**: 91-105
5 **Lynn SJ**, Nash MR. Truth in memory: ramifications for psychotherapy and hypnotherapy. *Am J Clin Hypn* 1994; **36**: 194-208
6 **Erickson MH**. The interspersal hypnotic technique for symptom correction and pain control. *Am J Clin Hypn* 1966; **8**: 198-209
7 **Forrest DW**. Hypnotism: A History. London, UK: Penguin, 1999
8 **Waxman D**. Hartland's Medical and Dental Hypnosis, 3rd Edition, London, UK: Harcourt Brace and Company Limited, 1998
9 **Integration of behavioral and relaxation approaches into the treatment of chronic pain and insomnia**. NIH Technology Assessment Panel on Integration of Behavioral and Relaxation Approaches into the Treatment of Chronic Pain and Insomnia. *JAMA* 1996; **276**: 313-318
10 **Richardson J**, Smith JE, McCall G, Pilkington K. Hypnosis for procedure-related pain and distress in pediatric cancer patients: a systematic review of effectiveness and methodology related to hypnosis interventions. *J Pain Symptom Manage* 2006; **31**: 70-84
11 **Uman LS**, Chambers CT, McGrath PJ, Kisely S. Psychological interventions for needle-related procedural pain and distress in children and adolescents. *Cochrane Database Syst Rev* 2006; CD005179
12 **Wilson S**, Maddison T, Roberts L, Greenfield S, Singh S. Systematic review: the effectiveness of hypnotherapy in the

management of irritable bowel syndrome. *Aliment Pharmacol Ther* 2006; **24**: 769-780
13 **Whitehead WE**. Hypnosis for irritable bowel syndrome: the empirical evidence of therapeutic effects. *Int J Clin Exp Hypn* 2006; **54**: 7-20
14 **Tan G**, Hammond DC, Joseph G. Hypnosis and irritable bowel syndrome: a review of efficacy and mechanism of action. *Am J Clin Hypn* 2005; **47**: 161-178
15 **Whorwell PJ**, Prior A, Faragher EB. Controlled trial of hypnotherapy in the treatment of severe refractory irritable-bowel syndrome. *Lancet* 1984; **2**: 1232-1234
16 **Whorwell PJ**. Review article: The history of hypnotherapy and its role in the irritable bowel syndrome. *Aliment Pharmacol Ther* 2005; **22**: 1061-1067
17 **Whorwell PJ**, Prior A, Colgan SM. Hypnotherapy in severe irritable bowel syndrome: further experience. *Gut* 1987; **28**: 423-425
18 **Harvey RF**, Hinton RA, Gunary RM, Barry RE. Individual and group hypnotherapy in treatment of refractory irritable bowel syndrome. *Lancet* 1989; **1**: 424-425
19 **Galovski TE**, Blanchard EB. The treatment of irritable bowel syndrome with hypnotherapy. *Appl Psychophysiol Biofeedback* 1998; **23**: 219-232
20 **Vidakovic-Vukic M**. Hypnotherapy in the treatment of irritable bowel syndrome: methods and results in Amsterdam. *Scand J Gastroenterol Suppl* 1999; **230**: 49-51
21 **Palsson OS**, Turner MJ, Johnson DA, Burnett CK, Whitehead WE. Hypnosis treatment for severe irritable bowel syndrome: investigation of mechanism and effects on symptoms. *Dig Dis Sci* 2002; **47**: 2605-2614
22 **Gonsalkorale WM**, Miller V, Afzal A, Whorwell PJ. Long term benefits of hypnotherapy for irritable bowel syndrome. *Gut* 2003; **52**: 1623-1629
23 **Gonsalkorale WM**, Houghton LA, Whorwell PJ. Hypnotherapy in irritable bowel syndrome: a large-scale audit of a clinical service with examination of factors influencing responsiveness. *Am J Gastroenterol* 2002; **97**: 954-961
24 **Calvert EL**, Houghton LA, Cooper P, Morris J, Whorwell PJ. Long-term improvement in functional dyspepsia using hypnotherapy. *Gastroenterology* 2002; **123**: 1778-1785
25 **Jones H**, Cooper P, Miller V, Brooks N, Whorwell PJ. Treatment of non-cardiac chest pain: a controlled trial of hypnotherapy. *Gut* 2006; **55**: 1403-1408
26 **Eichhorn R**, Tracktir J. The effect of hypnotically induced emotions upon gastric secretion. *Gastroenterology* 1955; **29**: 432-438
27 **Eichhorn R**, Tracktir J. The effect of hypnosis upon gastric secretion. *Gastroenterology* 1955; **29**: 417-421
28 **Kehoe M**, Ironside W. Studies on the experimental evocation of depressive responses using hypnosis. III. The secretory rate of total gastric acid with respect to various spontaneous experiences such as nausea, disgust, crying, and dyspnea. *Psychosom Med* 1964; **26**: 224-249
29 **Stacher G**, Berner P, Naske R, Schuster P, Bauer P, Starker H, Schulze D. Effect of hypnotic suggestion of relaxation on basal and betazole-stimulated gastric acid secretion. *Gastroenterology* 1975; **68**: 656-661
30 **Piper DW**, Greig M, Shinners J, Thomas J, Crawford J. Chronic gastric ulcer and stress. A comparison of an ulcer population with a control population regarding stressful events over a lifetime. *Digestion* 1978; **18**: 303-309
31 **Nasiry RW**, McIntosh JH, Byth K, Piper DW. Prognosis of chronic duodenal ulcer: a prospective study of the effects of demographic and environmental factors and ulcer healing. *Gut* 1987; **28**: 533-540
32 **Klein KB**, Spiegel D. Modulation of gastric acid secretion by hypnosis. *Gastroenterology* 1989; **96**: 1383-1387
33 **Beaugerie L**, Burger AJ, Cadranel JF, Lamy P, Gendre JP, Le Quintrec Y. Modulation of orocaecal transit time by hypnosis. *Gut* 1991; **32**: 393-394
34 **Bond JH Jr**, Levitt MD, Prentiss R. Investigation of small bowel transit time in man utilizing pulmonary hydrogen (H2) measurements. *J Lab Clin Med* 1975; **85**: 546-555
35 **La Brooy SJ**, Male PJ, Beavis AK, Misiewicz JJ. Assessment of the reproducibility of the lactulose H2 breath test as a measure of mouth to caecum transit time. *Gut* 1983; **24**: 893-896
36 **Chiarioni G**, Vantini I, De Iorio F, Benini L. Prokinetic effect of gut-oriented hypnosis on gastric emptying. *Aliment Pharmacol Ther* 2006; **23**: 1241-1249
37 **Benini L**, Castellani G, Sembenini C, Bardelli E, Caliari S, Volino C, Vantini I. Gastric emptying of solid meals in achalasic patients after successful pneumatic dilatation of the cardia. *Dig Dis Sci* 1994; **39**: 733-737
38 **Jian R**, Ducrot F, Ruskone A, Chaussade S, Rambaud JC, Modigliani R, Rain JD, Bernier JJ. Symptomatic, radionuclide and therapeutic assessment of chronic idiopathic dyspepsia. A double-blind placebo-controlled evaluation of cisapride. *Dig Dis Sci* 1989; **34**: 657-664
39 **Nightingale SL**. New warnings added to cisapride labeling. *JAMA* 1998; **280**: 410-412
40 **Hartland J**. Further observations on the use of "ego-strengthening" techniques. *Am J Clin Hypn* 1971; **14**: 1-8
41 **Colgan SM**, Faragher EB, Whorwell PJ. Controlled trial of hypnotherapy in relapse prevention of duodenal ulceration. *Lancet* 1988; **1**: 1299-1300
42 **Levi S**, Beardshall K, Swift I, Foulkes W, Playford R, Ghosh P, Calam J. Antral Helicobacter pylori, hypergastrinaemia, and duodenal ulcers: effect of eradicating the organism. *BMJ* 1989; **299**: 1504-1505
43 **Tack J**, Talley NJ, Camilleri M, Holtmann G, Hu P, Malagelada JR, Stanghellini V. Functional gastroduodenal disorders. *Gastroenterology* 2006; **130**: 1466-1479
44 **Locke GR 3rd**. Nonulcer dyspepsia: what it is and what it is not. *Mayo Clin Proc* 1999; **74**: 1011-1014; quiz 1015
45 **Talley NJ**, Boyce P, Jones M. Dyspepsia and health care seeking in a community: How important are psychological factors? *Dig Dis Sci* 1998; **43**: 1016-1022
46 **Moayyedi P**, Delaney BC, Vakil N, Forman D, Talley NJ. The efficacy of proton pump inhibitors in nonulcer dyspepsia: a systematic review and economic analysis. *Gastroenterology* 2004; **127**: 1329-1337
47 **Galmiche JP**, Clouse RE, Balint A, Cook IJ, Kahrilas PJ, Paterson WG, Smout AJ. Functional esophageal disorders. *Gastroenterology* 2006; **130**: 1459-1465
48 **Chambers J**, Bass C. Chest pain with normal coronary anatomy: a review of natural history and possible etiologic factors. *Prog Cardiovasc Dis* 1990; **33**: 161-184
49 **Clouse RE**, Carney RM. The psychological profile of non-cardiac chest pain patients. *Eur J Gastroenterol Hepatol* 1995; **7**: 1160-1165
50 **Numans ME**, Lau J, de Wit NJ, Bonis PA. Short-term treatment with proton-pump inhibitors as a test for gastroesophageal reflux disease: a meta-analysis of diagnostic test characteristics. *Ann Intern Med* 2004; **140**: 518-527
51 **Jackson JL**, O'Malley PG, Tomkins G, Balden E, Santoro J, Kroenke K. Treatment of functional gastrointestinal disorders with antidepressant medications: a meta-analysis. *Am J Med* 2000; **108**: 65-72
52 **Miller V**, Jones H, Whorwell PJ. Hypnotherapy for non-cardiac chest pain: long-term follow-up. *Gut* 2007; **56**: 1643
53 **Sarnelli G**, Caenepeel P, Geypens B, Janssens J, Tack J. Symptoms associated with impaired gastric emptying of solids and liquids in functional dyspepsia. *Am J Gastroenterol* 2003; **98**: 783-788
54 **Rhee PL**, Kim YH, Son HJ, Kim JJ, Koh KC, Paik SW, Rhee JC, Choi KW. Evaluation of individual symptoms cannot predict presence of gastric hypersensitivity in functional dyspepsia. *Dig Dis Sci* 2000; **45**: 1680-1684
55 **Tougas G**. The autonomic nervous system in functional bowel disorders. *Gut* 2000; **47** Suppl 4: iv78-iv80; discussion iv87

56 **Wallace RK**, Benson H, Wilson AF. A wakeful hypometabolic physiologic state. *Am J Physiol* 1971; **221**: 795-799

57 **Goldman MC**. Gastric secretion during a medical interview. *Psychosom Med* 1963; **25**: 351-356

58 **Thompson DG**, Richelson E, Malagelada JR. Perturbation of gastric emptying and duodenal motility through the central nervous system. *Gastroenterology* 1982; **83**: 1200-1206

59 **Stanghellini V**, Malagelada JR, Zinsmeister AR, Go VL, Kao PC. Stress-induced gastroduodenal motor disturbances in humans: possible humoral mechanisms. *Gastroenterology* 1983; **85**: 83-91

60 **Cann PA**, Read NW, Cammack J, Childs H, Holden S, Kashman R, Longmore J, Nix S, Simms N, Swallow K, Weller J. Psychological stress and the passage of a standard meal through the stomach and small intestine in man. *Gut* 1983; **24**: 236-240

61 **Lea R**, Houghton LA, Calvert EL, Larder S, Gonsalkorale WM, Whelan V, Randles J, Cooper P, Cruickshanks P, Miller V, Whorwell PJ. Gut-focused hypnotherapy normalizes disordered rectal sensitivity in patients with irritable bowel syndrome. *Aliment Pharmacol Ther* 2003; **17**: 635-642

62 **Mayer EA**, Naliboff BD, Craig AD. Neuroimaging of the brain-gut axis: from basic understanding to treatment of functional GI disorders. *Gastroenterology* 2006; **131**: 1925-1942

63 **Gregory LJ**, Yaguez L, Williams SC, Altmann C, Coen SJ, Ng V, Brammer MJ, Thompson DG, Aziz Q. Cognitive modulation of the cerebral processing of human oesophageal sensation using functional magnetic resonance imaging. *Gut* 2003; **52**: 1671-1677

64 **Meier W**, Klucken M, Soyka D, Bromm B. Hypnotic hypo- and hyperalgesia: divergent effects on pain ratings and pain-related cerebral potentials. *Pain* 1993; **53**: 175-181

65 **Faymonville ME**, Laureys S, Degueldre C, DelFiore G, Luxen A, Franck G, Lamy M, Maquet P. Neural mechanisms of antinociceptive effects of hypnosis. *Anesthesiology* 2000; **92**: 1257-1267

66 **Hilgard ER**, Morgan AH, Macdonald H. Pain and dissociation in the cold pressor test: a study of hypnotic analgesia with "hidden reports" through automatic key pressing and automatic talking. *J Abnorm Psychol* 1975; **84**: 280-289

67 **Levy RL**, Olden KW, Naliboff BD, Bradley LA, Francisconi C, Drossman DA, Creed F. Psychosocial aspects of the functional gastrointestinal disorders. *Gastroenterology* 2006; **130**: 1447-1458

68 **Palsson OS**, Whitehead WE. Hypnosis for non-cardiac chest pain. *Gut* 2006; **55**: 1381-1384

69 **Gonsalkorale WM**, Toner BB, Whorwell PJ. Cognitive change in patients undergoing hypnotherapy for irritable bowel syndrome. *J Psychosom Res* 2004; **56**: 271-278

70 **Musial F**, Klosterhalfen S, Enck P. Placebo responses in patients with gastrointestinal disorders. *World J Gastroenterol* 2007; **13**: 3425-3429

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REVIEW

Interstitial cells of Cajal in the gut - A gastroenterologist's point of view

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Abstract

Alterations of normal function of interstitial cells of Cajal (ICC) are reported in many intestinal disorders. Diagnosis of their involvement is rare (infrequent), but necessary to propose a specific treatment. This article reviews the place of ICC in the pathogenesis of achalasia, gastroesophageal reflux disease, infantile hypertrophic pyloric stenosis, chronic intestinal pseudo-obstruction and slow transit constipation. Moreover we discuss the role of the Cajal cells in the development of stromal tumors of the gastrointestinal tract.

Key words: Cajal cells; c-kit; Intestinal motility; Achalasia; Gastrointestinal stromal tumor

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INTRODUCTION

Digestive motility is highly coordinated and consists of local, non-propulsive mixing (segmental) and propulsive (peristaltic) movements. Mixing movements are produced by intrinsic pacemakers generating rhythmic contractions and peristalsis by intrinsic excitatory and inhibitory neural reflex pathways[1,2].

Even in the absence of stimulation, most regions of the gastrointestinal tract can generate some spontaneous electrical and mechanical activity. Recordings made from isolated muscle cells in the gastrointestinal tract show a regular discharge recorded as plateau and slow potentials. These pacemaker potentials are generated by a specialized population of cells, known as interstitial cells of Cajal (ICC)[3].

Together with the enteric nervous system, composed of both the myenteric (inter-muscular) plexus and the submucosal plexus, the ICC plays a major role in gastrointestinal motility[4]. The ICC was firstly described by Cajal SR in 1911[5]. He characterized "interstitial neurons" as "primitive accessory components that could modify smooth muscle contraction, subject themselves to regulation from principal neurons". Cajal provided detailed pictures of methylene blue-stained networks of interstitial cells, which were described as spindle shaped or stellate cells with long, ramified cell processes and large, oval, nuclei with sparse perinuclear cytoplasm, and intercalated between autonomic nerve endings and smooth muscle cells[5].

ICC constitutes networks that are widely distributed within the submucosal, intra-muscular and inter-muscular layers of the gastrointestinal tract from the lower esophagus to the internal anal sphincter.

These cells are defined by the expression of the CD117 (c-kit) protein which is a membrane receptor with tyrosine kinase activity[3,4,6].

In the past decade, knowledge of the role of ICC in the digestive physiology and pathology has progressed. In this review, we highlight some of these advances which could have clinical impact either in pathogenesis or treatment.

ESOPHAGUS

Achalasia

Achalasia is characterized by relaxation failure of lower

esophageal sphincter (LES) and lack of peristaltic contraction of esophageal body[7]. The etiology of this disorder is unknown and may be "idiopathic" or secondary to malignancy (local invasion or a paraneoplastic manifestation).

In primary or idiopathic achalasia, the failure of deglutitive inhibition is responsible for aperistalsis. This dysfunction is due to a loss of inhibitory nerves and progressive degeneration of ganglion cells containing vasoactive intestinal peptide (VIP) and nitric oxide (NO). Hypertensive LES is thought to result from a combination of the lack of tonic inhibitory nitrergic influence and an unopposed cholinergic activity.

The mechanism of inflammatory process responsible of these alterations is unclear. It is suggested to be an autoimmune disorder induced by a viral or food antigen in a patient genetically predisposed to the disease[8,9]. ICC involvement in achalasia is debated[10,11].

Electronic microscope studies of muscle coat of LES in seven patients with achalasia showed that muscle wall components (nerve endings, smooth muscle cells, ICC and connective tissue) were modified. ICC ultrastructure was altered, namely clear cytoplasm, fewer mitochondria, and scarce smooth endoplasmic reticulum. A reduced number of contacts between nerves and ICC were reported. Specific changes in smooth muscle cells were also documented, whereas the nerve endings had a normal ultrastructure. Alterations in older patients were more pronounced[12]. Since the LES components specifically altered in achalasia are the nerve endings and ICC, they are regarded as principally responsible for abnormal motility[12].

Achalasia is uncommon among pediatric population and some authors consider it as a different entity. Rare familial forms, combining early onset achalasia, alacrymia, ACTH insensitivity and dysautonomia, are known as Allgrove's syndrome or "four A" syndrome. Allgrove's syndrome is inherited in an autosomal recessive mode and may express in adulthood. Massive loss of neural elements and neuronal NO synthase as well as a marked fibrotic process of the muscle layers of the cardia have been observed in this syndrome[13]. ICC in cardia was also markedly decreased or absent while ICC (and neural structures) were preserved in pylorus[13].

Gastro-esophageal reflux disease (GERD)

GERD is a highly prevalent condition. Typical symptoms of heartburn and acid regurgitation are encountered in 15%-20% of the general population[14].

GERD represents the most common cause of esophagitis that may be complicated with esophageal ulcers, peptic stenosis and Barrett's esophagus, which carries a high risk of esophageal adenocarcinoma[14].

The role of the ICC in inhibitory transmission in the LES is still discussed.

In W/W_v mutant mice (lack of ICC) LOS pressure was lower than wild-type mice but a normal swallow still induced LOS relaxation, arguing against the role of ICC in inhibitory transmission[15]. Another study demonstrated that in W/W_v animals, cholinergic and nitrergic neurotransmission is greatly reduced pleading for the role of ICC in mediating neural inputs[16]. However enteric neurons, varicose processes, and the ability to release neurotransmitters are not reduced, and smooth muscle cells demonstrate responsiveness to exogenous transmitters[16].

Loss of ICC during development or in pathologic conditions would significantly compromise the ability of GI muscles to generate typical motor reflexes[17].

Esophagitis itself may be at the origin of an alteration of normal function of the Cajal cells: in advanced stages of GERD, inflammatory changes in the esophageal wall will also involve the ICC. That way, the more severe the esophagitis, the more severe is the ICC impairment. This destruction leads to loss of effective contraction of esophagus, maintaining reflux and thus aggravating the symptoms[18].

STOMACH

Gastroparesis

Delayed gastric emptying can be secondary to muscular, neural, humoral causes or use of anticholinergic and opiates medicines. In the absence of an identified cause, gastroparesis is termed as idiopathic[19]. Clinical features of gastroparesis are frequently indistinguishable from true mechanical obstruction and severity of symptoms is variable. Most patients present with early satiety, nausea, and abdominal pain. In some cases, symptoms can be highly incapacitating: chronic abdominal pain and vomiting leading to dehydration, electrolyte imbalance, nutritional impairment and weight loss[20,21].

ICC is involved in regulation of gastric emptying by generating slow waves.

A decrease in ICC density ranged from 60% to 100% depending on the area investigated was demonstrated in histologic studies of stomach of type 1 diabetic patients[6]. The number of immunopositive cells for c-kit was significantly decreased in the corpus and antrum of the gastroparesis patients compared with control tissues[21]. The loss of intra muscular ICC and associated nerves in the gastric fundus could explain the low basal gastric tone and increased compliance of the stomach. The hypomotility of the antrum can also be explained by the absence of slow wave generation by the ICC[21,22].

Infantile hypertrophic pyloric stenosis

This is a congenital disorder characterized by functional gastric-outlet obstruction. Dysfunction of pyloric inhibition has been implicated in the pathophysiology of hypertrophic pyloric stenosis. Normal inhibition process is mediated by peptidergic and NO enteric nerves and also may involve ICC. Although myenteric neurons appear normal, those innervating the circular-muscle layer of the pyloric sphincter lack NO synthetase[21]. In children with hypertrophic pyloric stenosis, there was a significant decrease in the number of ICC[22,23]. The following observations were made using electron microscopy in gastric specimens from patients with pyloric stenosis versus normal controls[24]. Muscle cells were primarily in a

proliferative phase and exhibited very few gap junctions between smooth muscle cells or ICC: (1) Near absence of nerve fibers containing large granular vesicles in the circular muscle layer; (2) Fewer nerve cell bodies in the myenteric plexus and lower total number of ganglia; (3) Decreased number of ICC. These findings may plead for a role of ICC in the pathogenesis.

SMALL INTESTINE AND COLON

Idiopathic chronic intestinal pseudo-obstruction (CIIP)

CIIP is characterized by defective gastrointestinal propulsion together with symptoms and signs of bowel obstruction in the absence of any lesions or mechanical obstacle[25]. CIIP is regarded as a neuropathy, myopathy or both[26,27].

A possible role played by the ICC is demonstrated by the alterations in ICC network reported in patients with CIIP. Electron microscopy and immunochemistry studies showed a decreased number of ICCs along with structural abnormalities such as loss of processes and damaged intracellular cytoskeleton and organelles[28].

Slow transit constipation (STC)

This is a very prevalent motility problem, but its mechanisms are unclear. Studies found that ICC density in the colon of patients with constipation was significantly decreased compared with those of normal patients[29]. Expression of *c-kit* mRNA and c-kit protein was significantly decreased in the colon of STC, suggesting that the c-kit signal pathway may play an important role in ICC reduction in STC[30-32].

Since slow-transit constipation is secondary to problems with the ENS, ICC, or smooth muscle cells, replacement of the missing or defective cells would be an attractive way of treatment[31]. Growing precursors of the defective cells from stem cells should be easy, but the distribution of the cells to their proper locations is still problematic[31,32]. For the moment this is a promise of genetic treatment.

TUMORS OF GASTROINTESTINAL TRACT

Gastrointestinal stromal tumors (GISTs)

GISTs have been recognized as a biologically distinctive tumor type, different from smooth muscle and neural tumors of the gastrointestinal tract. They constitute the majority of gastrointestinal mesenchymal tumors[33].

GISTs originate from the ICC. Their origin from the ICC has been proven by their immunophenotypic (CD117 positive) and ultrastructural resemblance and also by the presence of an embryonic smooth muscle myosin similar to the one present in the ICC[33-36] (Table 1). Approximately 80% of GISTs also express CD34.

Annual incidence of clinically detected new cases of GISTs in the United States has increased to 5000-6000 per year due to better diagnosis, and incidence is rising. Uncommonly, GISTs arise in families, and in these patients germline mutations of c-kit have been identified particularly in exons 11 and 13. A diffuse hyperplasia of the ICC, which is regarded as a pre-neoplastic lesion is noted in these patients[33]. The patients with exon 11 mutations develop cutaneous mastocytosis with or without cutaneous hyperpigmentation, but those with exon 13 mutations do not have these features[33,34]. The tumors under 3 cm in diameter are mostly benign, but all GISTs have a malignant potential[35].

Table 1 Immunohistochemical analysis of GI mesenchymal tumors[35,36]

Tumor	Positive immunohistochemical staining
GIST	CD 117
	CD 34
Malignant GIST	Ki 67
Smooth muscle tumor	Smooth muscle actin
	Desmin
Schwannoma	S100
Glomus tumor	Smooth muscle actin
	Vimentin

The majority of GISTs occurs in the stomach (60%-70%), small intestine (20%-30%) and only 10% or less in the esophagus, colon and rectum, and they affect mainly middle aged patients. Similar tumors, sometimes known as extra-gastrointestinal stromal tumors (E-GIST), may arise in the omentum, mesentery, or retroperitoneum and at least one case of pancreatic tumor was described[37,38]. The presence of ICC in normal pancreas was demonstrated recently[39].

The symptoms may vary from none or slight abdominal discomfort to brisk gastrointestinal hemorrhage, perforation or obstruction.

Imatinib mesylate, a synthetic tyrosine kinase inhibitor developed for the use in the management of interferon resistant chronic myeloid leukemia (CML), was shown to be effective against a number of other tyrosine kinases including c-kit and platelet derived growth factor (PDGF) and now it is considered to be the drug of choice for metastatic and inoperable GIST's[33,34].

CONCLUSION

Knowledge on the role of ICC in gastrointestinal disorders is increasing. However, with the exception of GIST's, no major breakthrough has been made in treatment. Further studies may provide new treatments.

REFERENCES

1 **Stevens RJ**, Publicover NG, Smith TK. Induction and organization of Ca2+ waves by enteric neural reflexes. *Nature* 1999; **399**: 62-66
2 **Wood JD**. Mixing and moving in the gut. *Gut* 1999; **45**: 333-334
3 **Ward SM**. Interstitial cells of Cajal in enteric neurotransmission. *Gut* 2000; **47** Suppl 4: iv40-iv43; discussion iv52
4 **Takaki M**. Gut pacemaker cells: the interstitial cells of Cajal (ICC). *J Smooth Muscle Res* 2003; **39**: 137-161
5 **Cajal SR**. Histology of the nervous system of man and

vertebrates (translated by N Swanson and LW Swanson), New York: Oxford University Press, 1995: 891-942
6 **Long QL**, Fang DC, Shi HT, Luo YH. Gastro-electric dysrhythm and lack of gastric interstitial cells of cajal. *World J Gastroenterol* 2004; **10**: 1227-1230
7 **Sifrim D**, Janssens J, Vantrappen G. Failing deglutitive inhibition in primary esophageal motility disorders. *Gastroenterology* 1994; **106**: 875-882
8 **Robertson CS**, Martin BA, Atkinson M. Varicella-zoster virus DNA in the oesophageal myenteric plexus in achalasia. *Gut* 1993; **34**: 299-302
9 **Metman EH**, Lagasse JP, Pic P, Picon L, Danquechin Dorval E, Goudeau A. [Varicella and primary achalasia of the lower esophageal sphincter] *Gastroenterol Clin Biol* 1996; **20**: 1138-1139
10 **Ward SM**, Morris G, Reese L, Wang XY, Sanders KM. Interstitial cells of Cajal mediate enteric inhibitory neurotransmission in the lower esophageal and pyloric sphincters. *Gastroenterology* 1998; **115**: 314-329
11 **Sanders KM**, Ward SM, Daniel EE. ICC in neurotransmission: hard to swallow a lack of involvement. *Gastroenterology* 2002; **122**: 1185-1186; author reply 1186-1187
12 **Faussone-Pellegrini MS**, Cortesini C. The muscle coat of the lower esophageal sphincter in patients with achalasia and hypertensive sphincter. An electron microscopic study. *J Submicrosc Cytol* 1985; **17**: 673-685
13 **Metman EH**, Debbabi S, Negreanu L. Troubles moteurs de l' œsophage, Encyclopédie medico-chirurgicale. *Elsevier* 2006; **4**: 1-19
14 **Richter JE**. Gastrooesophageal reflux disease. *Best Pract Res Clin Gastroenterol* 2007; **21**: 609-631
15 **Dickens EJ**, Edwards FR, Hirst GD. Selective knockout of intramuscular interstitial cells reveals their role in the generation of slow waves in mouse stomach. *J Physiol* 2001; **531**: 827-833
16 **Ward SM**, Beckett EA, Wang X, Baker F, Khoyi M, Sanders KM. Interstitial cells of Cajal mediate cholinergic neurotransmission from enteric motor neurons. *J Neurosci* 2000; **20**: 1393-1403
17 **Ward SM**, Sanders KM. Physiology and pathophysiology of the interstitial cell of Cajal: from bench to bedside. I. Functional development and plasticity of interstitial cells of Cajal networks. *Am J Physiol Gastrointest Liver Physiol* 2001; **281**: G602-G611
18 **Shafik A**, El-Sibai O, Shafik I, Shafik A. Electroesophagogram in gastroesophageal reflux disease with a new theory on the pathogenesis of its electric changes. *BMC Surg* 2004; **4**: 13
19 **Forster J**, Damjanov I, Lin Z, Sarosiek I, Wetzel P, McCallum RW. Absence of the interstitial cells of Cajal in patients with gastroparesis and correlation with clinical findings. *J Gastrointest Surg* 2005; **9**: 102-108
20 **Hirst GD**, Edwards FR. Role of interstitial cells of Cajal in the control of gastric motility. *J Pharmacol Sci* 2004; **96**: 1-10
21 **Ibba Manneschi L**, Pacini S, Corsani L, Bechi P, Faussone-Pellegrini MS. Interstititал cells of Cajal in the human stomach: distribution and relationship with enteric innervation. *Histol Histopathol* 2004; **19**: 1153-1164
22 **Ordog T**, Redelman D, Horvath VJ, Miller LJ, Horowitz B, Sanders KM. Quantitative analysis by flow cytometry of interstitial cells of Cajal, pacemakers, and mediators of neurotransmission in the gastrointestinal tract. *Cytometry A* 2004; **62**: 139-149
23 **Vanderwinden JM**, Rumessen JJ. Interstitial cells of Cajal in human gut and gastrointestinal disease. *Microsc Res Tech* 1999; **47**: 344-360
24 **Langer JC**, Berezin I, Daniel EE. Hypertrophic pyloric stenosis: ultrastructural abnormalities of enteric nerves and the interstitial cells of Cajal. *J Pediatr Surg* 1995; **30**: 1535-1543
25 **De Giorgio R**, Sarnelli G, Corinaldesi R, Stanghellini V. Advances in our understanding of the pathology of chronic intestinal pseudo-obstruction. *Gut* 2004; **53**: 1549-1552
26 **Coulie B**, Camilleri M. Intestinal pseudo-obstruction. *Annu Rev Med* 1999; **50**: 37-55
27 **De Giorgio R**, Guerrini S, Barbara G, Cremon C, Stanghellini V, Corinaldesi R. New insights into human enteric neuropathies. *Neurogastroenterol Motil* 2004; **16** Suppl 1: 143-147
28 **Feldstein AE**, Miller SM, El-Youssef M, Rodeberg D, Lindor NM, Burgart LJ, Szurszewski JH, Farrugia G. Chronic intestinal pseudoobstruction associated with altered interstitial cells of cajal networks. *J Pediatr Gastroenterol Nutr* 2003; **36**: 492-497
29 **Basilisco G**, Gebbia C, Peracchi M, Velio P, Conte D, Bresolin N, Nobile-Orazio E. Cerebellar degeneration and hearing loss in a patient with idiopathic myenteric ganglionitis. *Eur J Gastroenterol Hepatol* 2005; **17**: 449-452
30 **Tong WD**, Liu BH, Zhang LY, Xiong RP, Liu P, Zhang SB. Expression of c-kit messenger ribonucleic acid and c-kit protein in sigmoid colon of patients with slow transit constipation. *Int J Colorectal Dis* 2005; **20**: 363-367
31 **Schiller LR**. New and emerging treatment options for chronic constipation. *Rev Gastroenterol Disord* 2004; **4** Suppl 2: S43-S51
32 **Rao SS**. Constipation: evaluation and treatment. *Gastroenterol Clin North Am* 2003; **32**: 659-683
33 **D'Amato G**, Steinert DM, McAuliffe JC, Trent JC. Update on the biology and therapy of gastrointestinal stromal tumors. *Cancer Control* 2005; **12**: 44-56
34 **de Silva CM**, Reid R. Gastrointestinal stromal tumors (GIST): C-kit mutations, CD117 expression, differential diagnosis and targeted cancer therapy with Imatinib. *Pathol Oncol Res* 2003; **9**: 13-19
35 **Hwang JH**, Kimmey MB. The incidental upper gastrointestinal subepithelial mass. *Gastroenterology* 2004; **126**: 301-307
36 **Ando N**, Goto H, Niwa Y, Hirooka Y, Ohmiya N, Nagasaka T, Hayakawa T. The diagnosis of GI stromal tumors with EUS-guided fine needle aspiration with immunohistochemical analysis. *Gastrointest Endosc* 2002; **55**: 37-43
37 **Nakagawa M**, Akasaka Y, Kanai T, Yamashita T, Kuroda M, Takayama H, Miyazawa N. Extragastrointestinal stromal tumor of the greater omentum: case report and review of the literature. *Hepatogastroenterology* 2003; **50**: 691-695
38 **Yamaura K**, Kato K, Miyazawa M, Haba Y, Muramatsu A, Miyata K, Koide N. Stromal tumor of the pancreas with expression of c-kit protein: report of a case. *J Gastroenterol Hepatol* 2004; **19**: 467-470
39 **Popescu LM**, Hinescu ME, Ionescu N, Ciontea SM, Cretoiu D, Ardelean C. Interstitial cells of Cajal in pancreas. *J Cell Mol Med* 2005; **9**: 169-190

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REVIEW

Current status of intrahepatic cholangiocarcinoma

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Abstract

Intrahepatic cholangiocarcinoma (ICC) is a rare primary liver cancer with a global increasing trend in recent years. Symptoms tend to be vague and insidious in development, often are diagnosed at an advanced stage when only palliative approaches can be used with a median survival rate of months. Comparing with HCC, ICC tends to spread to lymph nodes early, and is rarely limited to the regional lymph nodes, with a frequent postoperative recurrence. Surgery is the only choice of curative therapy for ICC, but recently no consensus has been established for operation. Thus, more data from multiple centers and more cases are needed. Generally speaking, current adjunctive therapy cannot clearly improve survival. Further research is needed to find more effective radio- and chemotherapeutic regimens.

Key words: Intrahepatic cholangiocarcinoma; Lymph node metastasis; Liver transplantation; Adjunctive therapy

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EPIDEMIOLOGY

Intrahepatic cholangiocarcinoma (ICC) is a rare malignant tumor which arises from the epithelial cells of intrahepatic bile ducts (beyond the second order bile ducts). The incidence of ICC is reported to be only about 10% of primary liver cancers. But, recent studies from several countries have indicated that the incidence of ICC is increasing which cannot be solely explained by reclassification and improved detection[1-10]. The rate of ICC for males is greater than that for females; but ICC is less distinct than hepatocellular carcinoma and usually occurs after the sixth decade of life[1-10]. A recent study reported that in addition to the established risk factors such choledochal cysts, chronic cholangitis, inflammatory bowel disease, primary sclerosing cholangitis (PSC) parasitic infections, drug or toxin exposure, and genetic risks, other conditions such as biliary cirrhosis, cholelithiasis, alcoholic liver disease, nonspecific cirrhosis, are significantly associated with ICC[11]. The incidence of diabetes, thyrotoxicosis, chronic pancreatitis, obesity, chronic nonalcoholic liver disease, HCV/HBV infection, chronic typhoid carrier state and smoking, is increasing, suggesting that these conditions might partly explain the trends of ICC in incidence[12]. However, many tumors arise in the absence of any known predisposition[13-17]. Despite the global increase, regional, racial, ethnic, gender and age variations occur. Moreover, it was reported that the incidence of ICC has decreased in Denmark[18,19]. ICC has the worst prognosis of any tumor arising in the liver; its 5-year survival is poor, and accompanied by a high recurrence rate. The overall 5-year survival rate ranges 13%-42%[20-22]. Chu *et al*[23] showed that the median survival after conservative therapy and hepatic resection is 1.8 mo and 12.2 mo, respectively.

DIAGNOSIS

Recent advances have been made in diagnosis of ICC with MRCP combined MRI, CT, positron-emission tomography scanning (PET) with [F-18] fluorodeoxyglucose (FDG), virtual three-dimensional images and optical coherence tomography (OCT), a high-resolution imaging technique that produces cross-sectional images *in vivo*[24,25], endoscopic retrograde cholangiography (ERCP) with brush cytology and biopsy, endoscopic ultrasound with guided fine-needle aspiration, advanced cytological tests including fluorescent *in situ* hybridization or

digital image analysis (DIA), cholangioscopy (peroral cholangioscopy, percutaneous cholangioscopy, transpapillary cholangioscopy)[26,27]. Sandwich enzyme-linked immunosorbent assay can show a 71% sensitivity and 90% specificity for new tumor markers in serum and bile including genomic and proteomic markers [such as CA199, CEA and mucin5, subtypes A and C (MUC5AC)][28]. On the other hand, most patients present too late to be diagnosed at an advanced stage when only palliative approaches can be used with a median survival of months.

Macroscopic aspect

ICC is defined as a kind of tumor originating from the second branch (segmental branch) or the proximal branch of bile duct[29] and further classified into hilar type and peripheral type. The former arises from the large intrahepatic biliary epithelium (segmental branches) having histological features of a papillary epithelial component or a large tubular component. The latter arises from small biliary epithelium (smaller than segmental branches) with histological features of small-sized glands in a fibrotic background, closely packed, somewhat distorted small ducts, and cordlike structure, but lacking large glands, and Shinichi Aishima, *etc*. It was recently reported that ICC is associated with different predispositions when arising from different levels of the biliary tree and likely to show an aggressive course even in cases of a small tumor arising from the large biliary duct[30,31].

Histological aspect

ICC, arising from cholangiocytes, is a moderately- to well-differentiated tubular adenocarcinoma. Papillary adenocarcinoma, signet-ring carcinoma, squamous cell or muco-epidermoid carcinoma and lymphoepithelioma-like forms are rare histological variants. The most outstanding histological feature is the presence of abundant desmoplastic stroma in ICC compared with HCC, leading to a low diagnostic yield of random biopsies. Desmoplasia may also cause capsular retraction. According to the degree of stromal desmoplasia in the tumor and Kajiyama, Kiyoshi, *etc*, ICC is microscopically categorized into scirrhous-type (SICC) and nonscirrhous-type (NSICC). The frequencies of lymphatic permeation, perineural invasion and the proliferative activity measured by MIB-1 immunostaining, monoclonal antibody specific for Ki-67 [a nuclear antigen expressed throughout the cell cycle (G_1, S-G_2 and M), but absent in quiescent cells (G_0)], were significantly higher in SICC than in NSICC, and serosal invasion, vascular invasion, lymph nodes metastases also tend to be more frequent in SICC and are closely related to the prognosis[32].

Classification

Three types of ICC have been established using the TNM staging system and the classification system established by the Liver Cancer Study Group of Japan: mass-forming type (MF), periductal-infiltration type (PI) and intraductal growth type (IG). MF type forms a definite round-shaped mass with an expansive growth pattern, but without fibrous capsule, and locates in the liver parenchyma. The border between cancerous and non-cancerous portions is distinct, and this type of ICC does not invade the major branch of the portal triad. PI type is defined as a mass extending longitudinally along the bile duct, occasionally involves the surrounding blood vessels and/or hepatic parenchyma, often resulting in dilatation of the peripheral bile duct. IG type proliferates towards the lumen of bile duct papillary or like a tumor thrombus, occasionally involving superficial extension. This type of ICC is usually detected in a thick bile duct[29].

Table 1 Staging system for ICC proposed by Liver Cancer Study Group of Japan

T1: Meet requirements (single nodule, tumor 2 cm or less and no portal vein, hepatic vein and serous membrane invasion)			
T2: Meet two of the three requirements			
T3: Meet one of the three requirements			
T4: Meet none of the three requirements			
N1: No metastasis to lymph node			
N2: Metastasis to any lymph nodes			
M0: No distant metastasis			
M1: Positive distant metastasis			
Stage Ⅰ	T1	N0	M0
Stage Ⅱ	T2	N0	M0
Stage Ⅲ	T3	N0	M0
Stage ⅣA	T4	N0	M0
	Or any T	N1	M0
Stage ⅣB	Any T	Any N	M1

Staging system

ICC is a rare type of primary liver cancer, accounting only for 5%-10% of all liver cancers, and has a low resectability rate. So the International Union against Cancer (UICC) defined the TNM staging system solely from clinical experience in treating HCC[33]. Based on the distinct difference in the mechanism and biologic behavior between HCC and ICC, the Liver Cancer Study Group of Japan has proposed a new TNM staging system for the MF type of ICC (Table 1)[34]. Serosal invasion is not a T-factor component in the UICC tumor staging system. Uenishi *et al*[35] retrospectively analyzed sixty-three patients who underwent hepatic resection for mass-forming intrahepatic cholangiocarcinoma between January 1983 and December 2003, and found that that serosal invasion has no impact on survival of patients after hepatic resection for MF type of ICC. Another staging system used for MF type of ICC, defined a solitary tumor without vascular invasion as stage Ⅰ, a solitary tumor with vascular invasion as stage Ⅱ, multiple tumor with or without vascular invasion as stage ⅢA, tumor with regional lymph node metastasis as stage ⅢB, tumor with distant metastases as stage Ⅳ. In this system, tumor size is excluded from T factor. It is likely that the influence of tumor size on its prognosis cannot be evaluated because the number of small tumors is too small[36].

Table 2 Lymph node groups by tumor location

	N1	N2	N3
Right lobe	Hepatoduodenal ligament	Along left gastric artery Along common hepatic artery Along celiac artery Posterior surface of pancreas head	Distant
Left lobe	Right cardiac region	Along left gastric artery	
	Lesser curvature of stomach	Along common hepatic artery	
		Along celiac artery	Distant
	Hepatoduodenal ligament	Posterior surface of pancreas head	

N3 distant: Abdominal aorta, root of the mesentery, inferior vena cava, *etc.*

Spreading mode

The spreading modes of ICC, such as sinusoidal invasion, spreading along duct walls and periductal tissue, growth replacing the biliary epithelium or intraductal growth, spreading along Glisson's sheath (lymphatic involvement, perineural or intraneural invasion, permeation of the portal connective tissue and vascular involvement) have been reported[37-40]. Nakajima *et al*[37] reported that sinusoidal invasion and portal vein invasion are the most frequent mode of intrahepatic spread. Different macroscopic types of ICC have different modes of spread. The MF type of ICC tends to invade the liver *via* the portal vein system and Glisson's sheath when the tumor increases in size with a frequent remnant hepatic recurrence. The PI type of ICC has a tendency to infiltrate making it difficult to get clear margins during hepatectomy, and to spread along Glisson's sheath *via* lymphatic vessels, thus invading connective tissue and major vessels at the hilum and hepatoduodenal ligament. The IG type of ICC has an extremely favorable prognosis after surgical resection compared with the other two types. Moreover, this type of ICC has a lower rate of lymphatic or intrahepatic metastasis and recurrence after curative surgical resection[37-39]. Yamamoto *et al*[40] suggested that anatomic and extensive hepatectomy is a rational procedure for MF type of ICC, and hepatectomy with extrahepatic duct excision and hilar lymph node resection is a rational procedure for IP and MF types of ICC with biliary invasion. The Liver Cancer Study Group of Japan has proposed a criterion for the invasion degrees of ICC: (1) no tumor invasion of the portal vein, hepatic vein, or bile duct; (2) tumor invasion distal to the second branch of the portal vein or bile duct and/or invasion of a branch of the hepatic vein; (3) tumor invasion of the second branch of the portal vein or the bile duct, the major hepatic veins and/or the short hepatic veins; (4) tumor invasion of the first branch of the portal vein or of the bile duct and tumor invasion of the inferior vena cava[29].

Lymph node metastasis

The most outstanding pattern of ICC compared with HCC is early lymphatic spread. The findings in the majority recent literature indicate that lymph node status is an important prognostic factor for patients undergoing hepatic resection[41-47]. Yet nodal status does not affect survival after aggressive surgical treatment in patients with ICC[48], and some long-term survivors with positive lymph nodes have also been reported[49-51]. It was reported that the rate of metastasis for ICC to hilar lymph nodes is about 50%[41,42,52]. Nakagawa *et al*[43] reported that the positive rate of lymph nodes in patients with lymph node dissection is 47%, 33%, 17%, 13%, 10%, 3%, respectively. Regarding the pattern of lymph node spread, the Liver Cancer Study Group of Japan has proposed a classification of regional lymph nodes in liver cancer (Table 2) and three major routes of lymphatic spread of ICC: hepatoduodenal route, cardiac route (through the lesser omentum to the cardiac portion of the stomach and the gastric lesser curvature), and diaphragmatic route[48]. Hepatoduodenal ligament is the most common site of nodal metastasis in ICC patients irrespective of the tumor location. Almost all patients are involved in positive lymph nodes of the hepatoduodenal ligament or along the common hepatic artery, lymph nodes are also found in about half of the patients involving the left lobe of liver[43,44,53-56]. Nozaki *et al*[57] reported that extensive lymph node metastasis was observed in most patients, only 3 (20%) of 15 patients with lymph node metastasis had regional lymph node metastasis. Shimada *et al*[47] has reported the similar observations, suggesting that lymph node metastasis of ICC is rarely limited to the regional lymph nodes.

Surgical treatment

Surgery is the only choice of curative therapy for ICC. However, only a few patients are suitable for surgery. Good results depend on comprehensive preoperative evaluation, patient selection and discreet operation.

EVALUATION

Assessment of resectability

Tumors that are medically fit for hepatic resection must be completely resected with negative histological margins, no evidence of metastases, disseminated disease, and extensive lymphadenopathy. The following factors must be considered. (1) Biliary tract invasion: bilateral involvement of hepatic ducts to the level of the secondary biliary radicals, atrophy of one liver lobe with contralateral secondary biliary radical involvement is a contraindication to resection. (2) Lymph node metastasis: Inoue *et al*[46] reported that the outcome of 16 patients with lymph node metastasis, accounting for 31.4% of all patients, was quite poor. Their median survival time was 14.1 mo and none of them survived 5 years except for one patient with the IG type of ICC, suggesting that the presence of lymph node metastasis in the MF type of ICC is a sign of non-curability. However, a longer survival time (over 5 years) in ICC patients with lymph node metastasis has been described[54,58]. (3) Vessel invasion: Based on some

centers' support for resection of ICC with vascular reconstruction *en bloc*, involvement of the main hepatic artery or portal vein is the relative contraindication to resection[40,59]. (4) Intrahepatic metastasis: Intrahepatic metastasis in the remaining liver is considered unfit for hepatic resection, and disseminated disease should not undergo hepatectomy. (5) Hepatic functional reserve: It is important to accurately estimate liver reserve function before hepatectomy to avoid postoperative liver failure. Methods to evaluate liver function, including routine examinations of aminotransferase, bilirubin, albumin, prothrombin time, Child-Pugh classification, and hepatic imaging providing volumetric information, indocyanine green (ICG) test. The indocyanine green retention rate at 15 min [ICG (R15)] has recently been considered a sensitive marker for liver reserve function. Nevertheless, it remains imperfect. Moreover, how to evaluate the maximal hepatic resection volume according to liver reserve function remains controversial. Trimethadione (TMO) tolerance test can show the Child-Pugh score in evaluation of cirrhosis. Hepatic 99mTc-diethylenetriamine pentaacetic acid-galactosyl-human serum albumin (99mTc-GSA) clearance test can show postoperative hepatic function and liver stiffness assessed quantitatively with a tactile sensor. The combination of Child-Pugh score, presence of ascites, serum bilirubin levels, indocyanine green retention (ICG R15) value, and remnant liver CT volumetry as well as age, diabetes, cardiopulmonary function, and general performance need to be taken into consideration preoperatively. Other factors affecting respectability include the size and extent of the tumor[60].

Evaluation modalities

Ultrasound can diagnose biliary dilatation and suspected cholangiocarcinoma by localizing the site of obstruction and excluding gallstones. Color Doppler can detect tumor-induced compression/thrombosis of the portal vein or hepatic artery. However, ultrasound is non-specific, often misses small perihilar, extrahepatic, and periampullary tumors, and is not good at defining the extent of tumor.

CT can detect biliary dilatation, intrahepatic cholangiocarcinoma greater than 1 cm in diameter, small liver metastases, lymphadenopathy, biliary obstruction, suspected perihilar tumor or tumor involving the portal venous/arterial system. However, CT can only establish the resectability in 60% of cases[61], and cannot usually define the extent of cholangiocar-cinoma because of the sclerosis and fibrosis of surrounding tissue. In addition, CT cannot accurately differentiate ICC from PSC[62].

MRI along with MRCP can detect ICC and assess preoperative ICC patients by investigating all involved structures, such as the bile ducts, vessels, and hepatic parenchyma, which are important factors for prognosis. Some new tissue-specific MR contrast agents with hepatobiliary and reticuloendothelial cell affinity, such as gadobenate, and ultra-small iron-oxide (USPIO) particles contrast agents with lymph node specificity can be used to detect and assess tumor invasion[63-65]. MRCP may have some potential advantages over CT in identifying intrahepatic mass lesions, and can provide a three-dimensional computerized reconstruction of the biliary tree allowing assessment of bile ducts both above and below a stricture. The non-invasively acquired cholangiographic images obtained by MRCP are comparable with invasive cholangiographies (ERCP and PTC), high positive and negative predictive values for detecting the level and features of biliary obstructions[61,66-68]. Owing to its intrinsically high tissue contrast and multiplanar capability, MRCP is superior to ERCP for defining the anatomy of tumor and assessing its respectability. However, the tendency of MRCP to understage the extent of cholangiocarcinoma has been reported[69]. MRI is not superior to CT in identifying lymph node metastasis.

PET scanning with the focal accumulation of nucleotide tracer 18-fluorodeoxyglucose (FDG) is an emerging staging technique for many cancers. This technique can detect nodular cholangiocarcinoma as small as 1 cm in diameter, but is less sensitive to infiltrating tumors[70]. FDG-PET has a higher specificity for lymphadenopathy than CT, although there is no difference in sensitivity between them[71,72]. In a retrospective study, 21 patients with ICC underwent CT, MRI and PET for lymph node metastasis, which were concordant in 16 patients and discordant in 5 patients (positive FDG-PET in three, positive CT and MRI in two). Moreover, PET may has some superiority over CT and MRI in detecting distant metastases[71].

The above non-invasive techniques may be complementary and sometimes are all necessary as part of surgical assessment depending on the clinical situation. Furthermore, invasive modalities are also needed sometimes to assess the resectability and predict the prognosis. In most cases, ERCP/PTC is replaced by MRCP, but ERCP with OCT can provide more information for surgical plan[21]. Another advantage of these techniques over MRCP is that washing, brushing and intraductal biopsies can be obtained for cytopathologic analysis, adding some new cytological tests, such as DIA, fluorescent *in situ* hybridization, so that the sensitivity increases, especially to patients with PSC or apparent biliary obstruction[73,74]. But, negative cytology from brushings does not exclude malignancy. Preoperative (percutaneous choledochoscope) and intraoperative vholedochoscope with biopsy can help to make an early diagnosis. Blood vessel involvement is an important prognosis factor. As a means of evaluating vascular invasion, hypovascular or hypervascular lesion, concomitant vascular resection and reconstruction, angiography should be reserved in some cases. Percutaneous transhepatic portography (PTP) and retrograde selective hepatic venography should be selected. Virtual 3D is a new kind of technique for constructing three-dimensional virtual images of the portal vein, hepatic artery, and bile ducts. On account of it, accurate knowledge of partial anatomy can be gotten. Preoperative planning for complex biliary

surgery especially lesions invading the hepatic hilum may be improved[75]. ICC in patients with lymphadenopathy is often missed on preoperative imaging. Endoscopic ultrasound can be useful in identifying local lymph node enlargement and allows a good view of distal extrahepatic biliary tree and vasculature[26]. The sensitivity of fine needle aspiration of the tumor mass or its surrounding lymph nodes and endoscopic ultrasound is greater than ERCP with brushings in detecting malignancy[26,27,76]. Endoscopic ultrasound-guided regional lymph node sampling can be performed in early disease to assess the respectability or eligibility for transplantation[77]. However, endoscopic aspiration of hilar masses is not recommended because of the potential of tumor seeding. Laparoscopy is gradually replaced by ultrasonography and other imaging studies, but has identified a third case of peritoneal and superficial liver metastases[51,78,79].

OPERATION

Hepatectomy

It was recently reported that aggressive surgical strategies in the treatment of ICC can significantly increase the survival of ICC patients[14,80,81]. Yamamoto *et al*[82] and Ohashi *et al*[83] suggested that anatomic and extensive hepatectomy is the rational procedure for mass-forming ICC, while hepatectomy with extrahepatic duct excision, and hilar lymph nodal resection is the rational procedure for infiltrating ICC. The 3- and 5-year survival rates of ICC patients after curative resection (n = 56, 53% and 50%, respectively) were significantly higher than those of patients after non-curative resection (n = 67, 7% and 2% respectively, $P < 0.0001$). In 54 patients followed-up after curative resection, the rate of recurrence after surgery was 46%. The rate of recurrence was significantly higher in patients with various mass-forming ICC tumors ($P = 0.039$) than in those with other types of tumors or tumors > 3 cm in diameter than in those with tumors > 3 cm or < less 3 cm ($P = 0.006$)[84]. Kim *et al*[85] reported that the median survival time after non-curative resection is 3.0 mo. Chu *et al*[22] showed the the median survival time is 1.8 mo and 2.9 mo, respectively, after conservative management and palliative operations. Only a curative resection can prolong survival. ICC has no characteristic symptoms at its early stage and is often at its advanced stage when it is diagnosed. Consequently, the resectability rate is usually low, extended hepatectomy possibly in combination with resection of other structures (e.g. extrahepatic bile duct, portal vein and inferior vena cava) is generally required. Wu *et al*[86] described a case of initially unresectable, locally advanced intrahepatic cholangiocarcinoma that showed a remarkable regression after transcatheter arterial chemoembolization with degradable starch microspheres, allowing for subsequent successful curative resection. In a retrospectively study, Yamamoto *et al*[40] allocated 83 patients who had undergone resection to a standard surgery group (n = 56), in which the patients underwent hepatectomy alone or hepatectomy with bile duct resection, and an extended surgery group (n = 27), in which the patients underwent the standard operation combined with vessel resection and/or pancreatectomy. The 5-year survival rate was significantly higher in the standard surgery group (30%) than in the extended surgery group (10%, $P = 0.0061$). So they concluded that extended surgery does not improve the curative resection rate or the surgical outcome of ICC[40].

Lymphadenectomy

ICC frequently demonstrates lymphatic spread. Lymph node metastasis is a significant prognostic factor for IHCC. Whether lymph nodes are dissected, and what is the extent of dissection remain the two important questions to be solved. No consensus has been reached concerning the indications and value of lymph node dissection for ICC. Hepatectomy with extensive lymph node dissection is the standard operation for intrahepatic cholangiocarcinoma in Japan. However, lymph node dissection may not always be effective in reducing tumor recurrence. Chu *et al*[22] and Shimada *et al*[47] that lymph node dissection alone is not likely to improve the prognosis without further control of liver metastases. However, there are reported cases of long-term survival after extended surgical resection of intrahepatic cholangiocarcinoma with extensive lymph node metastasis[56,87].

Transplantation

Pichlmayr *et al*[88] reported that the median survival time of 18 patients with IHCC after liver transplantation was 5.0 mo, and the 1-year survival rate was 13.9%. Casavilla *et al*[89] performed liver transplantation for patients with unresectable tumor (n = 12) or advanced cirrhosis (n = 8) and found that the mortality within 30 d was 7.4%. Overall, the tumor-free survival rates were 64% and 57%, respectively at 1 year, 34% and 34%, respectively at 3 years, and 26% and 27%, respectively at 5 years after operation. About 59.3% patients experienced tumor recurrence. When patients with positive margins, multiple tumors, and lymph node involvement were excluded, the patient survival rate was 74%, 64% and 62%, at 1, 3, and 5 years, respectively after operation. A Mayo Clinic group[90] used preoperative irradiation and chemotherapy for patients with unresectable cholangiocarcinoma above the cystic duct without intrahepatic or extrahepatic metastases. Patients initially received external-beam irradiation plus bolus fluorouracil (5-FU), followed by brachytherapy with iridium and concomitant protracted venous infusion of 5-FU. 5-FU was then administered continuously through an ambulatory infusion pump until OLT. After irradiation, patients underwent an exploratory laparotomy to exclude metastatic disease. The patients have a median follow-up time of 44 mo (range 17-83 mo, 7 of 9 patients > 36 mo). Only 1 patient developed tumor relapse. The group concluded that OLT in combination with preoperative irradiation and chemotherapy is associated with prolonged disease-free, and overall survival in highly selected patients with early-stage cholangiocarcinoma[90]. A comparison of recent series is shown in Table 3.

Table 3 Comparison of recent series

Authors	Yr	Countries	Procedure (patients)	Prognosis (%)				Tumor recurrence (%)
				Median (mo)	1 yr	3 yr	5 yr	
Pichlmayr *et al*[88]	1995	Germany	Hepatic resection (32)	12.8				
			Liver transplantation (18)	5.0				
Casavilla *et al*[89]	1997	America	Hepatic resection (34)		60	37	31	56
			Liver transplantation (20)		70	29	18	55
Chu *et al*[22]	1997	Hong Kong, China	Conservative management (15)	1.8				
			Palliative operation (23)	2.9				
			Hepatic resection (39)	12.2	57.3	23.9	15.9	
Madariaga *et al*[98]	1998	Japan	Hepatic resection (34)	19	67	40	35	
Meyer *et al*[99]	2000	America	Liver transplantation (207)		72	48 (2 yr)	23	51
Kawarada *et al*[100]	2001	Japan	hepatic resection (37)	31.5	54.1	34	23.9	
Fu *et al*[101]	2004	China	Palliative or curative operation (79)	11.9	49.4	17.3	9.6	
Robles *et al*[102]	2004	Spain	Liver transplantation (23)		77	65	42	35
Lang *et al*[80]	2005	Germany	R0-resection (complete tumor removal) (16)	46	94	82		37.5
			R1-resection (microscopic tumor at the cutting margin) (11)	5	22	0		
Ghali *et al*[103]	2005	Canada	Liver transplantation (10)		90	80	20	80
Urahashi *et al*[104]	2007	Japan	HPD (hepatectomy with pancreatoduodenectomy) (12)		42	33	33	75
De Oliveira *et al*[105]	2007	America	Hepatic resection (R0-resection)	80			63	
			Hepatic resection (overall)	28			40	
Becker *et al*[106]	2008	America	Liver transplantation (280)		74		38	

Adjunctive therapy

Recurrence of ICC is due to failure in surgery, warranting consideration of adjuvant treatments. Neither adjuvant nor neoadjuvant therapy, however, has been shown to improve survival. Roayaie *et al*[91] performed chemo-radiation therapy for postoperative patients with positive resection margins or nodal invasion and did not find any difference in the actuarial disease-free survival between the patients with or without adjuvant chemo-radiation. Sanz-Altamira *et al*[92] used 5-fluorouracil, leucovorin, and carboplatin in patients with unresectable biliary tree carcinoma and found that 21% of the patients had significant responses. Ando *et al*[93] treated an IHCC patient with postoperative recurrence of multiple liver metastases, and a complete response was noted 1 year after the patient underwent 4 courses of hepatic arterial infusion therapy *via* a subcutaneously implanted injection port and received cisplatin. The research of Furuse *et al*[94] showed that, of the twenty-four patients not amenable to surgery, three had a response rate of 12.5%, thirteen had a stable disease, seven had a progressive disease, and one was not evaluated. Lee *et al*[95] treated 24 patients immunohistochemically proven cholangiocarcinoma patients with gemcitabine and cisplatin. Of these 24 patients, 5 had a partial response, 12 had a stable disease, and 7 had a progressive disease during treatment. These patients had a median survival time of 9.30 mo. In a study by Feisthammel *et al*[96], the response rate was 10% for patients with inoperable intrahepatic cholangiocarcinoma (n = 17) or gallbladder cancer (n = 13) after treatment with irinotecan followed by folinic acid and 5-FU, and an additional 10% of patients had a stable disease. The median overall survival time of was 166 d and 273 d, respectively and median progression-free survival time of intrahepatic cholangiocarcinoma and gallbladder cancer patients was 166-273 d, and 84-159 d, respectively. These results suggest that the present therapy is a useful option for advanced IHCC. Rai *et al*[97] reported a 59-year old lady who underwent orthotopic liver transplantation (OLT) for intrahepatic cholangiocarcinoma recurrence 13 mo after transplantation in spite of adjuvant chemotherapy. She survived 18 mo after her recurrent tumor was treated with radiofrequency ablation, suggesting that radiofrequency ablation can be used in treatment of recurrent tumor after liver transplantation.

REFERENCES

1 **Patel T**. Increasing incidence and mortality of primary intrahepatic cholangiocarcinoma in the United States. *Hepatology* 2001; **33**: 1353-1357

2 **Patel T**. Worldwide trends in mortality from biliary tract malignancies. *BMC Cancer* 2002; **2**: 10

3 **Shaib Y**, El-Serag HB. The epidemiology of cholangiocarcinoma. *Semin Liver Dis* 2004; **24**: 115-125

4 **Shaib YH**, Davila JA, McGlynn K, El-Serag HB. Rising incidence of intrahepatic cholangiocarcinoma in the United States: a true increase? *J Hepatol* 2004; **40**: 472-477

5 **Khan SA**, Taylor-Robinson SD, Toledano MB, Beck A, Elliott P, Thomas HC. Changing international trends in mortality rates for liver, biliary and pancreatic tumours. *J Hepatol* 2002; **37**: 806-813

6 **Taylor-Robinson SD**, Toledano MB, Arora S, Keegan TJ, Hargreaves S, Beck A, Khan SA, Elliott P, Thomas HC. Increase in mortality rates from intrahepatic cholangiocarcinoma in England and Wales 1968-1998. *Gut* 2001; **48**: 816-820

7 **Okuda K**, Nakanuma Y, Miyazaki M. Cholangiocarcinoma: recent progress. Part 1: epidemiology and etiology. *J Gastroenterol Hepatol* 2002; **17**: 1049-1055

8 **Mouzas IA**, Dimoulios P, Vlachonikolis IG, Skordilis P, Zoras O, Kouroumalis E. Increasing incidence of cholangiocarcinoma in Crete 1992-2000. *Anticancer Res* 2002; **22**: 3637-3641

9 **Wood R**, Brewster DH, Fraser LA, Brown H, Hayes PC, Garden OJ. Do increases in mortality from intrahepatic cholangiocarcinoma reflect a genuine increase in risk? Insights from cancer registry data in Scotland. *Eur J Cancer* 2003; **39**: 2087-2092

10 **Kato I**, Kuroishi T, Tominaga S. Descriptive epidemiology of subsites of cancers of the liver, biliary tract and pancreas in Japan. *Jpn J Clin Oncol* 1990; **20**: 232-237

11 **Sorensen HT**, Friis S, Olsen JH, Thulstrup AM, Mellemkjaer L, Linet M, Trichopoulos D, Vilstrup H, Olsen J. Risk of liver and other types of cancer in patients with cirrhosis: a nationwide cohort study in Denmark. *Hepatology* 1998; **28**: 921-925

12 **Welzel TM**, Graubard BI, El-Serag HB, Shaib YH, Hsing AW, Davila JA, McGlynn KA. Risk factors for intrahepatic and extrahepatic cholangiocarcinoma in the United States: a population-based case-control study. *Clin Gastroenterol Hepatol* 2007; **5**: 1221-1228

13 **Lazaridis KN**, Gores GJ. Cholangiocarcinoma. *Gastroenterology* 2005; **128**: 1655-1667

14 **Khan SA**, Thomas HC, Davidson BR, Taylor-Robinson SD. Cholangiocarcinoma. *Lancet* 2005; **366**: 1303-1314

15 **Oh SW**, Yoon YS, Shin SA. Effects of excess weight on cancer incidences depending on cancer sites and histologic findings among men: Korea National Health Insurance Corporation Study. *J Clin Oncol* 2005; **23**: 4742-4754

16 **Yamamoto S**, Kubo S, Hai S, Uenishi T, Yamamoto T, Shuto T, Takemura S, Tanaka H, Yamazaki O, Hirohashi K, Tanaka T. Hepatitis C virus infection as a likely etiology of intrahepatic cholangiocarcinoma. *Cancer Sci* 2004; **95**: 592-595

17 **Shin HR**, Lee CU, Park HJ, Seol SY, Chung JM, Choi HC, Ahn YO, Shigemastu T. Hepatitis B and C virus, Clonorchis sinensis for the risk of liver cancer: a case-control study in Pusan, Korea. *Int J Epidemiol* 1996; **25**: 933-940

18 **Jepsen P**, Vilstrup H, Tarone RE, Friis S, Sorensen HT. Incidence rates of intra- and extrahepatic cholangiocarcinomas in Denmark from 1978 through 2002. *J Natl Cancer Inst* 2007; **99**: 895-897

19 **McLean L**, Patel T. Racial and ethnic variations in the epidemiology of intrahepatic cholangiocarcinoma in the United States. *Liver Int* 2006; **26**: 1047-1053

20 **Lieser MJ**, Barry MK, Rowland C, Ilstrup DM, Nagorney DM. Surgical management of intrahepatic cholangiocarcinoma: a 31-year experience. *J Hepatobiliary Pancreat Surg* 1998; **5**: 41-47

21 **Valverde A**, Bonhomme N, Farges O, Sauvanet A, Flejou JF, Belghiti J. Resection of intrahepatic cholangiocarcinoma: a Western experience. *J Hepatobiliary Pancreat Surg* 1999; **6**: 122-127

22 **Chu KM**, Fan ST. Intrahepatic cholangiocarcinoma in Hong Kong. *J Hepatobiliary Pancreat Surg* 1999; **6**: 149-153

23 **Chu KM**, Lai EC, Al-Hadeedi S, Arcilla CE Jr, Lo CM, Liu CL, Fan ST, Wong J. Intrahepatic cholangiocarcinoma. *World J Surg* 1997; **21**: 301-305; discussion 305-306

24 **Poneros JM**, Tearney GJ, Shiskov M, Kelsey PB, Lauwers GY, Nishioka NS, Bouma BE. Optical coherence tomography of the biliary tree during ERCP. *Gastrointest Endosc* 2002; **55**: 84-88

25 **Slattery JM**, Sahani DV. What is the current state-of-the-art imaging for detection and staging of cholangiocarcinoma? *Oncologist* 2006; **11**: 913-922

26 **Bardales RH**, Stelow EB, Mallery S, Lai R, Stanley MW. Review of endoscopic ultrasound-guided fine-needle aspiration cytology. *Diagn Cytopathol* 2006; **34**: 140-175

27 **Crowe DR**, Eloubeidi MA, Chhieng DC, Jhala NC, Jhala D, Eltoum IA. Fine-needle aspiration biopsy of hepatic lesions: computerized tomographic-guided versus endoscopic ultrasound-guided FNA. *Cancer* 2006; **108**: 180-185

28 **Patel T**, Singh P. Cholangiocarcinoma: emerging approaches to a challenging cancer. *Curr Opin Gastroenterol* 2007; **23**: 317-323

29 **Liver Cancer Study Group of Japan**. Intrahepatic cholangiocarcinoma, macroscopic typing. In: Okamoto E (eds) Classification of primary liver cancer. Tokyo: Kanehara, 1997: 6-7

30 **Aishima S**, Kuroda Y, Nishihara Y, Iguchi T, Taguchi K, Taketomi A, Maehara Y, Tsuneyoshi M. Proposal of progression model for intrahepatic cholangiocarcinoma: clinicopathologic differences between hilar type and peripheral type. *Am J Surg Pathol* 2007; **31**: 1059-1067

31 **Isaji S**, Kawarada Y, Taoka H, Tabata M, Suzuki H, Yokoi H. Clinicopathological features and outcome of hepatic resection for intrahepatic cholangiocarcinoma in Japan. *J Hepatobiliary Pancreat Surg* 1999; **6**: 108-116

32 **Kajiyama K**, Maeda T, Takenaka K, Sugimachi K, Tsuneyoshi M. The significance of stromal desmoplasia in intrahepatic cholangiocarcinoma: a special reference of 'scirrhous-type' and 'nonscirrhous-type' growth. *Am J Surg Pathol* 1999; **23**: 892-902

33 **Sobin LH**, Wittekin C. UICC TNM classification of malignant tumors, 5th ed. New York: Wiley-Liss, 1997

34 **The Liver Cancer Study Group of Japan**. General rules for the clinical and pathological study of primary liver cancer. 2nd ed. Tokyo: Kanehara, 2003

35 **Uenishi T**, Yamazaki O, Yamamoto T, Hirohashi K, Tanaka H, Tanaka S, Hai S, Kubo S. Serosal invasion in TNM staging of mass-forming intrahepatic cholangiocarcinoma. *J Hepatobiliary Pancreat Surg* 2005; **12**: 479-483

36 **Okabayashi T**, Yamamoto J, Kosuge T, Shimada K, Yamasaki S, Takayama T, Makuuchi M. A new staging system for mass-forming intrahepatic cholangiocarcinoma: analysis of preoperative and postoperative variables. *Cancer* 2001; **92**: 2374-2383

37 **Nakajima T**, Kondo Y, Miyazaki M, Okui K. A histopathologic study of 102 cases of intrahepatic cholangiocarcinoma: histologic classification and modes of spreading. *Hum Pathol* 1988; **19**: 1228-1234

38 **Weinbren K**, Mutum SS. Pathological aspects of cholangiocarcinoma. *J Pathol* 1983; **139**: 217-238

39 **Sasaki A**, Aramaki M, Kawano K, Morii Y, Nakashima K, Yoshida T, Kitano S. Intrahepatic peripheral cholangiocarcinoma: mode of spread and choice of surgical treatment. *Br J Surg* 1998; **85**: 1206-1209

40 **Yamamoto M**, Takasaki K, Yoshikawa T. Extended resection for intrahepatic cholangiocarcinoma in Japan. *J Hepatobiliary Pancreat Surg* 1999; **6**: 117-121

41 **Washburn WK**, Lewis WD, Jenkins RL. Aggressive surgical resection for cholangiocarcinoma. *Arch Surg* 1995; **130**: 270-276

42 **Chou FF**, Sheen-Chen SM, Chen CL, Chen YS, Chen MC. Prognostic factors of resectable intrahepatic cholangiocarcinoma. *J Surg Oncol* 1995; **59**: 40-44

43 **Nakagawa T**, Kamiyama T, Kurauchi N, Matsushita M, Nakanishi K, Kamachi H, Kudo T, Todo S. Number of lymph node metastases is a significant prognostic factor in intrahepatic cholangiocarcinoma. *World J Surg* 2005; **29**: 728-733

44 **Yamamoto M**, Takasaki K, Yoshikawa T. Lymph node metastasis in intrahepatic cholangiocarcinoma. *Jpn J Clin Oncol* 1999; **29**: 147-150

45 **Uenishi T**, Hirohashi K, Kubo S, Yamamoto T, Yamazaki O, Kinoshita H. Clinicopathological factors predicting outcome after resection of mass-forming intrahepatic cholangiocarcinoma. *Br J Surg* 2001; **88**: 969-974

46 **Inoue K**, Makuuchi M, Takayama T, Torzilli G, Yamamoto J, Shimada K, Kosuge T, Yamasaki S, Konishi M, Kinoshita T, Miyagawa S, Kawasaki S. Long-term survival and prognostic factors in the surgical treatment of mass-forming type cholangiocarcinoma. *Surgery* 2000; **127**: 498-505

47 **Shimada M**, Yamashita Y, Aishima S, Shirabe K, Takenaka K, Sugimachi K. Value of lymph node dissection during resection of intrahepatic cholangiocarcinoma. *Br J Surg* 2001; **88**: 1463-1466

48 **Ohtsuka M**, Ito H, Kimura F, Shimizu H, Togawa A, Yoshidome H, Shimamura F, Shimizu Y, Miyazaki M. Extended hepatic resection and outcomes in intrahepatic cholangiocarcinoma. *J Hepatobiliary Pancreat Surg* 2003; **10**: 259-264

49 **Murakami Y**, Yokoyama T, Takesue Y, Hiyama E, Yokoyama Y, Kanehiro T, Uemura K, Matsuura Y. Long-term survival of peripheral intrahepatic cholangiocarcinoma with metastasis to the para-aortic lymph nodes. *Surgery* 2000; **127**: 105-106

50 **Yamamoto M**, Takasaki K, Imaizumi T, Ariizumi S, Matsumura N, Nakano M. A long-term survivor of intrahepatic cholangiocarcinoma with lymph node metastasis: a case report. *Jpn J Clin Oncol* 2002; **32**: 206-209

51 **Weber SM**, Jarnagin WR, Klimstra D, DeMatteo RP, Fong Y, Blumgart LH. Intrahepatic cholangiocarcinoma: resectability, recurrence pattern, and outcomes. *J Am Coll Surg* 2001; **193**: 384-391

52 **Nakeeb A**, Pitt HA, Sohn TA, Coleman J, Abrams RA, Piantadosi S, Hruban RH, Lillemoe KD, Yeo CJ, Cameron JL. Cholangiocarcinoma. A spectrum of intrahepatic, perihilar, and distal tumors. *Ann Surg* 1996; **224**: 463-473; discussion 473-475

53 **Liver Cancer Study Group of Japan**. Clinical and surgical findings. In: Liver Cancer Study Group of Japan, editors. Classification of Primary Liver Cancer. First English Edition. Tokyo: Kanehara Shuppan, 1997: 1-22

54 **Tsuji T**, Hiraoka T, Kanemitsu K, Takamori H, Tanabe D, Tashiro S. Lymphatic spreading pattern of intrahepatic cholangiocarcinoma. *Surgery* 2001; **129**: 401-407

55 **Okami J**, Dono K, Sakon M, Tsujie M, Hayashi N, Fujiwara Y, Nagano H, Umeshita K, Nakamori S, Monden M. Patterns of regional lymph node involvement in intrahepatic cholangiocarcinoma of the left lobe. *J Gastrointest Surg* 2003; **7**: 850-856

56 **Shirabe K**, Shimada M, Harimoto N, Sugimachi K, Yamashita Y, Tsujita E, Aishima S. Intrahepatic cholangiocarcinoma: its mode of spreading and therapeutic modalities. *Surgery* 2002; **131**: S159-S164

57 **Nozaki Y**, Yamamoto M, Ikai I, Yamamoto Y, Ozaki N, Fujii H, Nagahori K, Matsumoto Y, Yamaoka Y. Reconsideration of the lymph node metastasis pattern (N factor) from intrahepatic cholangiocarcinoma using the International Union Against Cancer TNM staging system for primary liver carcinoma. *Cancer* 1998; **83**: 1923-1929

58 **Isa T**, Kusano T, Shimoji H, Takeshima Y, Muto Y, Furukawa M. Predictive factors for long-term survival in patients with intrahepatic cholangiocarcinoma. *Am J Surg* 2001; **181**: 507-511

59 **Nakagohri T**, Konishi M, Inoue K, Oda T, Kinoshita T. Extended right hepatic lobectomy with resection of inferior vena cava and portal vein for intrahepatic cholangiocarcinoma. *J Hepatobiliary Pancreat Surg* 2000; **7**: 599-602

60 **Shimada K**, Sano T, Sakamoto Y, Esaki M, Kosuge T, Ojima H. Surgical outcomes of the mass-forming plus periductal infiltrating types of intrahepatic cholangiocarcinoma: a comparative study with the typical mass-forming type of intrahepatic cholangiocarcinoma. *World J Surg* 2007; **31**: 2016-2022

61 **Zhang Y**, Uchida M, Abe T, Nishimura H, Hayabuchi N, Nakashima Y. Intrahepatic peripheral cholangiocarcinoma: comparison of dynamic CT and dynamic MRI. *J Comput Assist Tomogr* 1999; **23**: 670-677

62 **Bhuiya MR**, Nimura Y, Kamiya J, Kondo S, Fukata S, Hayakawa N, Shionoya S. Clinicopathologic studies on perineural invasion of bile duct carcinoma. *Ann Surg* 1992; **215**: 344-349

63 **Braga HJ**, Imam K, Bluemke DA. MR imaging of intrahepatic cholangiocarcinoma: use of ferumoxides for lesion localization and extension. *AJR Am J Roentgenol* 2001; **177**: 111-114

64 **Harisinghani MG**, Barentsz J, Hahn PF, Deserno WM, Tabatabaei S, van de Kaa CH, de la Rosette J, Weissleder R. Noninvasive detection of clinically occult lymph-node metastases in prostate cancer. *N Engl J Med* 2003; **348**: 2491-2499

65 **Peterson MS**, Murakami T, Baron RL. MR imaging patterns of gadolinium retention within liver neoplasms. *Abdom Imaging* 1998; **23**: 592-599

66 **Yeh TS**, Jan YY, Tseng JH, Chiu CT, Chen TC, Hwang TL, Chen MF. Malignant perihilar biliary obstruction: magnetic resonance cholangiopancreatographic findings. *Am J Gastroenterol* 2000; **95**: 432-440

67 **Manfredi R**, Barbaro B, Masselli G, Vecchioli A, Marano P. Magnetic resonance imaging of cholangiocarcinoma. *Semin Liver Dis* 2004; **24**: 155-164

68 **Manfredi R**, Brizi MG, Masselli G, Vecchioli A, Marano P. [Malignant biliary hilar stenosis: MR cholangiography compared with direct cholangiography] *Radiol Med* 2001; **102**: 48-54

69 **Zidi SH**, Prat F, Le Guen O, Rondeau Y, Pelletier G. Performance characteristics of magnetic resonance cholangiography in the staging of malignant hilar strictures. *Gut* 2000; **46**: 103-106

70 **Anderson CD**, Rice MH, Pinson CW, Chapman WC, Chari RS, Delbeke D. Fluorodeoxyglucose PET imaging in the evaluation of gallbladder carcinoma and cholangiocarcinoma. *J Gastrointest Surg* 2004; **8**: 90-97

71 **Kim YJ**, Yun M, Lee WJ, Kim KS, Lee JD. Usefulness of 18F-FDG PET in intrahepatic cholangiocarcinoma. *Eur J Nucl Med Mol Imaging* 2003; **30**: 1467-1472

72 **Grobmyer SR**, Wang L, Gonen M, Fong Y, Klimstra D, D'Angelica M, DeMatteo RP, Schwartz L, Blumgart LH, Jarnagin WR. Perihepatic lymph node assessment in patients undergoing partial hepatectomy for malignancy. *Ann Surg* 2006; **244**: 260-264

73 **Kipp BR**, Stadheim LM, Halling SA, Pochron NL, Harmsen S, Nagorney DM, Sebo TJ, Therneau TM, Gores GJ, de Groen PC, Baron TH, Levy MJ, Halling KC, Roberts LR. A comparison of routine cytology and fluorescence in situ hybridization for the detection of malignant bile duct strictures. *Am J Gastroenterol* 2004; **99**: 1675-1681

74 **Baron TH**, Harewood GC, Rumalla A, Pochron NL, Stadheim LM, Gores GJ, Therneau TM, De Groen PC, Sebo TJ, Salomao DR, Kipp BR. A prospective comparison of digital image analysis and routine cytology for the identification of malignancy in biliary tract strictures. *Clin Gastroenterol Hepatol* 2004; **2**: 214-219

75 **Endo I**, Shimada H, Takeda K, Fujii Y, Yoshida K, Morioka D, Sadatoshi S, Togo S, Bourquain H, Peitgen HO. Successful duct-to-duct biliary reconstruction after right hemihepatectomy. Operative planning using virtual 3D reconstructed images. *J Gastrointest Surg* 2007; **11**: 666-670

76 **Abu-Hamda EM**, Baron TH. Endoscopic management of cholangiocarcinoma. *Semin Liver Dis* 2004; **24**: 165-175

77 **Fritscher-Ravens A**, Broering DC, Sriram PV, Topalidis T, Jaeckle S, Thonke F, Soehendra N. EUS-guided fine-needle aspiration cytodiagnosis of hilar cholangiocarcinoma: a case series. *Gastrointest Endosc* 2000; **52**: 534-540

78 **Corvera CU**, Weber SM, Jarnagin WR. Role of laparoscopy in the evaluation of biliary tract cancer. *Surg Oncol Clin N Am* 2002; **11**: 877-891

79 **Yeh CN**, Jan YY, Yeh TS, Hwang TL, Chen MF. Hepatic resection of the intraductal papillary type of peripheral cholangiocarcinoma. *Ann Surg Oncol* 2004; **11**: 606-611

80 **Lang H**, Sotiropoulos GC, Frühauf NR, Dömland M, Paul A, Kind EM, Malagó M, Broelsch CE. Extended hepatectomy for intrahepatic cholangiocellular carcinoma (ICC): when is it worthwhile? Single center experience with 27 resections in 50 patients over a 5-year period. *Ann Surg* 2005; **241**: 134-143

81 **Neuhaus P**, Jonas S, Settmacher U, Thelen A, Benckert C, Lopez-Hänninen E, Hintze RE. Surgical management of proximal bile duct cancer: extended right lobe resection

increases resectability and radicality. *Langenbecks Arch Surg* 2003; **388**: 194-200
82 **Yamamoto J**, Kosuge T, Takayama T, Shimada K, Makuuchi M, Yoshida J, Sakamoto M, Hirohashi S, Yamasaki S, Hasegawa H. Surgical treatment of intrahepatic cholangiocarcinoma: four patients surviving more than five years. *Surgery* 1992; **111**: 617-622
83 **Ohashi K**, Nakajima Y, Tsutsumi M, Kanehiro H, Fukuoka T, Hisanaga M, Taki J, Nakae D, Konishi Y, Nakano H. Clinical characteristics and proliferating activity of intrahepatic cholangiocarcinoma. *J Gastroenterol Hepatol* 1994; **9**: 442-446
84 **Yamamoto M**, Takasaki K, Otsubo T, Katsuragawa H, Katagiri S. Recurrence after surgical resection of intrahepatic cholangiocarcinoma. *J Hepatobiliary Pancreat Surg* 2001; **8**: 154-157
85 **Kim HJ**, Yun SS, Jung KH, Kwun WH, Choi JH. Intrahepatic cholangiocarcinoma in Korea. *J Hepatobiliary Pancreat Surg* 1999; **6**: 142-148
86 **Wu Y**, Saiura A, Yamamoto J, Koga R, Asahara S, Kamei A, Takano K, Ikari T, Seki M, Yamaguchi T, Muto T. Locally advanced intrahepatic cholangiocarcinoma successfully resected after transcatheter arterial chemoembolization with degradable starch microspheres: report of a case. *Hepatogastroenterology* 2007; **54**: 1345-1347
87 **Asakura H**, Ohtsuka M, Ito H, Kimura F, Ambiru S, Shimizu H, Togawa A, Yoshidome H, Kato A, Miyazaki M. Long-term survival after extended surgical resection of intrahepatic cholangiocarcinoma with extensive lymph node metastasis. *Hepatogastroenterology* 2005; **52**: 722-724
88 **Pichlmayr R**, Lamesch P, Weimann A, Tusch G, Ringe B. Surgical treatment of cholangiocellular carcinoma. *World J Surg* 1995; **19**: 83-88
89 **Casavilla FA**, Marsh JW, Iwatsuki S, Todo S, Lee RG, Madariaga JR, Pinna A, Dvorchik I, Fung JJ, Starzl TE. Hepatic resection and transplantation for peripheral cholangiocarcinoma. *J Am Coll Surg* 1997; **185**: 429-436
90 **De Vreede I**, Steers JL, Burch PA, Rosen CB, Gunderson LL, Haddock MG, Burgart L, Gores GJ. Prolonged disease-free survival after orthotopic liver transplantation plus adjuvant chemoirradiation for cholangiocarcinoma. *Liver Transpl* 2000; **6**: 309-316
91 **Roayaie S**, Guarrera JV, Ye MQ, Thung SN, Emre S, Fishbein TM, Guy SR, Sheiner PA, Miller CM, Schwartz ME. Aggressive surgical treatment of intrahepatic cholangiocarcinoma: predictors of outcomes. *J Am Coll Surg* 1998; **187**: 365-372
92 **Sanz-Altamira PM**, Ferrante K, Jenkins RL, Lewis WD, Huberman MS, Stuart KE. A phase II trial of 5-fluorouracil, leucovorin, and carboplatin in patients with unresectable biliary tree carcinoma. *Cancer* 1998; **82**: 2321-2325
93 **Ando E**, Tanaka M, Yamashita F, Fukumori K, Sumie S, Yano Y, Sata M. Chemotherapy for hepatocellular carcinoma with portal hypertension due to tumor thrombus. *J Clin Gastroenterol* 2000; **31**: 247-249
94 **Furuse J**, Okusaka T, Funakoshi A, Yamao K, Nagase M, Ishii H, Nakachi K, Ueno H, Ikeda M, Morizane C, Horikawa Y, Mizuno N. Early phase II study of uracil-tegafur plus doxorubicin in patients with unresectable advanced biliary tract cancer. *Jpn J Clin Oncol* 2006; **36**: 552-556
95 **Lee GW**, Kang JH, Kim HG, Lee JS, Lee JS, Jang JS. Combination chemotherapy with gemcitabine and cisplatin as first-line treatment for immunohistochemically proven cholangiocarcinoma. *Am J Clin Oncol* 2006; **29**: 127-131
96 **Feisthammel J**, Schoppmeyer K, Mossner J, Schulze M, Caca K, Wiedmann M. Irinotecan with 5-FU/FA in advanced biliary tract adenocarcinomas: a multicenter phase II trial. *Am J Clin Oncol* 2007; **30**: 319-324
97 **Rai R**, Manas D, Rose J. Radiofrequency ablation of recurrent cholangiocarcinoma after orthotopic liver transplantation - a case report. *World J Gastroenterol* 2005; **11**: 612-613
98 **Madariaga JR**, Iwatsuki S, Todo S, Lee RG, Irish W, Starzl TE. Liver resection for hilar and peripheral cholangiocarcinomas: a study of 62 cases. *Ann Surg* 1998; **227**: 70-79
99 **Meyer CG**, Penn I, James L. Liver transplantation for cholangiocarcinoma: results in 207 patients. *Transplantation* 2000; **69**: 1633-1637
100 **Kawarada Y**, Yamagiwa K, Das BC. Analysis of the relationships between clinicopathologic factors and survival time in intrahepatic cholangiocarcinoma. *Am J Surg* 2002; **183**: 679-685
101 **Fu XH**, Tang ZH, Zong M, Yang GS, Yao XP, Wu MC. Clinicopathologic features, diagnosis and surgical treatment of intrahepatic cholangiocarcinoma in 104 patients. *Hepatobiliary Pancreat Dis Int* 2004; **3**: 279-283
102 **Robles R**, Figueras J, Turrion VS, Margarit C, Moya A, Varo E, Calleja J, Valdivieso A, Valdecasas JC, Lopez P, Gomez M, de Vicente E, Loinaz C, Santoyo J, Fleitas M, Bernardos A, Llado L, Ramirez P, Bueno FS, Jaurrieta E, Parrilla P. Spanish experience in liver transplantation for hilar and peripheral cholangiocarcinoma. *Ann Surg* 2004; **239**: 265-271
103 **Ghali P**, Marotta PJ, Yoshida EM, Bain VG, Marleau D, Peltekian K, Metrakos P, Deschenes M. Liver transplantation for incidental cholangiocarcinoma: analysis of the Canadian experience. *Liver Transpl* 2005; **11**: 1412-1416
104 **Urahashi T**, Yamamoto M, Ohtsubo T, Katsuragawa H, Katagiri S, Takasaki K. Hepatopancreatoduodenectomy could be allowed for patients with advanced intrahepatic cholangiocarcinoma. *Hepatogastroenterology* 2007; **54**: 346-349
105 **De Oliveira ML**, Cunningham SC, Cameron JL, Kamangar F, Winter JM, Lillemoe KD, Choti MA, Yeo CJ, Schulick RD. Cholangiocarcinoma: thirty-one-year experience with 564 patients at a single institution. *Ann Surg* 2007; **245**: 755-762
106 **Becker NS**, Rodriguez JA, Barshes NR, O'Mahony CA, Goss JA, Aloia TA. Outcomes analysis for 280 patients with cholangiocarcinoma treated with liver transplantation over an 18-year period. *J Gastrointest Surg* 2008; **12**: 117-122

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TOPIC HIGHLIGHT

Akio Inui, MD, PhD, Professor, *Series Editor*

What's new about ghrelin in 2008?

Akio Inui

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9th NPY Meeting was held for the first time in Japan in March 2008, which was organized by Akio Inui, Professor and Chairman, Department of Psychosomatic Internal Medicine, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima, Japan.

This TOPIC HIGHLIGHT is the "ghrelin version" of the proceedings of 9th NPY Meeting and presents examples of the critical interplay in ghrelin-NPY pathway in response to environmental, pharmacological and genetic challenges in the stomach and hypothalamus. Other major topics in connection with NPY and related peptides are published in the special issue of Nutrition, thus together representing a comprehensive review of state of the art knowledge of the operation in regulating multiple physiological functions in the periphery and CNS.

These papers are written by leaders in their respective scientific fields by the use of various approaches and models to examine the secretion and action of ghrelin, including manometric and force transducer methods to measure physiological and ghrelin peptides-induced GI motility, electrophysiology to measure neuronal activity in NPY neurons, NPY receptor knockout and other animal models to examine appetite, secretion of stomach ghrelin and leptin or gastric acid, and human studies in relation to *Helicobacter pylori*.

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TOPIC HIGHLIGHT

Akio Inui, MD, PhD, Professor, *Series Editor*

Effects of ghrelin on interdigestive contractions of the rat gastrointestinal tract

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Abstract

Ghrelin causes interdigestive contractions of the stomach in rats. However, it remains unknown whether ghrelin causes interdigestive contractions in the small intestine. Four strain gauge transducers were implanted on the antrum, duodenum, proximal and distal jejunum. After an overnight fast, gastrointestinal (GI) contractions were recorded in freely moving conscious rats. Spontaneous phase Ⅲ-like contractions were observed at every 13-16 min in rat GI tract. The fasted motor patterns were replaced by the fed motor pattern immediately after food intake. Two minutes after finishing the spontaneous phase Ⅲ-like contractions in the antrum, acyl ghrelin (0.8, 2.4 and 8.0 μg/kg per min) was continuously infused for 30 min. Three-five minutes after the starting ghrelin infusion, augmented phase Ⅲ-like contractions were observed at the antrum, duodenum, and jejunum. Ghrelin infusion (0.8, 2.4 and 8.0 μg/kg per min) significantly increased motility index of phase Ⅲ-like contractions at the antrum and jejunum in a dose dependent manner, compared to that of saline injection. Thus, it is likely that exogenously administered ghrelin causes phase Ⅲ-like contraction at the antrum, which migrates to the duodenum and jejunum. The possible role of 5-HT, in addition to ghrelin, in mediating intestinal migrating motor complex (MMC), is discussed.

Key words: Phase Ⅲ-like contractions; Strain gage transducers; Motility index

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GHRELIN AND INTERDIGESTIVE GASTRIC MOTILITY

In the interdigestive state, the stomach and small intestine show a remarkable motor pattern, known as the migrating motor complex (MMC)[1]. MMC consists of three phases; phase I (period of motor quiescence), phase Ⅱ (period of irregular low amplitude contractions) and phase Ⅲ (period of regular high amplitude contractions). In humans and dogs, MMC is usually observed every 90-120 min in the interdigestive state. In contrast, in rats, MMC cycle is less than 20 min and not so regular, compared to humans and dogs[2,3]. Exogenously administered motilin does not induce phase Ⅲ-like contractions in rats. Motilin or its receptors are not found in rats[4].

Ghrelin, a 28-amino acid peptide, was discovered as the endogenous ligand for growth hormone secretagogue receptor (GHS-R) from the rat stomach[5]. Because of a structural resemblance to motilin, ghrelin is known as the motilin-related peptide[6,7]. Ghrelin administration causes phase Ⅲ-like contraction at the antrum and duodenum in conscious rats[3,8]. We recently showed that gastric spontaneous phase Ⅲ-like contractions were abolished by ghrelin receptor antagonists[9]. This suggests that endogenous ghrelin regulates spontaneous phase Ⅲ-like contractions of the rat stomach.

However, it still remains unknown whether ghrelin regulates intestinal phase Ⅲ-like contractions in rats. In the current study, we investigated whether exogenously administered ghrelin stimulates phase Ⅲ-like contractions of gastrointestinal (GI) tract in conscious rats.

EFFECTS OF GHRELIN ON THE INTERDIGESTIVE GI CONTRACTIONS

Male Sprague-Dawley rats weighing 280-340 g were kept

in-group cages under conditions of controlled temperature (22-24℃), humidity and light (12 h light cycle starting at 7:00 am) with free access to laboratory chow and water. Protocols describing the use of rats were approved by the Institutional Animal Care and Use Committee of Zablocki VA Medical Center (Milwaukee) and carried out in accordance with the National Institute of Health "Guide for the Care and Use of Laboratory Animals". All efforts were made to minimize animal suffering and to reduce the number of animal in experiments.

After overnight fasting, the rats were anesthetized with intraperitoneal injection of pentobarbital sodium (45 mg/kg). Through a midline laparotomy, strain gauge transducers were implanted on the serosal surface of the antrum, duodenum and jejunum. Duodenal transducers were implanted at 5 cm distal from the pylorus. Jejunal transducers were implanted at 15 cm (the proximal jejunum: J-1) and 25 cm (the distal jejunum: J-2) distal from the pylorus, respectively. The wires from transducer were exteriorized through abdominal wall, ran under skin toward the back. Intravenous catheter was inserted into right jugular vein, and similarly exteriorized to the back, as previously reported[2,9]. The catheter was filled with heparinized saline (100 U/mL) to prevent coagulation. Wires and a catheter were protected by a protective jacket (Star Medical, Tokyo, Japan). After the surgery, rats were housed individual and were allowed to recover for one week before the experiments.

After the implantation of transducers, rats were given food once daily at 12:00 pm-16:00 pm, as previously reported[2]. Experiments of GI motility recording were started at 9:00 am every day. The wires from the transducer were connected to the recording system (PowerLab model 8SP, ADI instruments, Colorado Springs, CO). GI contractions were measured with free access to water in freely moving conscious rats. Spontaneous phase Ⅲ-like contractions were observed for 2-3 h. Phase Ⅲ-like contractions were defined as clustered potent contractions with amplitude of more than 4 g, as previously reported[10].

Fujino *et al*[3] reported that bolus injection of acyl ghrelin (1 g/rat; iv) induced phase Ⅲ-like contraction in conscious rats. In general, bolus injection of certain peptides abruptly increased its plasma level. In our previous study, acyl ghrelin (0.8 μg/kg per min) was continuously infused for 5 min and potent phase Ⅲ-like contractions were observed in the antrum in rats[9]. In our current study, acyl ghrelin (0.8, 2.4 and 8.0 μg/kg per min) was continuously infused for 30 min. Acyl ghrelin was purchased from Tocris Cookson (Ellisville, MO).

Motility index (MI), area under the curve, was calculated using a computer-assisted system (PowerLab, ADInstruments, Colorado Springs, CO). MI in GI tract was compared thirty minutes before and during the infusion of acyl ghrelin. Saline infused rats served as controls.

Results were shown as mean ± SE. ANOVA followed by student's *t*-test was used to assess the difference among groups. A P value < 0.05 was considered to be statistically significant.

It has been showed that spontaneous phase Ⅲ-like contractions are observed at 12-15 min intervals of the stomach[3,9,10] in conscious rats. In our current study, cyclic changes of contractions were detected in the antrum, duodenum, J-1 and J-2 including a quiescence period (phase Ⅰ-like contractions) followed by a grouping of strong contractions (phase Ⅲ-like contractions). Spontaneous phase Ⅲ-like contractions were observed at every 13-16 min in rat GI tract. The fasted motor patterns were replaced by the fed motor pattern immediately after food intake[11].

Two minutes after finishing the spontaneous phase Ⅲ-like contractions in the antrum, acyl ghrelin (0.8, 2.4 and 8.0 μg/kg per min) was continuously infused for 30 min. Three-five minutes after the starting ghrelin infusion, augmented phase Ⅲ-like contractions were observed at the antrum, duodenum, J-1 and J-2 (Figure 1).

Ghrelin infusion (0.8, 2.4 and 8.0 μg/kg per min) significantly increased MI of phase Ⅲ-like contractions at the antrum and jejunum compared to that of saline injection, in a dose-dependent manner (Figure 2).

It is well established that exogenously administered ghrelin causes phase Ⅲ-like contractions in the interdigestive state at the antrum and duodenum in rats[3]. However, it is not clear whether intestinal phase Ⅲ-like contractions are affected by ghrelin administration.

We evaluated the effects of peripherally infused ghrelin on gastrointestinal phase Ⅲ-like contractions in freely moving conscious rats. We demonstrated that ghrelin infusion induced phase Ⅲ-like contractions in the antrum, duodenum, proximal and distal jejunum in a dose-dependent manner (0.8-80 μg/kg per min). This suggests that gastric phase Ⅲ-like contractions induced by exogenously administered ghrelin migrate distally to the small intestine.

We have previously shown that GHS-R antagonists significantly inhibited spontaneous phase Ⅲ-like contractions in conscious rats[9], suggesting that endogenously released ghrelin regulates spontaneous phase Ⅲ-like contractions. We also showed the correlation between the plasma ghrelin levels and occurrence of gastric phase Ⅰ- and Ⅲ-like contractions of the antrum[9]. However, it is not clear whether intestinal phase Ⅲ-like contractions are regulated by endogenously released ghrelin. Previous report showed that ghrelin stimulates motility in the rat small intestine and that the stimulatory effect of ghrelin is mediated *via* cholinergic neurons of the myenteric plexus[12].

Our recent study showed that GHS-R antagonists inhibited phase Ⅲ-like contractions at the antrum, but not the duodenum and the jejunum[11]. Previous studies also showed that GHS-R antagonists did not affect phase Ⅲ-like contractions in the duodenum[8].

It is likely that exogenously administered ghrelin (a pharmacological dose of ghrelin) causes phase-Ⅲ like contraction at the antrum, which migrates to the jejunum. In contrast, endogenously released ghrelin causes spontaneous phase Ⅲ-like contractions at the antrum, which do not migrate to the small intestine.

It has been demonstrated that 5-HT is involved in mediating interdigestive contractions of the small intes-

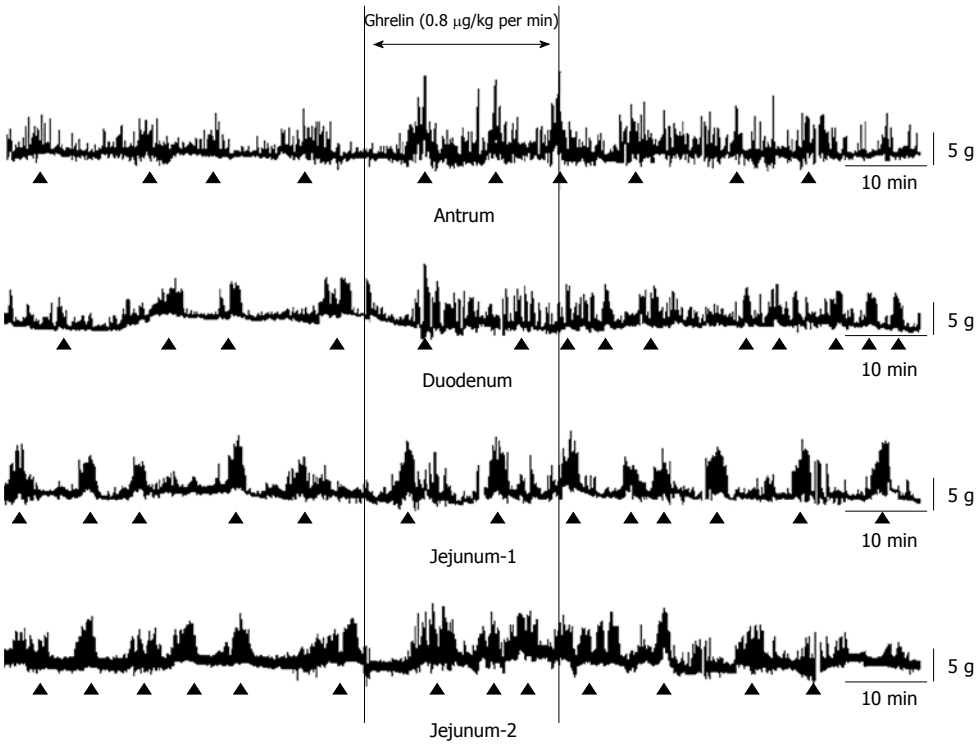

Figure 1 Effect of ghrelin infusion (0.8 μg/kg per min) on spontaneous phase Ⅲ-like contractions in conscious rats. Two minutes after finishing the spontaneous phase Ⅲ-like contractions in the antrum, acyl ghrelin (0.8 μg/kg per min) was continuously infused for 30 min. Three-five minutes after starting the ghrelin infusion, phase Ⅲ-like contractions were observed in response to ghrelin infusion at the antrum, duodenum, jejunum-1 and jejunum-2 (▲ indicates phase Ⅲ-like contractions).

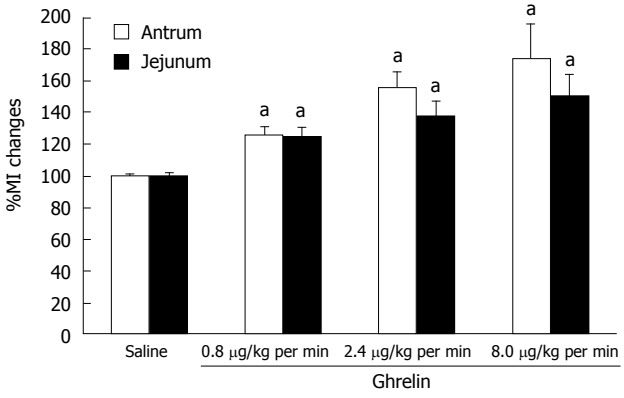

Figure 2 Effect of ghrelin on % changes of MI of phase Ⅲ-like contractions of GI tract ($^aP < 0.05$ *vs* saline, $n = 5$). Ghrelin infusion (0.8, 2.4 and 8.0 μg/kg) dose-dependently increased MI of phase Ⅲ-like contractions of the antrum and jejunum ($^aP < 0.05$ *vs* saline, $n = 5$).

tine in rats[13]. Subcutaneous or intravenous administration of 5-HT can induce intestinal migrating myoelectrical activity in rats[14,15]. Intestinal migrating myoelectrical activity was reduced by a 5-HT_3 antagonist, but not by a 5-HT_4 antagonist in conscious rats[15]. However, others showed that intestinal migrating myoelectrical activity was reduced by a 5-HT_4 antagonist, as well as a 5-HT_3 antagonist[16].

Our recent study showed that phase Ⅲ-like contractions at the jejunum, not the antrum and duodenum, were significantly attenuated by 5-HT_4 antagonists. In contrast, 5-HT_3 antagonists did not affect phase Ⅲ-like contractions in all of upper GI tract[11]. These suggest that spontaneous phase Ⅲ-like contractions at the jejunum is mediated *via* 5-HT_4 receptors, but not 5-HT_3 receptors.

5-HT_3 receptors[17] and 5-HT_4 receptors[18] are located on the cholinergic neurons of the myenteric plexus as well as sensory neurons of the intestinal mucosa[19]. Nerve endings of sensory neurons may well be the targets for the 5-HT released from enterochromaffin (EC) cells[20]. It is generally accepted that 5-HT stimulates intrinsic nerve fibers *via* 5-HT_4 receptors[21], while 5-HT stimulates extrinsic nerve fibers *via* 5-HT_3 receptors[19,22] in rats.

5-HT_3 receptors are located on the nerve terminal of vagal afferent of the duodenal mucosa in rats[23]. 5-HT released by mucosal stimuli initiates peristalsis by activating 5-HT_4 receptors on sensory CGRP neurons of the rat colon *in vitro*[24]. These suggest that 5-HT_4 receptors play a major role in mediating an intrinsic neural reflex. It is conceivable that luminally released 5-HT from duodenal EC cells initially stimulates duodenal phase Ⅲ-like contractions *via* 5-HT_4 receptors located on intrinsic primary afferent neurons (IPAN).

It has been shown that ghrelin receptors are synthesized in vagal afferent neurons and transported to the afferent terminal. This is the major pathway conveying ghrelin signals for starvation and growth hormone secretion to the brain[25]. Blockade of the gastric vagal neuron by vagotomy or perivagal application of capsaicin abolished ghrelin-induced feeding, GH secretion, and activation of NPY-producing and GHRH-producing neurons[25]. Ghrelin-induced acid secretion is also abolished by bilateral vagotomy[26].

Our recent study showed that spontaneous phase Ⅲ-like contractions were completely disappeared in vagotomized rats[11]. These results suggest that ghrelin-induced spontaneous phase-Ⅲ like is mediated *via* vagal pathways.

Spontaneous phase Ⅲ-like contractions are mainly regulated by ghrelin in the antrum, while spontaneous phase Ⅲ-like contractions are regulated by 5-HT in the jejunum. Released ghrelin from the gastric mucosa initi-

ates gastric phase Ⅲ-like contractions *via* vagal dependent pathways. Released 5-HT from intestinal EC cells induces intestinal phase Ⅲ-like contractions *via* IPAN in rats.

REFERENCES

1 **Szurszewski JH**. A migrating electric complex of canine small intestine. *Am J Physiol* 1969; **217**: 1757-1763
2 **Ariga H**, Imai K, Chen C, Mantyh C, Pappas TN, Takahashi T. Fixed feeding potentiates interdigestive gastric motor activity in rats: importance of eating habits for maintaining interdigestive MMC. *Am J Physiol Gastrointest Liver Physiol* 2008; **294**: G655-G659
3 **Fujino K**, Inui A, Asakawa A, Kihara N, Fujimura M, Fujimiya M. Ghrelin induces fasted motor activity of the gastrointestinal tract in conscious fed rats. *J Physiol* 2003; **550**: 227-240
4 **Depoortere I**, De Winter B, Thijs T, De Man J, Pelckmans P, Peeters T. Comparison of the gastroprokinetic effects of ghrelin, GHRP-6 and motilin in rats in vivo and in vitro. *Eur J Pharmacol* 2005; **515**: 160-168
5 **Kojima M**, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature* 1999; **402**: 656-660
6 **Tomasetto C**, Karam SM, Ribieras S, Masson R, Lefebvre O, Staub A, Alexander G, Chenard MP, Rio MC. Identification and characterization of a novel gastric peptide hormone: the motilin-related peptide. *Gastroenterology* 2000; **119**: 395-405
7 **Asakawa A**, Inui A, Kaga T, Yuzuriha H, Nagata T, Ueno N, Makino S, Fujimiya M, Niijima A, Fujino MA, Kasuga M. Ghrelin is an appetite-stimulatory signal from stomach with structural resemblance to motilin. *Gastroenterology* 2001; **120**: 337-345
8 **Wang Y**, Dong L, Cheng Y, Zhao P. Effects of ghrelin on feeding regulation and interdigestive migrating complex in rats. *Scand J Gastroenterol* 2007; **42**: 447-453
9 **Ariga H**, Tsukamoto K, Chen C, Mantyh C, Pappas TN, Takahashi T. Endogenous acyl ghrelin is involved in mediating spontaneous phase III-like contractions of the rat stomach. *Neurogastroenterol Motil* 2007; **19**: 675-680
10 **Tatewaki M**, Harris M, Uemura K, Ueno T, Hoshino E, Shiotani A, Pappas TN, Takahashi T. Dual effects of acupuncture on gastric motility in conscious rats. *Am J Physiol Regul Integr Comp Physiol* 2003; **285**: R862-R872
11 **Taniguchi H**, Ariga H, Zheng J, Ludwig K, Mantyh C, Pappas TN, Takahashi T. Endogenous ghrelin and 5-HT regulate interdigestive gastrointestinal contractions in conscious rats. *Am J Physiol Gastrointest Liver Physiol* 2008; **295**: G403-G411
12 **Edholm T**, Levin F, Hellstrom PM, Schmidt PT. Ghrelin stimulates motility in the small intestine of rats through intrinsic cholinergic neurons. *Regul Pept* 2004; **121**: 25-30
13 **Pineiro-Carrero VM**, Clench MH, Davis RH, Andres JM, Franzini DA, Mathias JR. Intestinal motility changes in rats after enteric serotonergic neuron destruction. *Am J Physiol* 1991; **260**: G232-G239
14 **Sagrada A**, Brancaccio N, Schiavone A. 5-Hydroxytryptamine affects rat migrating myoelectric complexes through different receptor subtypes: evidence from 5-hydroxytryptophan administration. *Life Sci* 1990; **46**: 1207-1216
15 **Lordal M**, Hellstrom PM. Serotonin stimulates migrating myoelectric complex via 5-HT3-receptors dependent on cholinergic pathways in rat small intestine. *Neurogastroenterol Motil* 1999; **11**: 1-10
16 **Axelsson LG**, Wallin B, Gillberg PG, Sjoberg B, Soderberg C, Hellstrom PM. Regulatory role of 5-HT and muscarinic receptor antagonists on the migrating myoelectric complex in rats. *Eur J Pharmacol* 2003; **467**: 211-218
17 **Miyata K**, Kamato T, Nishida A, Ito H, Yuki H, Yamano M, Tsutsumi R, Katsuyama Y, Honda K. Role of the serotonin3 receptor in stress-induced defecation. *J Pharmacol Exp Ther* 1992; **261**: 297-303
18 **Talley NJ**. Serotoninergic neuroenteric modulators. *Lancet* 2001; **358**: 2061-2068
19 **Gershon MD**. Review article: roles played by 5-hydroxytryptamine in the physiology of the bowel. *Aliment Pharmacol Ther* 1999; **13** Suppl 2: 15-30
20 **Berthoud HR**, Kressel M, Raybould HE, Neuhuber WL. Vagal sensors in the rat duodenal mucosa: distribution and structure as revealed by in vivo DiI-tracing. *Anat Embryol* (Berl) 1995; **191**: 203-212
21 **Foxx-Orenstein AE**, Kuemmerle JF, Grider JR. Distinct 5-HT receptors mediate the peristaltic reflex induced by mucosal stimuli in human and guinea pig intestine. *Gastroenterology* 1996; **111**: 1281-1290
22 **Blackshaw LA**, Grundy D. Effects of 5-hydroxytryptamine (5-HT) on the discharge of vagal mechanoreceptors and motility in the upper gastrointestinal tract of the ferret. *J Auton Nerv Syst* 1993; **45**: 51-59
23 **Glatzle J**, Sternini C, Robin C, Zittel TT, Wong H, Reeve JR Jr, Raybould HE. Expression of 5-HT3 receptors in the rat gastrointestinal tract. *Gastroenterology* 2002; **123**: 217-226
24 **Grider JR**, Kuemmerle JF, Jin JG. 5-HT released by mucosal stimuli initiates peristalsis by activating 5-HT4/5-HT1p receptors on sensory CGRP neurons. *Am J Physiol* 1996; **270**: G778-G782
25 **Date Y**, Murakami N, Toshinai K, Matsukura S, Niijima A, Matsuo H, Kangawa K, Nakazato M. The role of the gastric afferent vagal nerve in ghrelin-induced feeding and growth hormone secretion in rats. *Gastroenterology* 2002; **123**: 1120-1128
26 **Yakabi K**, Ro S, Onouhi T, Tanaka T, Ohno S, Miura S, Johno Y, Takayama K. Histamine mediates the stimulatory action of ghrelin on acid secretion in rat stomach. *Dig Dis Sci* 2006; **51**: 1313-1321

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TOPIC HIGHLIGHT

Akio Inui, MD, PhD, Professor, *Series Editor*

Growth hormone releasing peptide 2 reverses anorexia associated with chemotherapy with 5-fluorouracil in colon cancer cell-bearing mice

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Abstract

The cancer-associated anorexia-cachexia syndrome is observed in 80% of patients with advanced-stage cancer, and is one of the major obstacles in chemotherapy. Ghrelin is a orexigenic hormone that has been proposed to prevent anorexia. Aim of the study was to determine whether the addition of the ghrelin agonist growth hormone releasing peptide 2 (GHRP-2) to cytotoxic therapy with 5-fluorouracil (5-FU) prevents the anorexia associated with chemotherapy in cancer cachectic mice. Thirty-three BALB/c female tumour-bearing mice were randomized to receive a solution containing: (a) placebo; (b) GHRP-2; (c) 5-FU; or (d) 5-FU + GHRP-2. Ten BALB/c no tumour-bearing mice received placebo solution. Food intake and survival were checked. Six hours after the drug injection the cumulative food intake was significantly increased in mice treated with the combination of 5-FU + GHRP-2 *versus* the 5-FU alone (P = 0.0096). On day 3, the cumulative food intake of mice treated with GHRP-2, 5-FU and 5-FU + GHRP-2 significantly increased compared with naive and vehicle groups (P = 0.0007, P = 0.0038 and P = 0.0166, respectively). The median survival time was longer in 5-FU + GHRP-2 treated mice than in those with 5-FU, although it was not significant (18 d *versus* 15.5 d, P = 0.7). For the first time, we demonstrated that the addition of GHRP-2 to cytotoxic therapy with 5-FU improved appetite in tumour-bearing mice with anorexia/cachexia syndrome in early stage. These data suggest that GHRP-2 may improve the efficacy of therapy and the quality of life of cancer patients thank to the amelioration of their nutritional state.

Key words: GHS; Ghrelin; Cancer anorexia-cachexia syndrome; Food intake; Chemotherapy; Colon cancer cell line; Murine model

Perboni S, Bowers C, Kojima S, Asakawa A, Inui A. Growth hormone releasing peptide 2 reverses anorexia associated with chemotherapy with 5-fluorouracil in colon cancer cell-bearing mice. *World J Gastroenterol* 2008; 14(41): 6303-6305 Available from: URL: http://www.wjgnet.com/1007-9327/14/6303.asp DOI: http://dx.doi.org/10.3748/wjg.14.6303

CHEMOTHERAPY-INDUCED ANOREXIA

Chemotherapy is the most effective treatment for most cancer patients because of its systemic distribution. Despite the recent advances in the treatment, many patients do not respond to therapy, and die of their diseases. The cancer-associated anorexia-cachexia syndrome is observed in 80% of patients with advanced-stage cancer, and it is a very powerful prognostic indicator of poor outcome and poor quality of life. This syndrome is also one of the major obstacles in cancer chemotherapy[1].

Ghrelin is the natural ligand of growth hormone secretagogue receptor. Growth hormone releasing peptide 2 (GHRP-2) is a synthetic compound that acts as a potent agonist of GHS receptor[2]. Increasing evidences support that GHRP-2, like ghrelin[3], exerts orexigenic

proprieties. Administration of GHRP-2 has shown to increase food intake and body weight in rodents[4] and in healthy men[5]. Since ghrelin has a short half-life, the more stable GHRP-2 was preferred in this experiment.

Aim of the study was to determine whether the addition of GHRP-2 to cytotoxic therapy with 5-fluorouracil (5-FU) prevents anorexia associated with chemotherapy in cancer cachectic mice[6]. Secondary aims were to examine the chronic effects of GHRP-2 on reduced appetite and survival in this animal model.

MURINE MODEL OF CANCER

Six-weeks-old female BALB/c mice (19-24 g) were housed individually in equal plastic cages and received *ad libitum* standard diet and tap water in a regulated environment. The murine colon cancer cell line (colon 26) was supplied by Dr. Hayashi (Kitazato University, Kanagawa, Japan). Colon 26 cancer cells (5×10^5) were dissolved in a 100 μL volume of PBS containing 0.02% EDTA and then implanted in the abdominal cavity of female BALB/c mice by intraperitoneal administration. Seven days later, when mice manifested the first symptoms of anorexia and cachexia, the tumour-bearing mice were randomized in 4 groups, each of them composed by: (a) 11 mice receiving 5% glucoside solution + PBS (vehicle); (b) 12 mice receiving GHRP-2 + 5% glucoside solution; (c) 12 mice receiving 5FU + PBS; and (d) 15 mice receiving 5-FU + GHRP-2. Ten BALB/c no tumour-bearing mice received 5% glucoside solution + PBS (naïve). The day of randomisation was considered as day 0 of the experiment. GHRP-2 (DalaDβNalAlaTrpDPheLysNH2) was supplied by Prof. Bowers (Tulane University, New Orleans, USA). It was dissolved in PBS and was subcutaneously administered at dose of 10 μg/mouse daily. 5-fluorouracil (Kyowa Hakko Kogyo Co. Ldt, Tokyo, Japan) was dissolved in 5% glucoside solution and then intraperitoneally administered at dose of 100 mg/kg weekly. In the acute experiment, food intake and body weight were measured at 0, 1, 2, 4 and 6 h after the first injection of the drugs and then daily. Food intake was evaluated by subtracting uneaten food from initially pre-measured food and checking for food spillage. All the experiments were approved by the animal care committees at the Kobe University and the Kagoshima University (Japan). Results are expressed as mean ± SE. Analysis of variance (ANOVA) followed by Bonferroni's *t* test was used to assess the differences among groups. *P*-values < 0.05 were considered significant. The survival curves after tumour implantation were analyzed by Kaplan-Meier survival test.

GHRELIN AGONIST GHRP-2 REVERSES CHEMOTHERAPY-INDUCED ANOREXIA

The median survival time was longer in the group treated with 5-FU + GHRP-2 than in the group treated with 5-FU, although it was not significant (18 d *versus* 15.5 d, *P* = 0.7) as shown in Figure 1. At day 0, 6 h after the drug injection, the cumulative food intake was significantly

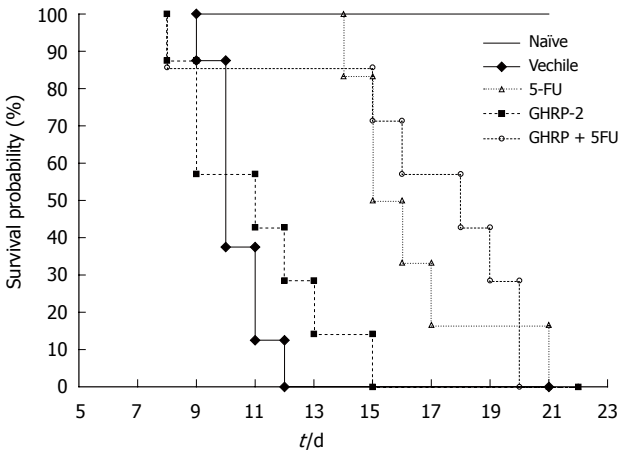

Figure 1 Kaplan-Meier curve of overall survival in colon 26-bearing mice (Naïve: 10 mice; Vehicle: 11 mice; GHRP-2: 12 mice; 5-FU: 12 mice; 5-FU + GHRP-2: 15 mice).

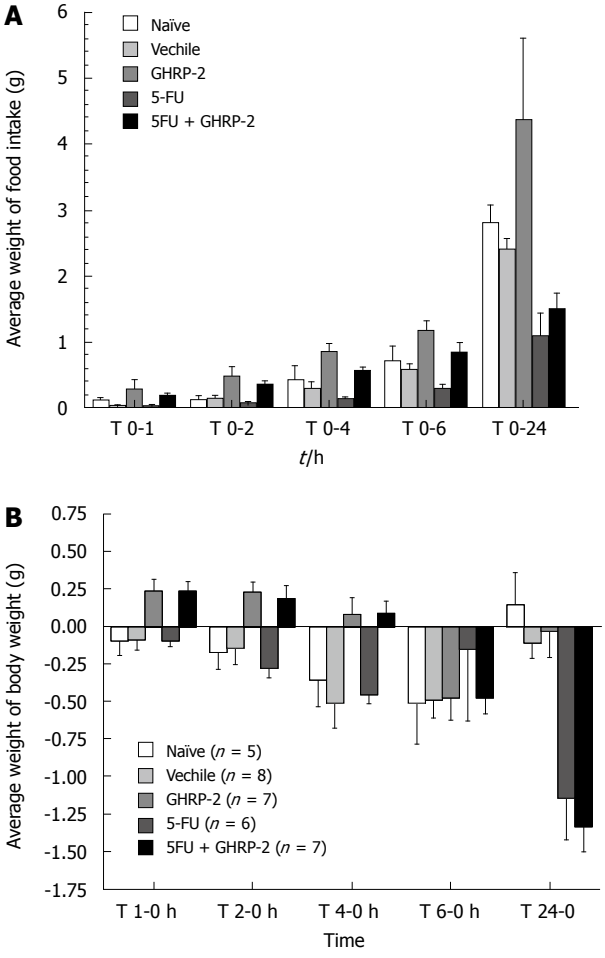

Figure 2 GHRP-2 reverses reduced food intake and body weight by 5-FU administration in colon 26-bearing mice-acute experiments. A: Food intake; B: Body weight.

increased in mice treated with the combination of 5-FU + GHRP-2 *versus* the 5-FU alone (*P* = 0.0096, Figure 2A). At day 0, 4 h after the drug injection, the cumulative body weight of the group treated with 5-FU + GHRP-2 showed a reduced loss of body weight compared with the group treated with 5-FU (*P* = 0.0074, Figure 2B). At day

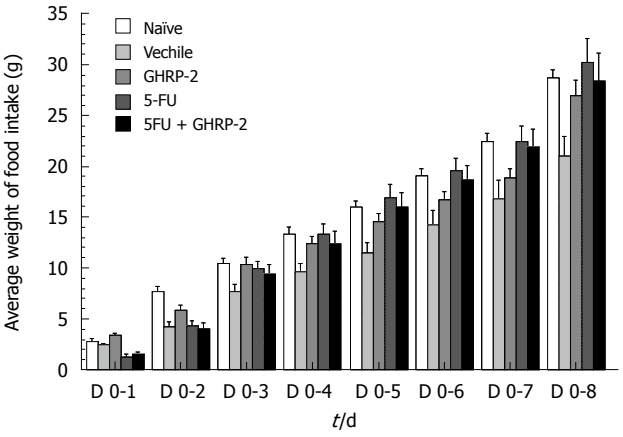

Figure 3 GHRP-2 increases cumulative food intake compared to vehicle controls in colon 26-bearing mice-chronic experiments.

2, the groups treated with 5-FU and 5-FU + GHRP-2 showed a higher loss of cumulative body weight compared with the group treated with GHRP-2 alone (P = 0.0003 and $P < 0.0001$, respectively, Figure 3). However, from day 3 to 5, the cumulative food intake of mice treated with 5-FU and 5-FU + GHRP-2 significantly increased compared with naive and vehicle groups (P = 0.0038 and P = 0.0166, respectively, Figure 3). The cumulative food intake was significantly increased in mice treated with GHRP-2 respect to naïve and vehicle groups throughout the observation period (P = 0.0007 and P = 0.0004, respectively, Figure 3). We could not follow the body weight changes since the tumour-bearing mice developed ascites after the inoculation of cancer cells into the abdominal cavity.

THERAPEUTIC ROLE OF GHRP-2 IN CANCER ANOREXIA-CACHEXIA

Up to 50% of cancer patients report changes in eating behavior at time of diagnosis, leading to weight loss[3]. This study suggests that the addition of GHRP-2 to cytotoxic therapy with 5-FU improved appetite in tumour-bearing mice with anorexia/cachexia syndrome in early stages, although a statistically significant improvement in survival was not achieved compared with mice treated with 5-FU. It is likely that GHRP-2 may overcome any resistance to the appetite-stimulating effects of ghrelin in cachectic animals and cancer patients with appetite loss. In our experiment GHRP-2 has shown to be a short-lasting, acute potent agent, which mimics the orexigenic effects of ghrelin[4,5]. In this study, we demonstrated for the first time that GHRP-2 reversed loss of food intake and body weight in tumour-bearing mice treated with chemotherapy. It is likely that preventing loss of appetite and weight associated with chemotherapy helps mice remain in a relatively balanced condition of electrolytes and hydration, thereby decreasing the side effect and increasing the efficacy of 5-FU. The characteristic of a long acting drug which allows to increased interval of time between two consecutive administrations of the drug, may be a further improve the quality of life in cancer patients.

This study has some limitations. The first is the difficulty to determinate if the survival and the weight loss experienced by tumour-bearing mice was due to tumour burden or to the efficacy or side effect of the 5-FU administration. Secondary, although GHRP-2 did increase the cumulative food intake in tumour-bearing mice, there was a lack of significant difference among groups in survival analysis which should be examined under various chemotherapy conditions and tumour models.

Due to the amelioration of the nutritional state, and of the side effects of chemotherapy, GHRP-2 may offer an interesting treatment for cachexia associated with cancer in order to improve the efficacy of therapy and the quality of life of cancer patients.

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REFERENCES

1 **Inui A**. Cancer anorexia-cachexia syndrome: current issues in research and management. *CA Cancer J Clin* 2002; **52**: 72-91
2 **Bowers CY**, Momany FA, Reynolds GA, Hong A. On the in vitro and in vivo activity of a new synthetic hexapeptide that acts on the pituitary to specifically release growth hormone. *Endocrinology* 1984; **114**: 1537-1545
3 **Neary NM**, Small CJ, Wren AM, Lee JL, Druce MR, Palmieri C, Frost GS, Ghatei MA, Coombes RC, Bloom SR. Ghrelin increases energy intake in cancer patients with impaired appetite: acute, randomized, placebo-controlled trial. *J Clin Endocrinol Metab* 2004; **89**: 2832-2836
4 **Tschop M**, Statnick MA, Suter TM, Heiman ML. GH-releasing peptide-2 increases fat mass in mice lacking NPY: indication for a crucial mediating role of hypothalamic agouti-related protein. *Endocrinology* 2002; **143**: 558-568
5 **Laferrere B**, Abraham C, Russell CD, Bowers CY. Growth hormone releasing peptide-2 (GHRP-2), like ghrelin, increases food intake in healthy men. *J Clin Endocrinol Metab* 2005; **90**: 611-614
6 **Seeliger H**, Guba M, Koehl GE, Doenecke A, Steinbauer M, Bruns CJ, Wagner C, Frank E, Jauch KW, Geissler EK. Blockage of 2-deoxy-D-ribose-induced angiogenesis with rapamycin counteracts a thymidine phosphorylase-based escape mechanism available for colon cancer under 5-fluorouracil therapy. *Clin Cancer Res* 2004; **10**: 1843-1852

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TOPIC HIGHLIGHT

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Characteristic features of ghrelin cells in the gastrointestinal tract and the regulation of stomach ghrelin expression and production

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Abstract

Ghrelin was isolated as an endogenous ligand for the GH secretagogue receptor from the rat stomach. Although physiological effects of ghrelin have been revealed by numerous studies, the regulation of stomach ghrelin remains obscure, and the factor that directly regulates ghrelin expression and production has not been identified. Here, we show some data regarding the characteristic features of ghrelin cells and the regulation of stomach ghrelin. In the gastrointestinal tract, ghrelin cells were identified as opened- and closed-type cells, and it was found that the number of ghrelin cells decreased from the stomach to the colon. The postnatal change in number of ghrelin cells in the stomach showed a sexually dimorphic pattern, indicating a role of estrogen in the regulation of stomach ghrelin. *In vitro* studies revealed that estrogen stimulated both ghrelin expression and production and that treatment with formestane, an aromatase (estrogen synthetase) inhibitor, decreased ghrelin expression level. On the other hand, leptin was found to inhibit both basal and estrogen-stimulated ghrelin expression. Moreover, both aromatase mRNA-expressing cells and leptin cells were found to be located close to ghrelin cells in the gastric mucosa. Furthermore, we found an inverse relationship between gastric ghrelin and leptin levels in a fasting state, and we revealed relative changes in expression of gastric ghrelin, estrogen and leptin in the postnatal rats. We propose that gastric estrogen and leptin directly regulate stomach ghrelin and that the balance control through gastric estrogen and leptin contributes to the altered ghrelin expression level in some physiological states.

Key words: Stomach; Estrogen; Leptin; Regulate; Ghrelin; Expression; Physiological state

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INTRODUCTION

Ghrelin, a 28-amino-acid peptide with an essential n-octanoyl modification on the third amino acid, was purified as an endogenous ligand for the growth hormone (GH) secretagogue receptor from rat stomach in 1999[1]. In initial studies, ghrelin was shown to stimulate GH release from the pituitary both *in vivo* and *in vitro*[1,2], and ghrelin was later found to be also deeply involved in the regulation of feeding behavior and energy homeostasis[3-7].

Ghrelin is predominantly produced in the stomach[1]. Date *et al*[8] further revealed that X/A-like cells in the stomach were responsible for ghrelin production. They also found that in the gastrointestinal tract, the greatest amount of ghrelin was in the gastric mucosa, and smaller amounts were in the small and large intestines[8]. However, the detailed distribution and morphological characteristics of ghrelin cells in the whole gastrointestinal tract have not been elucidated.

On the other hand, it has been reported that both the expression and secretion of ghrelin are mainly influenced by changes in energy balance, *i.e.* increased by fasting and decreased after refeeding[3,9-11]. In addition, many other factors such as leptin, insulin, somatostatin and vagal activity have also been shown to be involved in the regulation of stomach ghrelin[9,12-14]. However, the factor that directly regulates ghrelin expression or production remains unclear, and little is known about the regulation of gastric ghrelin expression in various physiological states.

Therefore, in this review, we summarize the data obtained by our group regarding the distribution and morphological characteristics of gastrointestinal ghrelin cells, postnatal changes in stomach ghrelin, and the regulation of stomach ghrelin in some physiological states.

DISTRIBUTION OF GHRELIN CELLS IN THE RAT GASTROINTESTINAL TRACT

It was found that plasma ghrelin-like immunoreactivity levels were reduced by 65% in totally gastrectomized patients, suggesting that the stomach is the major source of circulating ghrelin[15]. To investigate the morphological characteristics and distribution of ghrelin cells, we used anti-acylated rat ghrelin antiserum to detect ghrelin-immunopositive (ip) cells in the rat gastrointestinal tract in a previous study[16]. We found that ghrelin cells were present throughout the whole gastrointestinal mucosa from the stomach to the colon, and that they could be classified into two types, *i.e.* closed-type cells (Figure 1A) and lumen-contacted opened-type cells (Figure 1B). Interestingly, in ghrelin cells, des-acylated ghrelin was found to be mainly localized to the perinucleus, while acylated ghrelin was found to be distributed in the periphery of the cytoplasm. These findings suggest that des-acylated ghrelin, which is from the Golgi complex, undergoes acylation in secretory granules in the periphery of the cytoplasm.

Further morphometric analysis revealed that the largest number of ghrelin cells was in the stomach and the next-largest number was in the duodenum, and very small numbers of ghrelin cells were observed in the ileum, cecum and colon[16]. These findings are in agreement with the results of another study regarding gastrointestinal ghrelin content[8]. On the other hand, in the stomach, very few opened-type ghrelin cells were observed; but it was found that the percentages of opened-type ghrelin cells in all ghrelin cells in various regions of the gastrointestine gradually increased in the direction from the stomach to the lower intestine, being particularly high in the ileum, cecum and colon[16]. It is generally accepted that opened-type endocrine cells in the gastrointestinal tract are mainly regulated by luminal signals, whereas closed-type cells in the gastrointestinal tract receive modulation from hormones, neuronal stimulation or mechanical distension[17]. Therefore, the distinct distributions of opened- and closed-type ghrelin cells in the gastrointestine suggest that the ghrelin cells may be modulated by different stimulators and may play different physiological roles in various regions of the gastrointestinal tract.

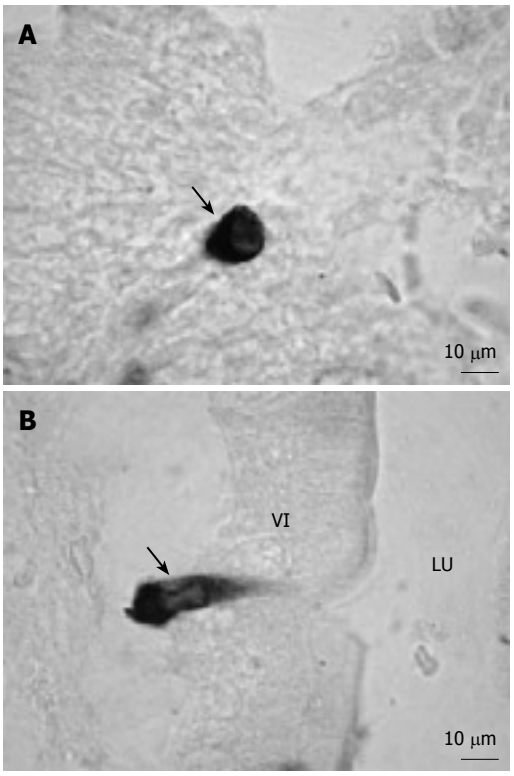

Figure 1 Representative microphotographs of ghrelin-ip cells in the rat gastrointestinal tract[16]. A: Closed-type ghrelin cells (arrow) were found in the crypt of the duodenum; B: Opened-type ghrelin cells (arrow) in contact with the lumen were found in the villi of the duodenum. Bar = 10 μm. VI: Villi; LU: Lumen.

POSTNATAL CHANGES IN STOMACH GHRELIN

In a recent study, the mRNA expression level of gastric ghrelin was shown to be elevated progressively during the second and third postnatal weeks[18]. Gualillo *et al*[19] also reported a gradual increase in the expression level of the gastric ghrelin gene after birth in the rat (up to 90 days). In agreement with these results, we found that gastric ghrelin expression was detectable just after birth in both male and female rats, and that expression levels of gastric ghrelin gradually increased during postnatal development (up to 8 wk of age) (Figure 2A and B).

Accordingly, ghrelin-ip and ghrelin mRNA-expressing cells were also observed just after birth[20]. At 1 wk of age, these ghrelin cells were mainly localized in the glandular base of the fundic gland, and then the distribution of ghrelin cells gradually extended from the glandular base to the glandular neck with increasing age in both sexes[20]. An interesting finding in that study was that two kinds of stained ghrelin cells, weakly stained and strongly stained cells, were found in female rats at the early stage of development (1 and 3 wk of age), whereas staining in most of the ghrelin cells was strong in male rats and 7-wk-old female rats[20]. With increasing age, weakly stained cells were replaced by

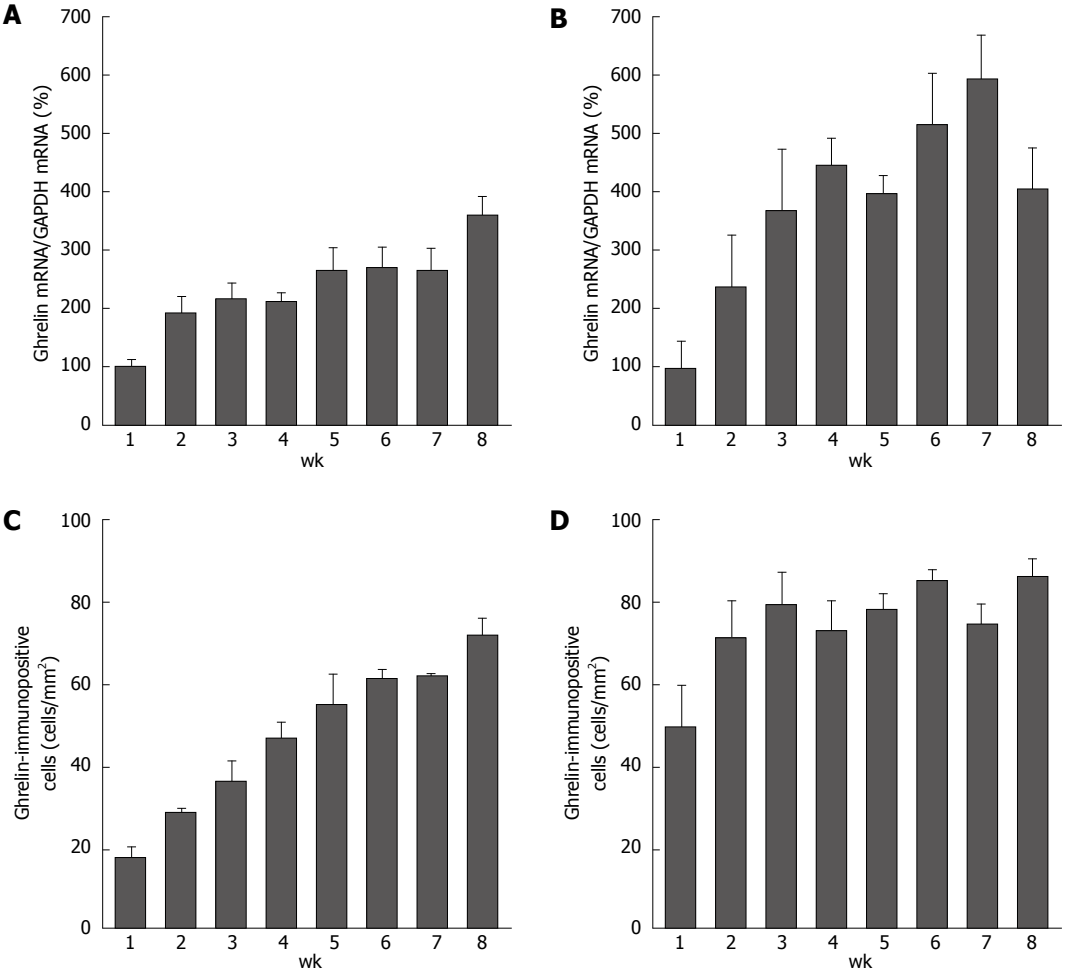

Figure 2 Changes in ghrelin mRNA levels and the densities of ghrelin-ip cells during the postneonatal period in the rat stomach[20]. The data in (A) and (B) are shown as the % of 1-wk-old (1 wk) rats (100%) and each bar represents the mean ± SE (n = 3). A: Ghrelin mRNA levels in the male stomach; B: Ghrelin mRNA levels in the female stomach; C: The densities of ghrelin-ip cells (cells/mm^2) in the male stomach; D: The densities of ghrelin-ip cells (cells/mm^2) in the female stomach.

strongly stained cells, resulting in almost no change in the density of ghrelin cells in female rats throughout the whole period of postnatal development (Figure 2D), in contrast to the increase in ghrelin mRNA expression level. On the other hand, in male rats, ghrelin cell density showed an age-dependent increase after birth (Figure 2C), and the increase was in concert with the increase in ghrelin expression level. The sexual dimorphism of ghrelin cell density suggests that ghrelin cells in female rats differentiate at an earlier stage of development than they do in male rats, and that sex steroids such as estrogen may be involved in the regulation of stomach ghrelin.

REGULATION OF STOMACH GHRELIN BY GASTRIC ESTROGEN AND LEPTIN IN SOME PHYSIOLOGICAL STATES

Regulatory role of gastric estrogen

A role of estrogen in the regulation of stomach ghrelin has also been suggested by several studies. In a previous study, the levels of gastric ghrelin mRNA and plasma ghrelin and the number of ghrelin cells were found to be transiently increased by ovariectomy in female rats[21]. In addition, it was found that ghrelin cells express estrogen receptor α (ERα)[21]. On the other hand, Ueyama *et al*[22] recently demonstrated that aromatase, an estrogen synthetase, is expressed in parietal cells of the rat stomach and that gastric parietal cells are capable of producing and secreting a substantial amount of estrogen. These findings indicate that gastric estrogen plays a role in the regulation of stomach ghrelin.

Therefore, in a previous study, using isolated stomach cells, which are rich in ghrelin cells, we determined the direct effect of estrogen on stomach ghrelin expression and production[23]. In that study, we found that estrogen treatment significantly stimulated ghrelin mRNA expression (Figure 3A) and production in a dose-dependent manner and that treatment of minced stomach tissue with formestane, an aromatase inhibitor, decreased ghrelin expression level[23]. Given that a significant increase in estrogen concentration in the portal vein compared with that in the artery was observed in intact rats, but not in gastrectomized rats[22], the concentration of gastric estrogen must be higher than that of plasma estrogen. Moreover, neither the gastric ghrelin expression nor the plasma

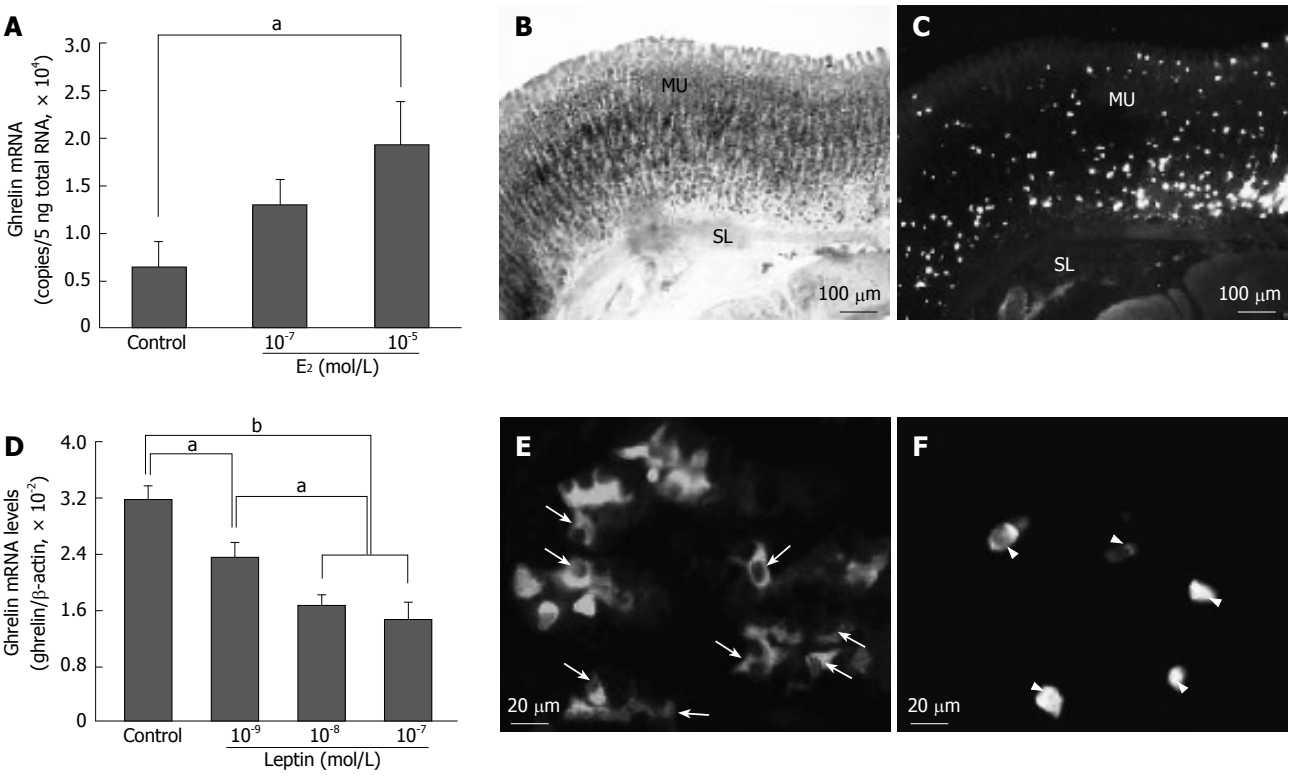

Figure 3 ***In vitro*** **effects of estrogen and leptin on ghrelin mRNA expression and distributions of aromatase mRNA-expressing, leptin-ip and ghrelin-ip cells in the gastric mucosa[23,27].** A: Changes in ghrelin mRNA expression after estrogen treatment; B: Aromatase mRNA-expressing cells were found in the glandular body of the fundic gland; C: Ghrelin-ip cells were found sporadically throughout the gastric mucosa; D: Changes in ghrelin mRNA expression after leptin treatment. Ghrelin mRNA level is expressed relative to β-actin mRNA level; E: Leptin-ip cells (arrow) were found in the lower half of the fundic gland; F: Ghrelin-ip cells (arrowhead) were found in the gastric mucosa. (A, D) Data are presented as mean ± SE. *n* = 3-4/group. $^{a}P < 0.05$; $^{b}P < 0.01$. Bar = 100 μm (B, C) and 20 μm (E, F) respectively. MU: Mucosa; SL: Smooth muscle layer.

ghrelin concentration was altered by gonadectomy[23]. Furthermore, ghrelin cells and aromatase mRNA-expressing cells were found to be located close together in the gastric mucosa (Figure 3B and C), suggesting that ghrelin cells are exposed to gastric estrogen. All of these results strongly suggest that estrogen produced in the stomach directly stimulates ghrelin expression and production in the rat stomach.

Regulatory role of gastric leptin

Reciprocal circadian rhythms in circulating ghrelin and leptin levels and antagonistic hypothalamus-mediated control of appetite by ghrelin and leptin have been revealed by several studies[3,6,24]; however, results of studies on the modulation of ghrelin expression by leptin are inconsistent. One group showed that leptin administration to rats for five days stimulated gastric ghrelin mRNA expression[11], whereas another group reported the opposite results[9]. Due to these conflicting results, the role of leptin in regulation of ghrelin expression remains unclear. On the other hand, leptin was initially thought to be adipocyte-derived[25], and it has also been identified in various tissues, including the stomach[26]. The fact that the release of gastric leptin is rapidly stimulated by food intake or CCK treatment suggests that gastric leptin is involved in the short-term control of energy balance[26], although adipocyte leptin is known to be a long-term regulator of energy balance.

In contrast to the stimulatory effect of estrogen, in another study, we found that leptin inhibits both basal (Figure 3D) and estrogen-stimulated ghrelin expression *in vitro*[27]. The fact that both long and short forms of the leptin receptor have been identified in the human and rat gastric mucosa[28,29] strongly suggests that the gastric epithelium could be a direct target of gastric leptin. Consistent with results of these studies, mRNAs of both OB-Ra and OB-Rb were found to be expressed in the rat gastric fundus, and no inhibitory effect of leptin on ghrelin expression was observed in leptin receptor-defective Zucker fatty (fa/fa) rats, indicating that leptin inhibits ghrelin expression *via* the leptin receptor[27]. Furthermore, it was revealed that leptin cells were mainly located in the lower half of the gastric mucosa, where most of the ghrelin cells were tightly surrounded by leptin cells (Figure 3E and F), suggesting that gastric leptin has a paracrine role in regulation of ghrelin cells, and that ghrelin cells may be exposed to a higher concentration of gastric leptin than that of plasma leptin since leptin infusion at 0.1 nmol/L, which can mimic the plasma leptin concentration under basal conditions in rats, has been shown to be incapable of suppressing ghrelin release from the isolated rat stomach[30].

Relative changes in gastric ghrelin, estrogen and leptin levels in some physiological states

Direct regulation of stomach ghrelin by gastric estrogen

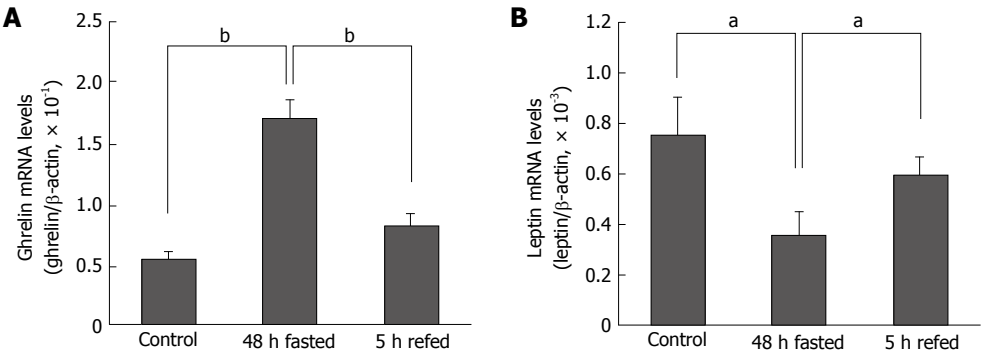

Figure 4 mRNA expression levels of gastric ghrelin and leptin under different feeding conditions[27]. (A) Effects of 48 h of fasting and 5 h of refeeding on gastric ghrelin expression; (B) Effects of 48 h of fasting and 5 h of refeeding on gastric leptin expression. Ghrelin and leptin mRNA levels are expressed relative to β-actin mRNA levels. Data are presented as mean ± SE. n = 3-4/group. $^{a}P < 0.05$; $^{b}P < 0.01$.

and leptin raises the possibility that gastric estrogen and leptin contribute to the altered ghrelin level in some physiological states. It is generally accepted that the most important physiological state for the regulation of stomach ghrelin is fasting. Results of numerous studies have shown that levels of both ghrelin expression and secretion are increased by fasting and decreased after refeeding[3,9-11]. Therefore, in a recent study, we determined the changes in gastric estrogen and leptin levels in relation to gastric ghrelin expression level under a fasting condition[27]. In that study, however, neither the expression level of gastric aromatase nor the concentration of estrogen in the portal vein was altered by fasting[27], although the expression level of gastric ghrelin was significantly elevated as predicted (Figure 4A). In contrast, both gastric leptin expression (Figure 4B) and concentrations were found to be significantly decreased by fasting[27]. In addition, refeeding of fasted animals induced an increase in gastric leptin expression level (Figure 4B), which was also opposite to the decreased gastric ghrelin expression level after food intake (Figure 4A).

In another study, relative changes in expressions of gastric ghrelin, aromatase and leptin in postnatal rats were investigated. We found that gastric ghrelin expression levels significantly increased from 2 wk of age to 4 wk of age. Similarly, gastric aromatase expression level was also significantly elevated in 4-wk-old rats compared with the level in 2-wk-old rats. In contrast, gastric leptin expression level decreased from 2 wk of age to 4 wk of age.

Although the mechanism underlying the fluctuations in gastric estrogen and leptin levels in a certain physiological state remains to be elucidated, these fluctuations in gastric estrogen and leptin levels together with the fact that estrogen and leptin produced in the stomach directly regulate gastric ghrelin expression led us to propose a regulatory model of stomach ghrelin expression in some physiological states. Under a basal (fed) condition, the expression of gastric ghrelin is maintained at a certain level due to a balance between positive regulation from gastric estrogen and negative regulation from gastric leptin. In postnatal development, gastric estrogen level increases and gastric leptin level decreases with increasing age, resulting in gradual elevation of ghrelin expression level during postnatal development. Similarly, under a fasting condition, when fasting reduces gastric leptin level with no change in gastric estrogen level, this balance is also broken by attenuated negative regulation due to decreased gastric leptin level and finally results in increased ghrelin expression. These two models do not, however, exclude the possibility of involvement of other factors such as neural control through the vagal nerve system or other hormones inside and outside the stomach such as gastric somatostatin and insulin. But, we believe that the balance control through gastric estrogen and leptin at least partially contributes to the altered ghrelin expression level in some physiological states.

CONCLUSION

In the gastrointestinal tract, ghrelin cells were identified as opened- and closed-type cells. The greatest number of ghrelin cells was found in the stomach, and it was found that the number of opened-type cells gradually increases in the direction from the stomach to the lower intestine. Stomach ghrelin cells in female rats differentiate at an earlier stage of development than they do in male rats. Gastric estrogen and leptin directly regulate ghrelin expression and production in the stomach, and the balance control through positive regulation from gastric estrogen and negative regulation from gastric leptin contributes to the altered ghrelin expression level in some physiological states. These findings provide new insights into the physiological regulation of stomach ghrelin, and may be important for the development of methods for controlling high ghrelin expression levels in some negative energy balance states, which directly contribute to increased food intake and adiposity. However, the regulatory mechanism of ghrelin is thought to be more complicated, and may involve other factors such as vagal activity and hormones outside the stomach. Further studies are necessary to elucidate the roles of these factors.

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REFERENCES

1 **Kojima M**, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature* 1999; **402**: 656-660
2 **Yamazaki M**, Nakamura K, Kobayashi H, Matsubara M, Hayashi Y, Kangawa K, Sakai T. Regulational effect of ghrelin on growth hormone secretion from perifused rat anterior pituitary cells. *J Neuroendocrinol* 2002; **14**: 156-162
3 **Tschop M**, Smiley DL, Heiman ML. Ghrelin induces adiposity in rodents. *Nature* 2000; **407**: 908-913
4 **Tschop M**, Weyer C, Tataranni PA, Devanarayan V, Ravussin E, Heiman ML. Circulating ghrelin levels are decreased in human obesity. *Diabetes* 2001; **50**: 707-709
5 **Wren AM**, Small CJ, Ward HL, Murphy KG, Dakin CL, Taheri S, Kennedy AR, Roberts GH, Morgan DG, Ghatei MA, Bloom SR. The novel hypothalamic peptide ghrelin stimulates food intake and growth hormone secretion. *Endocrinology* 2000; **141**: 4325-4328
6 **Nakazato M**, Murakami N, Date Y, Kojima M, Matsuo H, Kangawa K, Matsukura S. A role for ghrelin in the central regulation of feeding. *Nature* 2001; **409**: 194-198
7 **Shintani M**, Ogawa Y, Ebihara K, Aizawa-Abe M, Miyanaga F, Takaya K, Hayashi T, Inoue G, Hosoda K, Kojima M, Kangawa K, Nakao K. Ghrelin, an endogenous growth hormone secretagogue, is a novel orexigenic peptide that antagonizes leptin action through the activation of hypothalamic neuropeptide Y/Y1 receptor pathway. *Diabetes* 2001; **50**: 227-232
8 **Date Y**, Kojima M, Hosoda H, Sawaguchi A, Mondal MS, Suganuma T, Matsukura S, Kangawa K, Nakazato M. Ghrelin, a novel growth hormone-releasing acylated peptide, is synthesized in a distinct endocrine cell type in the gastrointestinal tracts of rats and humans. *Endocrinology* 2000; **141**: 4255-4261
9 **Asakawa A**, Inui A, Kaga T, Yuzuriha H, Nagata T, Ueno N, Makino S, Fujimiya M, Niijima A, Fujino MA, Kasuga M. Ghrelin is an appetite-stimulatory signal from stomach with structural resemblance to motilin. *Gastroenterology* 2001; **120**: 337-345
10 **Cummings DE**, Purnell JQ, Frayo RS, Schmidova K, Wisse BE, Weigle DS. A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. *Diabetes* 2001; **50**: 1714-1719
11 **Toshinai K**, Mondal MS, Nakazato M, Date Y, Murakami N, Kojima M, Kangawa K, Matsukura S. Upregulation of Ghrelin expression in the stomach upon fasting, insulin-induced hypoglycemia, and leptin administration. *Biochem Biophys Res Commun* 2001; **281**: 1220-1225
12 **Lucidi P**, Murdolo G, Di Loreto C, De Cicco A, Parlanti N, Fanelli C, Santeusanio F, Bolli GB, De Feo P. Ghrelin is not necessary for adequate hormonal counterregulation of insulin-induced hypoglycemia. *Diabetes* 2002; **51**: 2911-2914
13 **Shimada M**, Date Y, Mondal MS, Toshinai K, Shimbara T, Fukunaga K, Murakami N, Miyazato M, Kangawa K, Yoshimatsu H, Matsuo H, Nakazato M. Somatostatin suppresses ghrelin secretion from the rat stomach. *Biochem Biophys Res Commun* 2003; **302**: 520-525
14 **Williams DL**, Grill HJ, Cummings DE, Kaplan JM. Vagotomy dissociates short- and long-term controls of circulating ghrelin. *Endocrinology* 2003; **144**: 5184-5187
15 **Ariyasu H**, Takaya K, Tagami T, Ogawa Y, Hosoda K, Akamizu T, Suda M, Koh T, Natsui K, Toyooka S, Shirakami G, Usui T, Shimatsu A, Doi K, Hosoda H, Kojima M, Kangawa K, Nakao K. Stomach is a major source of circulating ghrelin, and feeding state determines plasma ghrelin-like immunoreactivity levels in humans. *J Clin Endocrinol Metab* 2001; **86**: 4753-4758
16 **Sakata I**, Nakamura K, Yamazaki M, Matsubara M, Hayashi Y, Kangawa K, Sakai T. Ghrelin-producing cells exist as two types of cells, closed- and opened-type cells, in the rat gastrointestinal tract. *Peptides* 2002; **23**: 531-536
17 **Solcia E**, Rindi G, Buffa R, Fiocca R, Capella C. Gastric endocrine cells: types, function and growth. *Regul Pept* 2000; **93**: 31-35
18 **Lee HM**, Wang G, Englander EW, Kojima M, Greeley GH Jr. Ghrelin, a new gastrointestinal endocrine peptide that stimulates insulin secretion: enteric distribution, ontogeny, influence of endocrine, and dietary manipulations. *Endocrinology* 2002; **143**: 185-190
19 **Gualillo O**, Caminos JE, Kojima M, Kangawa K, Arvat E, Ghigo E, Casanueva FF, Dieguez C. Gender and gonadal influences on ghrelin mRNA levels in rat stomach. *Eur J Endocrinol* 2001; **144**: 687-690
20 **Sakata I**, Tanaka T, Matsubara M, Yamazaki M, Tani S, Hayashi Y, Kangawa K, Sakai T. Postnatal changes in ghrelin mRNA expression and in ghrelin-producing cells in the rat stomach. *J Endocrinol* 2002; **174**: 463-471
21 **Matsubara M**, Sakata I, Wada R, Yamazaki M, Inoue K, Sakai T. Estrogen modulates ghrelin expression in the female rat stomach. *Peptides* 2004; **25**: 289-297
22 **Ueyama T**, Shirasawa N, Numazawa M, Yamada K, Shelangouski M, Ito T, Tsuruo Y. Gastric parietal cells: potent endocrine role in secreting estrogen as a possible regulator of gastro-hepatic axis. *Endocrinology* 2002; **143**: 3162-3170
23 **Sakata I**, Tanaka T, Yamazaki M, Tanizaki T, Zheng Z, Sakai T. Gastric estrogen directly induces ghrelin expression and production in the rat stomach. *J Endocrinol* 2006; **190**: 749-757
24 **Friedman JM**, Halaas JL. Leptin and the regulation of body weight in mammals. *Nature* 1998; **395**: 763-770
25 **Zhang Y**, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. *Nature* 1994; **372**: 425-432
26 **Bado A**, Levasseur S, Attoub S, Kermorgant S, Laigneau JP, Bortoluzzi MN, Moizo L, Lehy T, Guerre-Millo M, Le Marchand-Brustel Y, Lewin MJ. The stomach is a source of leptin. *Nature* 1998; **394**: 790-793
27 **Zhao Z**, Sakata I, Okubo Y, Koike K, Kangawa K, Sakai T. Gastric leptin, but not estrogen and somatostatin, contributes to the elevation of ghrelin mRNA expression level in fasted rats. *J Endocrinol* 2008; **196**: 529-538
28 **Sobhani I**, Bado A, Vissuzaine C, Buyse M, Kermorgant S, Laigneau JP, Attoub S, Lehy T, Henin D, Mignon M, Lewin MJ. Leptin secretion and leptin receptor in the human stomach. *Gut* 2000; **47**: 178-183
29 **Wang MY**, Zhou YT, Newgard CB, Unger RH. A novel leptin receptor isoform in rat. *FEBS Lett* 1996; **392**: 87-90
30 **Kamegai J**, Tamura H, Shimizu T, Ishii S, Sugihara H, Oikawa S. Effects of insulin, leptin, and glucagon on ghrelin secretion from isolated perfused rat stomach. *Regul Pept* 2004; **119**: 77-81

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TOPIC HIGHLIGHT

Akio Inui, MD, PhD, Professor, *Series Editor*

Feeding behavior and gene expression of appetite-related neuropeptides in mice lacking for neuropeptide Y Y5 receptor subclass

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Abstract

Neuropeptide Y (NPY) is a potent neurotransmitter for feeding. Besides NPY, orexigenic neuropeptides such as agouti-related protein (AgRP), and anorexigenic neuropeptides such as α-melatonin stimulating hormone (MSH) and cocaine-amphetamine-regulated transcript (CART) are also involved in central feeding regulation. During fasting, NPY and AgRP gene expressions are up-regulated and POMC and CART gene expressions are down-regulated in hypothalamus. Based on the network of peptidergic neurons, the former are involved in positive feeding regulation, and the latter are involved in negative feeding, which exert these feeding-regulated peptides especially in paraventricular nucleus (PVN). To clarify the compensatory mechanism of knock-out of NPY system on feeding, change in gene expressions of appetite-related neuropeptides and the feeding behavior was studied in NPY Y5-KO mice. Food intake was increased in Y5-KO mice. Fasting increased the amounts of food and water intake in the KO mice more profoundly. These data indicated the compensatory phenomenon of feeding behavior in Y5-KO mice. RT-PCR and ISH suggested that the compensation of feeding is due to change in gene expressions of AgRP, CART and POMC in hypothalamus. Thus, these findings indicated that the compensatory mechanism involves change in POMC/CART gene expression in arcuate nucleus (ARC). The POMC/CART gene expression is important for central compensatory regulation in feeding behavior.

Key words: Neuropeptide Y; Y5 receptor; Feeding; Arcuate nucleus; Knockout mice

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INTRODUCTION

Feeding behavior is a complicated process to regulate the body weight. The body weight is determined by balance between calorie intake mainly by feeding and energy consumption including exercise, body temperature and metabolism. Obesity derived from excess of food intake results in the metabolic syndrome, diabetes mellitus and associated cardiovascular diseases. Since hyperphagia contributes onset and progression of the metabolic syndrome and so on, central feeding regulation is an important issue to be clarified[1,2].

Feeding behavior is regulated strictly by more than 20 appetite-related neuropeptides which are expressed in feeding center in central hypothalamus[1-4]. The main regulation to control feeding are the opposing controls between the orexigenic NPY (neuropeptide Y)/AgRP (agouti-related protein) neurons and the anorexigenic POMC (proopiomelanocortin)/CART (cocaine-amphetamine-regulated transcript) neurons, which originate from the arcuate nucleus (ARC) to the paraventricular nucleus (PVN)[3-6]. The main pathway of two opposite neuron groups obtain and integrate nutritional informations, and communicate information regarding nutrient status and energy stores to the second-order neurons of feeding regulation in PVN[3,5,6]. Among these appetite-related neuropeptides, NPY is the endogenous, strongest orexigenic neuropeptide and fasting induces the most re-

markable increase in NPY gene expression in hypothalamus, indicating its principal and physiological relevance in feeding regulation[3-7].

We have been studying the regulatory mechanism of NPY gene expression in hypothalamus, so as to elucidate the mechanism of feeding-related regulation of NPY gene expression[8-11]. Leptin is an anti-obesity hormone derived from adipose tissue and reduces the NPY gene expression in hypothalamus. This inhibition of NPY gene expression by leptin is shown to be due to activation of SOCS3 in the NPY neurons in arcuate nucleus[11].

In contrast the NPY receptors have been classified into at least 6 subclasses (Y1-y6). Y1, Y2, Y4, Y5, and y6 receptors have been cloned and Y1, Y2 and Y5 receptors are mainly involved in central feeding regulation in hypothalamic ARC and PVN[12-19]. NPY-induced marked induction of feeding behavior intracerebroventricular (icv) is mediated through mainly Y1 and Y5 receptors in mammalians[13,15,16]. Interestingly, although activation of Y1 and Y5 receptors is involved in NPY-induced hyperphagia, the Y1-KO (knockout) and Y5-KO mice develop the late-onset obesity with increase in food intake and adiposity, while NPY-KO mice did not change the feeding behavior or body weight[17,20]. This implies a compensatory mechanism in feeding behavior in these KO mice, which is important for understanding the long-term treatment with the current Y1 and Y5 receptor antagonists for anti-obesity drugs. In addition the compensation against the orexigenic NPY system has not been elucidated to date yet. Therefore, first we chose the Y5-KO mice to investigate the central compensatory mechanism for knockout of NPY system, since the expression of Y5 receptor is much restricted in the brain[16,18,21]. Then we characterized change in the feeding behavior and gene expressions of various appetite-related neuropeptides in the hypothalamus of the NPY Y5-KO mice.

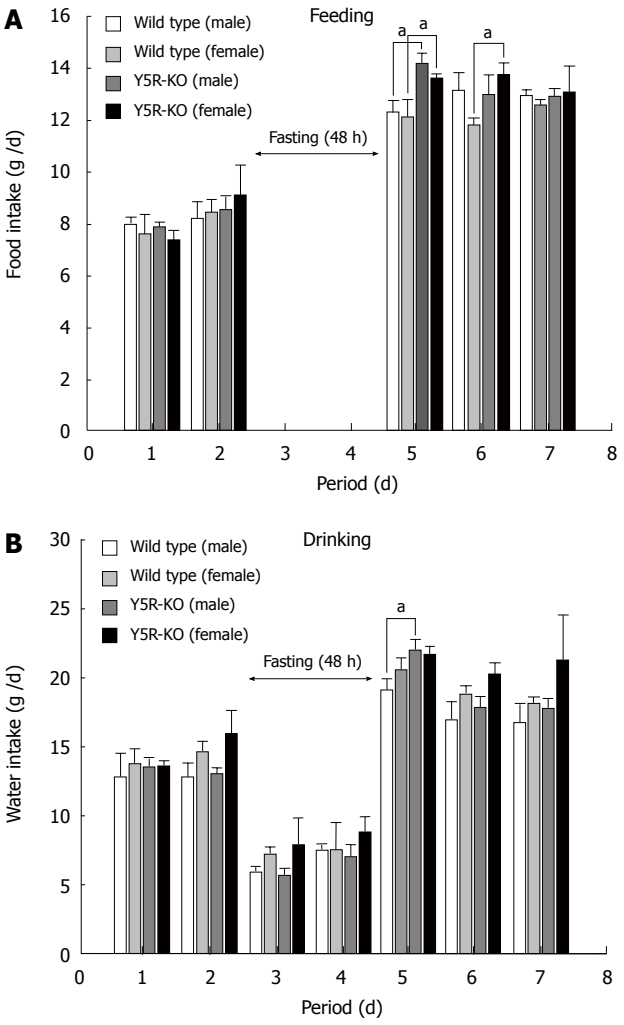

Figure 1 Fasting (48 h)-induced change in food intake (A) and water intake (B) in NPY Y5-KO mice. Fasting (48 h)-induced change in daily amounts of food intake and water intake in Y5-KO mice was measured and compared with those in wild-type (C57BL/6N) mice at 10 wk old. $^{a}P < 0.05$ (unpaired Student's *t* test).

FEEDING BEHAVIOR IN WILD-TYPE C57BL/6N MICE

NPY is the endogenous neuropeptide with most orexigenic potency in hypothalamus, and its injection icv or direct injection into PVN in hypothalamus produces marked increase in feeding behavior in rodents[13-16]. Fasting or central glucoprivation evoked by 2-deoxyglucose (2DG) induces eating with simultaneous increase in orexigenic NPY and AgRP gene expressions in ARC, followed by augmented NPY peptide release[7,8]. Since this fasting-induced food intake is significantly inhibited by selective Y1 or Y5 antagonists, the induction is mediated through Y1 and Y5 receptors (apparently, mainly through Y1 receptors in mice)[13,16]. This NPY/AgRP neurons are essential for feeding in adult mice[22].

In our experiments with male 10-wk-old wild-type mice, fasting for 48 h increased daily food intake and daily water intake by 53% and 50%, respectively (Figure 1). The increase in food intake followed by the concomitant increase in the orexigenic NPY and AgRP gene expressions and decrease in the anorexigenic POMC gene expression in ARC[4]. RT-PCR indicated that NPY and AgRP gene expression is increased remarkably and galanin gene expression is increased moderately, while orexin, MCH, CART or POMC mRNA did not change significantly. These findings suggested that fasting-induced food intake is involved markedly in NPY/AgRP gene expression and moderately in galanin gene expression in ARC in wild-type mice. NPY and AgRP coexist in the same neurons in ARC, and NPY and AgRP gene expressions were increased simultaneously and remarkably by fasting[4,7,8].

In contrast to the concept that the NPY system plays a pivotal role in central feeding regulation, the following studies have performed that in the NPY-KO and NPY Y1-KO mice their body weights or feeding behaviors did not change or that in NPY Y5-KO mice the body weight and food intake increased conversely[17,18,20]. This suggested the existence of a compensatory mechanism other than the NPY system. The existence of multiple

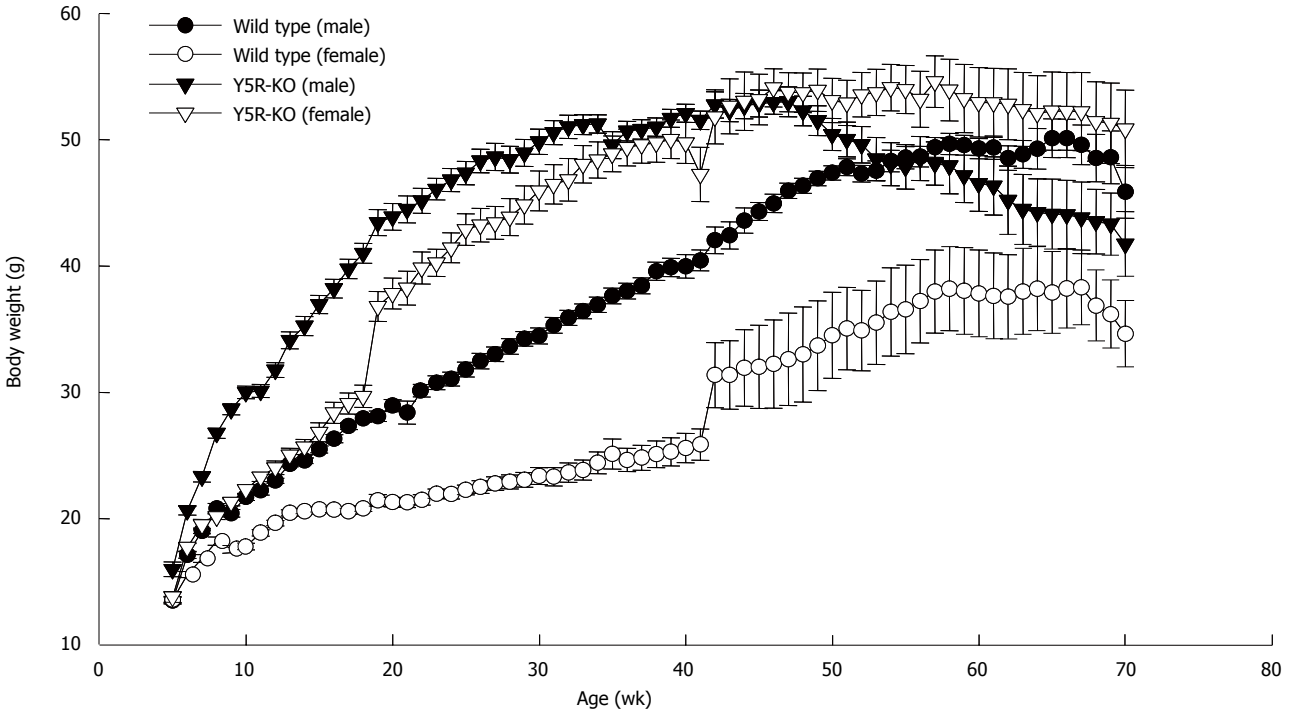

Figure 2 Growth curves of wild-type (C57BL/6N) and NPY Y5-KO mice from 4 to 69 wk old. Data are the mean ± SE. From 4 to 40 wk old, the body weight of Y5-KO mice was twice as much as that of wild-type mice.

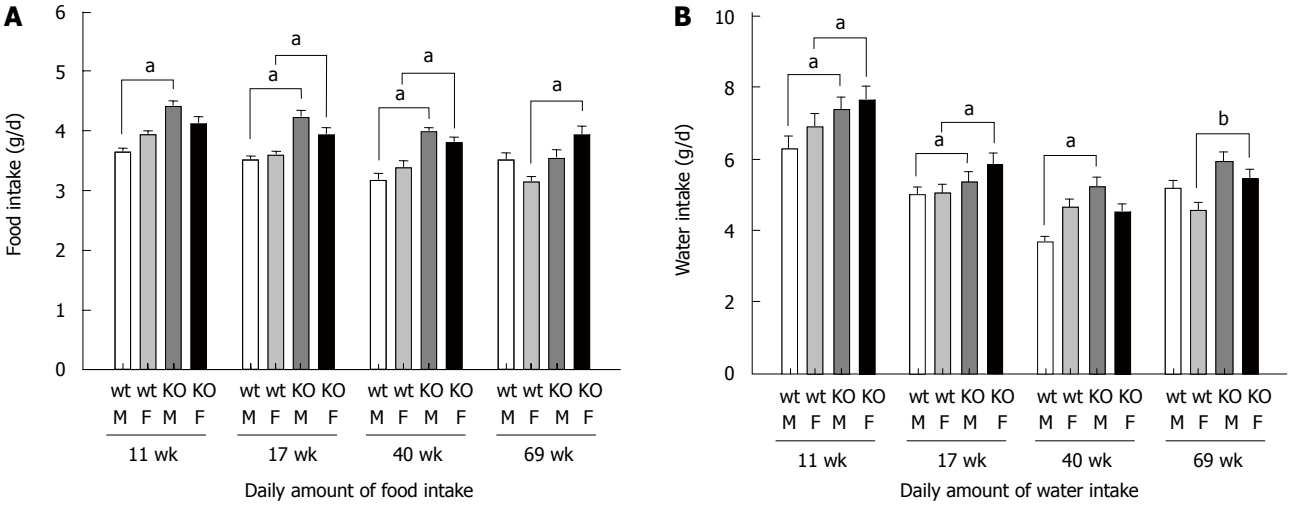

Figure 3 Change in feeding and drinking in NPY Y5-KO mice. Daily amounts of food intake (A) and water intake (B) in Y5-KO mice were measured and compared with those in wild-type (C57BL/6N) mice at the same age (from 11 to 69 wk old). $^{a}P < 0.05$, $^{b}P < 0.01$ (unpaired Student's *t* test). M: Male; F: Female.

feeding-regulatory systems is very important for the homeostasis of body weight. Therefore, we tried to elucidate the compensatory system except for NPY by using NPY Y5-KO mice.

COMPENSATORY FEEDING BEHAVIOR IN NPY Y5-KO MICE

As shown in Figure 2, growth curve of wild-type and NPY Y5-KO mice from 4 to 69 wk old. Although NPY is an orexigenic peptide, obviously Y5-KO mice have obese phenotype. From 4 to 40 wk old, the body weight of Y5-KO mice was twice as much as that of wild-type mice. The increase was observed in both male and female gender but more remarkably in female gender. Because NPY is related with regulation of release of female gonadotropins from pituitary glands, and because female in human being tends to be fatty after menopause, activation through Y5 receptors may be involved in suppression of onset and progression of obesity in female.

As shown in Figure 3, when Y5-KO mice were freely fed, their daily food intake and daily water intake were significantly higher than those of wild-type mice at any age. This suggested that overeating in Y5-KO mice produced obesity of the mice. Next, we measured the fasting (48 h)-induced change in food intake in Y5-KO mice

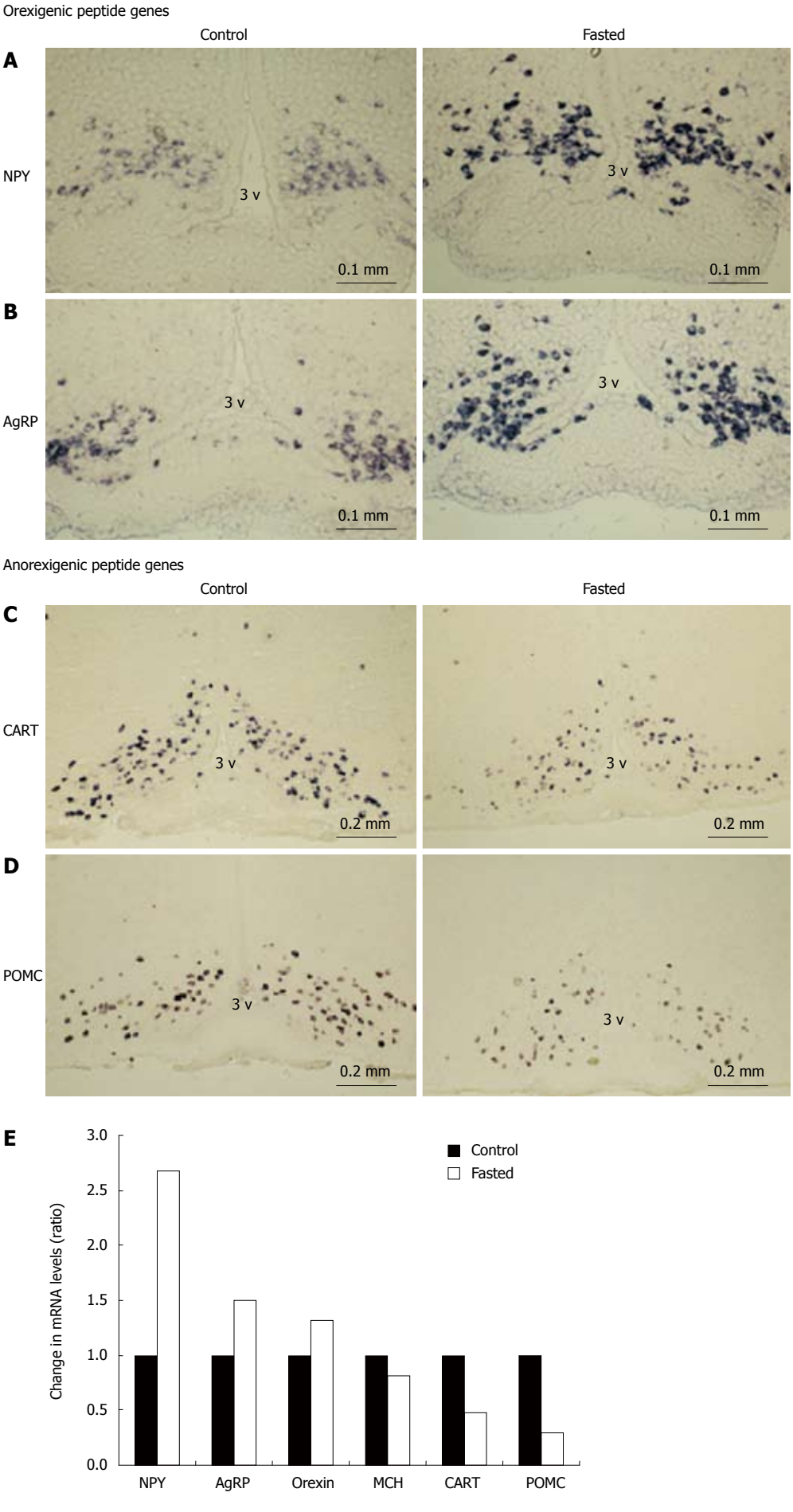

Figure 4 Effect of fasting on appetite-related gene expression in hypothalamus. Fasting (48 h)-induced change in gene expression of appetite-related genes (A: NPY; B: AgRP; C: CART; D: POMC) in Y5-KO mice was measured in the hypothalamus and change in signal intensity was quantitated in panel E at 10 wk old. 3v: Third ventricle; NPY: neuropeptide Y; AgRP: agouti-related protein; MCH: melanin-concentrating hormone; CART: cocaine-amphetamine-regulated transcript; POMC: proopiomelanocortin.

(Figure 1A). Compared with fasting-induced food intake in wild-type mice, obviously fasting-induced feeding was augmented by about 2 times. The fasting-induced water intake in Y5-KO mice was significantly increased following increase in food intake (Figure 1B). Thus, obesity in NPY Y5-KO mice is probably due to overeating *ad libitum* and on fasting.

To deny the possibility that obesity might be due to decrease in energy consumption, the decrease rate in body weight was studied during fasting (48 h) in Y5-KO mice. The body weght loss in 2 d of Y5-KO mice was almost the same to that in wild-type mice (data not shown). This indicated that the energy consumption rate is not changed in Y5-KO mice, but the obesity in Y5-KO mice is simply due to increased food intake *ad libitum* and on fasting.

CHANGE IN GENE EXPRESSION OF APPETITE-RELATED PEPTIDES IN HYPOTHALAMUS

The compensatory feeding behavior might be due to change in gene expressions of appetite-related neuropeptides other than NPY. Therefore, the change in gene expression of feeding-regulating peptides in hypothalamus was investigated, first, when NPY Y5-KO mice were fed freely. Under ordinary conditions in NPY Y5-KO mice the NPY and AgRP gene expressions were diminished probably due to disuse[4]. The decrease in NPY and AgRP gene expression which expresses in the same neuron group in ARC may be due to increased leptin level following obesity in Y5-KO mice. The gene expression of orexin, MCH, or CART was not changed in hypothalamus; but the POMC gene expression was significantly decreased by RT-PCR and ISH, suggesting that the synthesis of anorexigenic POMC-derived peptides such as -MSH is decreased. This decrease in POMC gene expression appears to be the principal cause of the compensatory overeating.

AUGMENTATION OF FASTING-INDUCED CHANGE IN GENE EXPRESSION IN NPY Y5-KO MICE

Fasting for 48 h produces augmented the fasting-induced food intake, with the concomitant increase in the orexigenic NPY and AgRP gene expressions and decrease in the anorexigenic POMC gene expression in ARC in wild-type mice[4]. Obviously the fasting-induced feeding behavior is augmented in Y5-KO mice (Figure 1). Next we investigated whether changes in gene expressions of appetite-regulated neuropeptides in hypothalamus are involved in 48 h-fasting-induced induced feeding behavior (Figure 4). NPY and AgRP gene expressions were induced by 48 h-fasting more profoundly in NPY Y5-KO mice than those in wild-type mice (Figure 4A and B). In contrast, CART and POMC gene expression were conversely decreased more markedly by fasting in Y5-KO mice. In contrast orexin and MCH (melanin-concentrating hormone) gene expressions were not changed by fasting in the mouse hypothalamus (Figure 4E). Thus, the augmentation of fasting-induced feeding behavior in NPY Y5-KO mice was accompanied by the concomitant fasting-induced changes in NPY/AgRP (both increase) and POMC/CART (both decrease) gene expression. Because the NPY system probably dysfunctions in NPY Y5-KO mice, the concomitant increase in AgRP gene expression and decrease in POMC and CART gene expression are the cause of fasting-induced augmentation of feeding behavior in NPY Y5-KO mice. The compensatory mechanism of feeding is probably due to overfunction of compensation by POMC and partly CART gene expressions which results in the late-onset obesity in Y5-KO mice.

CONCLUSION

Feeding behavior and energy balance are regulated in complicated manner by networks of neurons with classical neurotransmitters and appetite-related peptides. In this article, we showed that the compensatory feeding behavior occurs in NPY Y5-KO mice when the NPY system is probably inhibited, so that the late-onset obesity has appeared. This is probably due to the compensatory change in POMC gene expression. At present the mechanism of compensatory change in POMC/AgRP gene expression is still unknown, and remains to be clarified. This compensatory mechanism is not dependent on the technical procedure of knockout mice. Questions regarding when the compensatory mechanism was been completed, and whether the compensation might occur essential only in adult brain[22,23].

The investigation about the regulation of NPY, AgRP and POMC gene expression in hypothalamic nuclei is useful for elucidation of central feeding regulation and also for development of novel anti-obesity drugs in future.

REFERENCES

1 **Schwartz MW**, Woods SC, Porte D Jr, Seeley RJ, Baskin DG. Central nervous system control of food intake. *Nature* 2000; **404**: 661-671
2 **Morton GJ**, Cummings DE, Baskin DG, Barsh GS, Schwartz MW. Central nervous system control of food intake and body weight. *Nature* 2006; **443**: 289-295
3 **Murphy KG**, Bloom SR. Gut hormones and the regulation of energy homeostasis. *Nature* 2006; **444**: 854-859
4 **Higuchi H**, Yamaguchi T, Niki T. [Regulation of hypothalamic neuropeptide expression and feeding behavior in NPY-Y5 knockout (KO) mice] *Nippon Yakurigaku Zasshi* 2006; **127**: 92-96
5 **Kalra SP**, Kalra PS. Neuropeptide Y: a physiological orexigen modulated by the feedback action of ghrelin and leptin. *Endocrine* 2003; **22**: 49-56
6 **O'Rahilly S**, Yeo GS, Farooqi IS. Melanocortin receptors weigh in. *Nat Med* 2004; **10**: 351-352
7 **Bertile F**, Oudart H, Criscuolo F, Maho YL, Raclot T. Hypothalamic gene expression in long-term fasted rats: relationship with body fat. *Biochem Biophys Res Commun* 2003; **303**: 1106-1113

8 **Minami S**, Kamegai J, Sugihara H, Suzuki N, Higuchi H, Wakabayashi I. Central glucoprivation evoked by administration of 2-deoxy-D-glucose induces expression of the c-fos gene in a subpopulation of neuropeptide Y neurons in the rat hypothalamus. *Brain Res Mol Brain Res* 1995; **33**: 305-310
9 **Higuchi H**, Nakano K, Kim CH, Li BS, Kuo CH, Taira E, Miki N. Ca2+/calmodulin-dependent transcriptional activation of neuropeptide Y gene induced by membrane depolarization: determination of Ca(2+)- and cyclic AMP/phorbol 12-myristate 13-acetate-responsive elements. *J Neurochem* 1996; **66**: 1802-1809
10 **Muraoka O**, Xu B, Tsurumaki T, Akira S, Yamaguchi T, Higuchi H. Leptin-induced transactivation of NPY gene promoter mediated by JAK1, JAK2 and STAT3 in the neural cell lines. *Neurochem Int* 2003; **42**: 591-601
11 **Higuchi H**, Hasegawa A, Yamaguchi T. Transcriptional regulation of neuronal genes and its effect on neural functions: transcriptional regulation of neuropeptide Y gene by leptin and its effect on feeding. *J Pharmacol Sci* 2005; **98**: 225-231
12 **Woldbye DP**, Larsen PJ. The how and Y of eating. *Nat Med* 1998; **4**: 671-672
13 **Iyengar S**, Li DL, Simmons RM. Characterization of neuropeptide Y-induced feeding in mice: do Y1-Y6 receptor subtypes mediate feeding? *J Pharmacol Exp Ther* 1999; **289**: 1031-1040
14 **Yokosuka M**, Dube MG, Kalra PS, Kalra SP. The mPVN mediates blockade of NPY-induced feeding by a Y5 receptor antagonist: a c-FOS analysis. *Peptides* 2001; **22**: 507-514
15 **Gerald C**, Walker MW, Criscione L, Gustafson EL, Batzl-Hartmann C, Smith KE, Vaysse P, Durkin MM, Laz TM, Linemeyer DL, Schaffhauser AO, Whitebread S, Hofbauer KG, Taber RI, Branchek TA, Weinshank RL. A receptor subtype involved in neuropeptide-Y-induced food intake. *Nature* 1996; **382**: 168-171
16 **Kanatani A**, Mashiko S, Murai N, Sugimoto N, Ito J, Fukuroda T, Fukami T, Morin N, MacNeil DJ, Van der Ploeg LH, Saga Y, Nishimura S, Ihara M. Role of the Y1 receptor in the regulation of neuropeptide Y-mediated feeding: comparison of wild-type, Y1 receptor-deficient, and Y5 receptor-deficient mice. *Endocrinology* 2000; **141**: 1011-1016
17 **Marsh DJ**, Hollopeter G, Kafer KE, Palmiter RD. Role of the Y5 neuropeptide Y receptor in feeding and obesity. *Nat Med* 1998; **4**: 718-721
18 **Pedrazzini T**, Seydoux J, Kunstner P, Aubert JF, Grouzmann E, Beermann F, Brunner HR. Cardiovascular response, feeding behavior and locomotor activity in mice lacking the NPY Y1 receptor. *Nat Med* 1998; **4**: 722-726
19 **Naveilhan P**, Hassani H, Canals JM, Ekstrand AJ, Larefalk A, Chhajlani V, Arenas E, Gedda K, Svensson L, Thoren P, Ernfors P. Normal feeding behavior, body weight and leptin response require the neuropeptide Y Y2 receptor. *Nat Med* 1999; **5**: 1188-1193
20 **Erickson JC**, Clegg KE, Palmiter RD. Sensitivity to leptin and susceptibility to seizures of mice lacking neuropeptide Y. *Nature* 1996; **381**: 415-421
21 **Huang XF**, Han M, Storlien LH. The level of NPY receptor mRNA expression in diet-induced obese and resistant mice. *Brain Res Mol Brain Res* 2003; **115**: 21-28
22 **Luquet S**, Perez FA, Hnasko TS, Palmiter RD. NPY/AgRP neurons are essential for feeding in adult mice but can be ablated in neonates. *Science* 2005; **310**: 683-685
23 **Melnick I**, Pronchuk N, Cowley MA, Grove KL, Colmers WF. Developmental switch in neuropeptide Y and melanocortin effects in the paraventricular nucleus of the hypothalamus. *Neuron* 2007; **56**: 1103-1115

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TOPIC HIGHLIGHT

Akio Inui, MD, PhD, Professor, *Series Editor*

Different effects of ghrelin, des-acyl ghrelin and obestatin on gastroduodenal motility in conscious rats

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Abstract

Three peptides, ghrelin, des-acyl ghrelin and obestatin are derived from a common prohormone, preproghrelin by posttranslational processing, originating from endocrine cells in the stomach. To examine the effects of these peptides, we applied the manometric measurement of gastrointestinal motility in freely moving conscious rat models. Ghrelin exerts stimulatory effects on the motility of antrum and duodenum in both fed and fasted state of animals. Des-acyl ghrelin exerts inhibitory effects on the motility of antrum, but not on the motility of duodenum in the fasted state of animals. Obestatin exerts inhibitory effects on the motility of antrum and duodenum in the fed state, but not in the fasted state of animals. NPY Y2 or Y4 receptors in the brain may mediate the action of ghrelin, CRF type 2 receptors in the brain mediate the action of des-acyl ghrelin, whereas CRF type 1 and type 2 receptors in the brain mediate the action of obestatin. Vagal afferent pathways might be involved in the action of ghrelin, but not involved in the action of des-acyl ghrelin, whereas vagal afferent pathways might be partially involved in the action of obestatin.

Key words: Ghrelin; Des-acyl ghrelin; Obestatin; Gastrointestinal motility; Hypothalamus

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INTRODUCTION

Ghrelin, des-acyl ghrelin and obestatin are derived from a prohormone, preproghrelin by posttranslational processing. Ghrelin was first identified as endogenous ligand for growth hormone secretagogue receptors (GHS-R) with O-n-octanoyl acid modification at serine 3 position[1]. On the other hand, des-acyl ghrelin has no O-n-octanoyl acid modification[1]. Obestatin was predicted to be formed from preproghelin by a bioinformatic approach[2]. Obestatin was initially reported to be endogenous ligand for orphan G protein-coupled receptor GPR39[2]; however, recent studies have found no specific binding of obestatin to various types of GPR39-expressing cells[3-5]. Ghrelin is a potent stimulator of food intake and gastrointestinal motility[6], while des-acyl ghrelin exerts opposite effects on food intake and gastrointestinal motility[7]. The effects of obestatin on food intake and gastrointestinal motility have been controversial[8-13]. Very recently we have reported that obestatin exerts inhibitory action on gastroduodenal motility in the fed state of conscious rats[14]. Previous studies have shown that food intake and gastroduodenal motility are tightly related. For example, feeding stimulatory peptides such as NPY and ghrelin stimulate gastroduodenal motility[15,16], while feeding inhibitory peptides such as CRF and urocortin inhibit the gastroduodenal motility[17]. Here, we overview different effects of ghrelin, des-acyl ghrelin and obestatin on gastroduodenal motility by using freely moving conscious rat models.

MANOMETRIC MEASUREMENT OF GASTROINTESTINAL MOTILITY IN CONSCIOUS RATS

We developed freely moving conscious rat model to

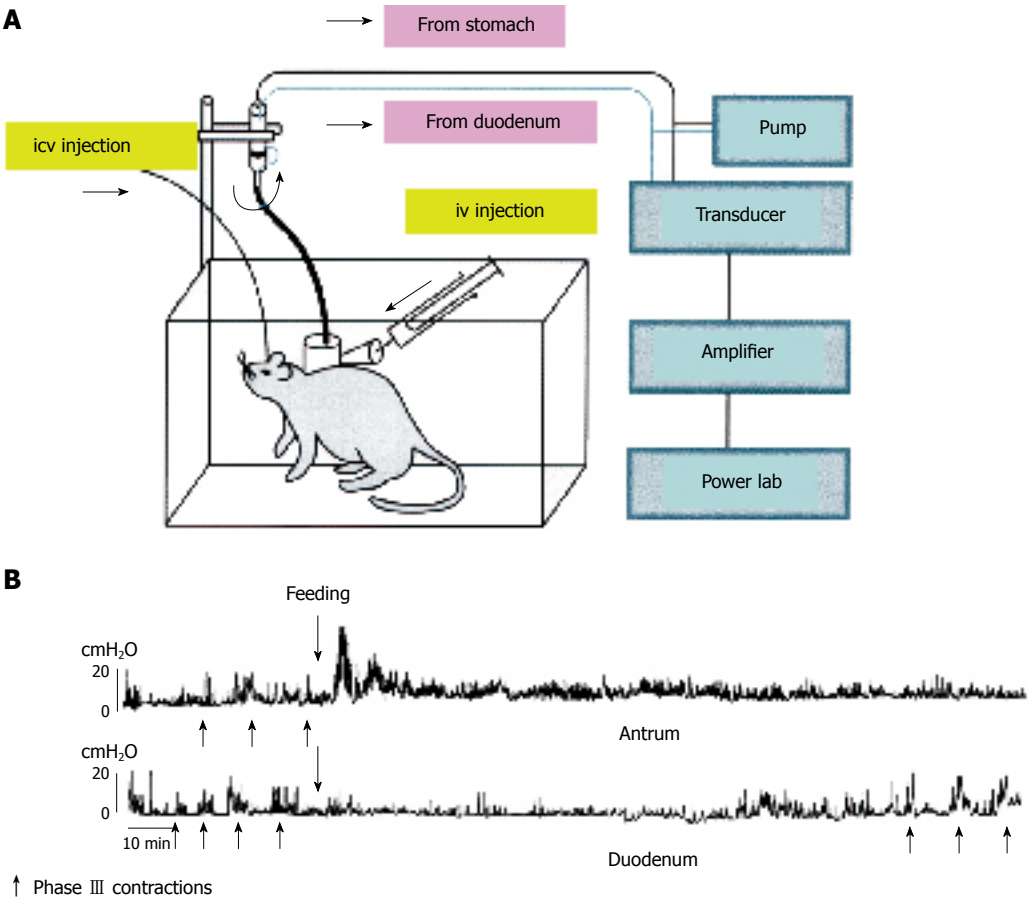
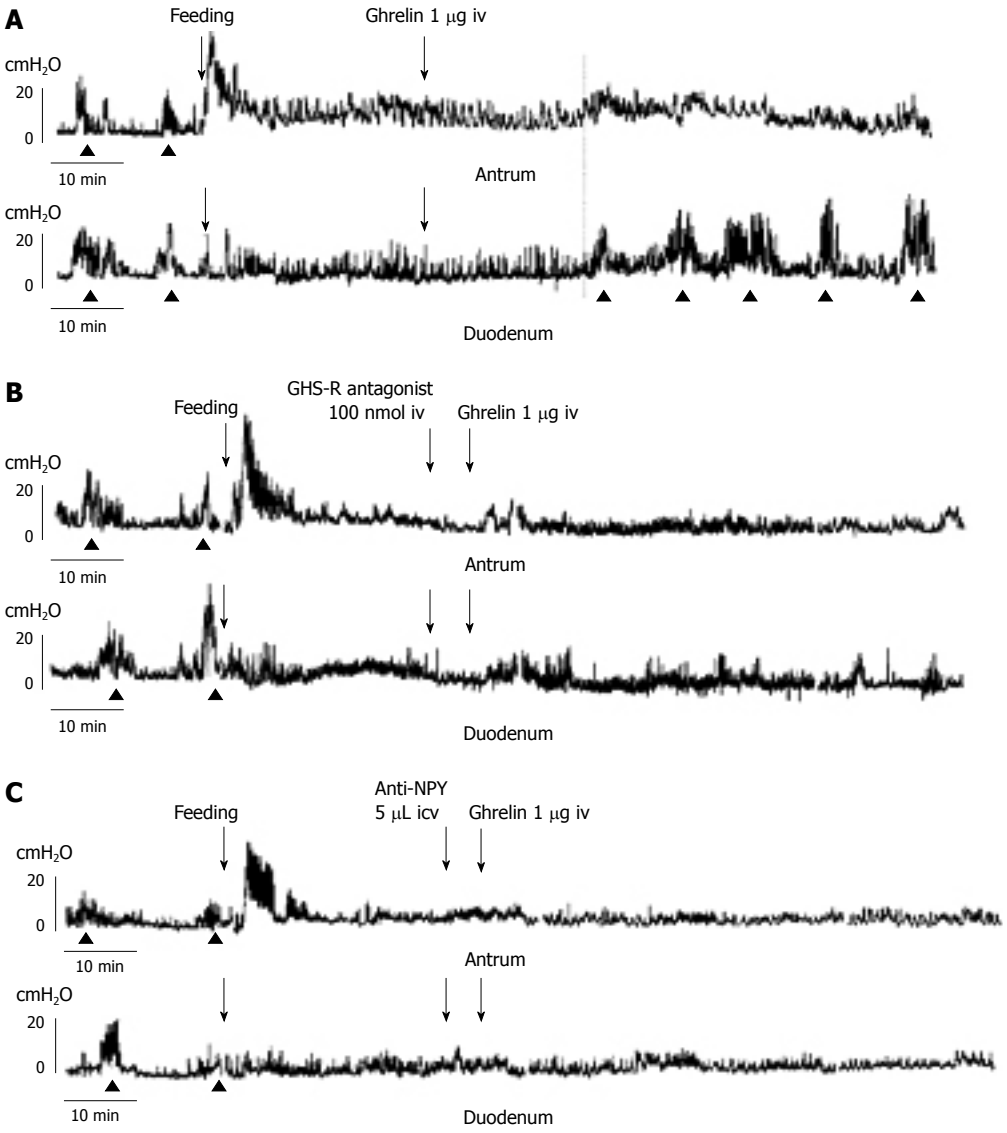


Figure 1 Measurement of gastrointestinal motility. A: Manometric measurement of gastroduo-denal motility in freely moving conscious rats; B: Fasted and fed motor activities in the antrum and duodenum. Phase Ⅲ-like contractions are indicated by arrows.

measure the gastrointestinal motility[15] (Figure 1A). This model permits the measurement of gastrointestinal motility in animals in the physiological fed and fasted states by a manometric method[15]. In the fasted state, the cyclic change of pressure waves were detected in both antrum and duodenum, including the quiescence period during which relatively low amplitude contractions occur (phase I-like contractions), followed by a grouping of strong contractions (phase Ⅲ-like contractions) (Figure 1B). The frequency of the onset of phase Ⅲ-like contractions was 5.3 ± 0.5/h (n = 6) in the antrum and 5.6 ± 0.8/h (n = 6) in the duodenum[16]. After food intake, such fasted motor pattern was disrupted and replaced by a fed motor pattern, which consisted of irregular contractions of high frequency (Figure 1B). The fed pattern continued for 85.7 ± 6.8 min (n = 5) in the duodenum and for more than 240 min in the antrum when rats were given 3 g of chow, and then replaced by the fasted motor pattern[14].

GHRELIN AND GASTRODUODENAL MOTILITY

Intracerebroventricular (icv) and intravenous (iv) injection of ghrelin stimulated the % motor index (%MI) in the antrum and induced the fasted motor activity in the duodenum when given in the fed state of animals[16] (Figure 2A). Icv and iv injection of ghrelin increased the frequency of phase Ⅲ-like contractions in both antrum and duodenum when given in the fasted state of animals[16]. The effects of iv injection of ghrelin on gastroduodenal motility were blocked by iv injection of GHS-R antagonist, but not by icv injection of GHS-R antagonist[16] (Figure 2B). In vagotomized animals, iv injection of ghrelin-induced the fasted motility in both antrum and duodenum when given in the fed state, iv injection of GHS-R antagonist completely blocked phase Ⅲ-like contractions in both antrum and duodenum[16]. Immunoneutralization of NPY in the brain blocked the stimulatory effects of ghrelin on the gastroduodenal motility[16] (Figure 2C). These results indicate that ghrelin released from the stomach may act on the ghrelin receptor on vagal afferent nerve terminals and NPY neurons in the brain may mediate the action of ghrelin on the gastroduodenal motility. *C-Fos* expression in the arcuate nucleus (ARC) in the hypothalamus and in the nucleus tractus solitarius (NTS) induced by intraperitoneal (ip) injection of ghrelin confirmed this effect (Figure 2D). Our previous study showed that immunoneutralization of NPY in the brain completely blocked the phase Ⅲ-like contractions in the duodenum of normal rats, and Y2 and Y4 receptor agonists induced the phase Ⅲ-like contractions in the duodenum when given in the fed state of animals[15]. Combined together, in normal animals ghrelin may stimulate gastroduodenal motility by activating the GHS-R on vagal afferent nerve terminals and affect NPY neurons in the hypothalamus, Y2 and/or Y4 receptors in the brain may mediate the action of ghrelin (Figure 3). Once the brain mechanism is eliminated by truncal vagotomy, ghrelin might be primarily involved in the regulation of fasted molility through GHS-R on the stomach and duodenum.



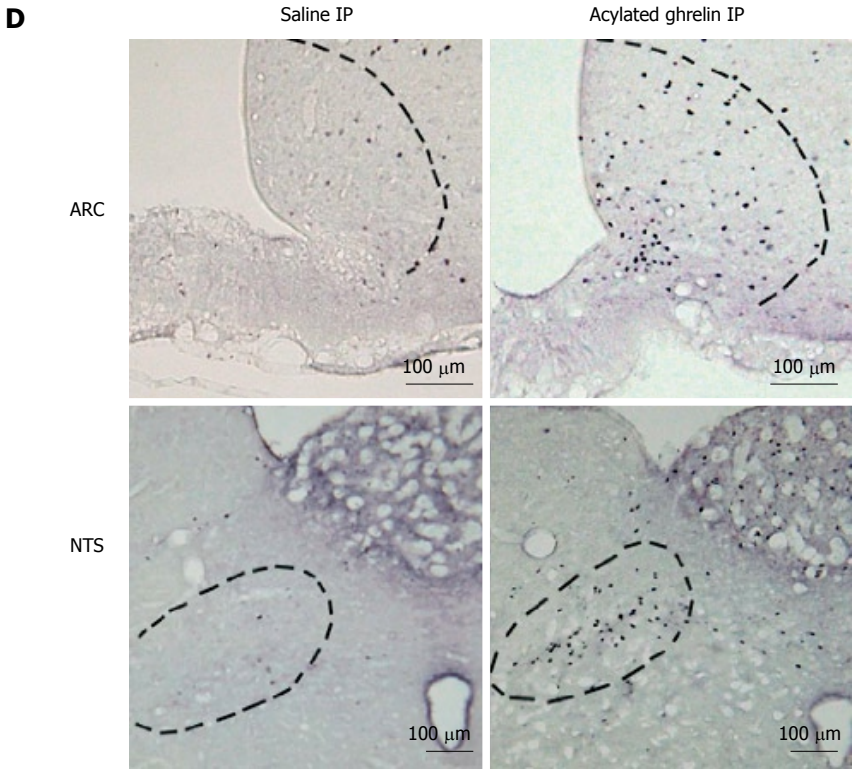
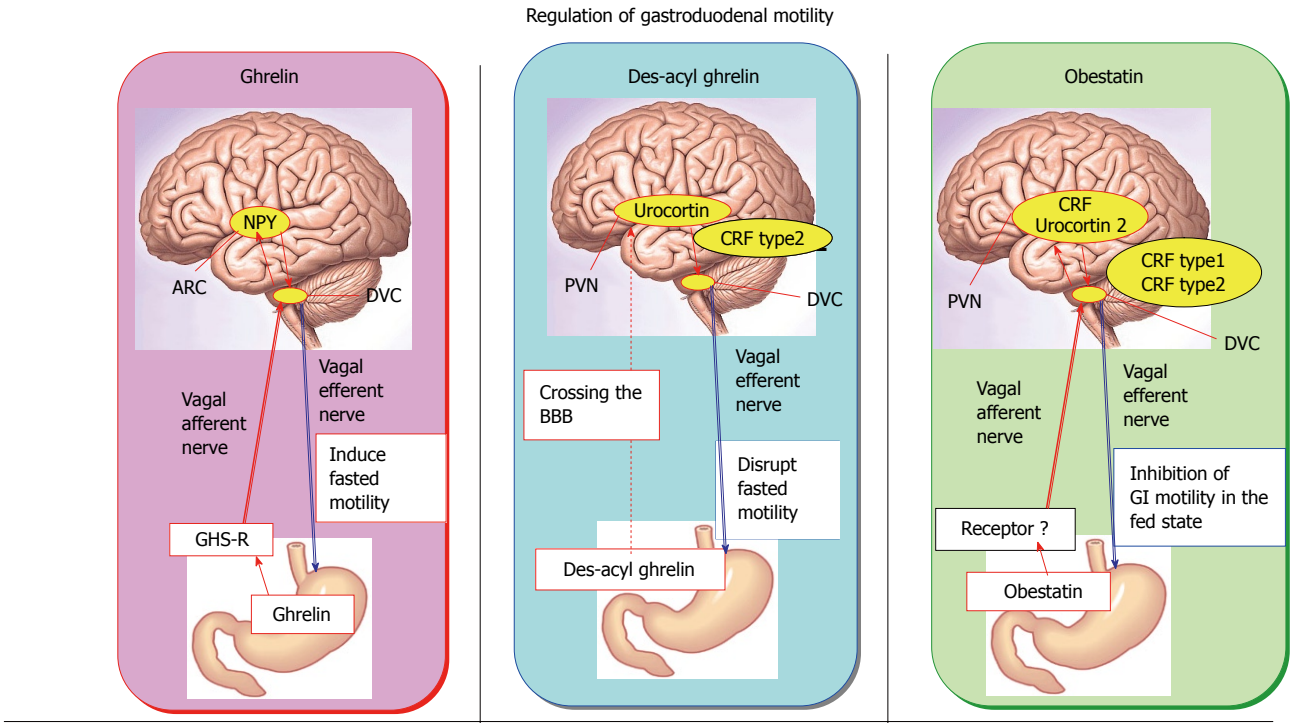


Figure 2 Ghrelin and gastroduodenal motility. A: Effects of iv injection of ghrelin on the fed motor activity of the antrum and duodenum. Iv injection of ghrelin induces the fasted pattern in the duodenum and increases the motor activity in the antrum; B: Iv injection of GHS-R antagonist completely blocks the effect of iv injection of ghrelin; C: Icv injection of NPY antiserum completely blocks the effect of iv injection of ghrelin; D: The density of c-Fos-positive cells in the arcuate nucleus (ARC) and nucleus tractus solitarius (NTS) increases with ip injection of ghrelin compared to saline-injected control.



	Ghrelin		Des-acyl ghrelin		Obestatin	
	Fasted state	Fed state	Fasted state	Fed state	Fasted state	Fed state
Stomach	↑	↑	↓	—	—	↓
Duodenum	↑	↑	↓	—	—	↓
Hypothalamus neuron	NPY		Urocortin		CRF, urocortin	
Brain receptor	Y2, Y4		CRF type 2		CRF type 1, type 2	
Vagal afferent	+		-		+	

Figure 3 Summary diagram of different effects of ghrelin, des-acyl ghrelin and obestatin on the gastroduodenal motility and brain mechanisms mediating the action of these peptide.

Human ghrelin has a structural resemblance to human motilin, and human ghrelin receptors exhibit a 50% identity with human motilin receptors[18]. Therefore, the role of ghrelin in the gastrointestinal motility is comparable with that of motilin[19,20]. Motilin originates from the endocrine cells in the duodenum[19], while ghrelin originates from the endocrine cells in the stomach[21], both of them are involved in the regulation of phase Ⅲ contractions in the gastrointestinal tracts. Motilin induces fasted motility in the stomach and duodenum when it is given peripherally, but not when given centrally[20,22], while ghrelin induces fasted motility in the duodenum when it is given both peripherally and centrally[16]. Since it is known that gastric acidification modulates the action of motilin[23], we examined the relationship between the effects of ghrelin on gastroduodenal motility and intragastric pH. The results showed that within 30 min after feeding, low intragastric pH (pH 2.5 ± 0.2) inhibited the effects iv injected ghrelin on gastroduodenal motility, and that this effect was reversed by an increase of intragastric pH (pH 5.4 ± 0.6) within 60 min after feeding, or by pretreatment of famotidine (intragastric pH 6.0-6.7)[16]. These results suggest that the sensitivity of the GHS-R in the gastrointestinal tract might be inhibited by low intragastric pH.

DES-ACYL GHRELIN AND GASTRODUODENAL MOTILITY

Central and peripheral administration of des-acyl ghrelin has been shown to significantly decrease food intake in food-deprived mice and decrease gastric emptying[6]. Transgenic mice with overexpression of the des-acyl ghrelin gene exhibited a decrease in body weight, food intake and fat mass weight accompanied by moderately decreased linear growth compared with their nontransgenic littermates[6]. In rats, des-acyl ghrelin injected intraperitoneally (ip) effectively decreased food intake in food-deprived rats, and decreased the dark-phase food intake in free-feeding rats, but failed to decrease the light-phase food intake in free-feeding rats[7].

Icv and iv injections of des-acyl ghrelin disrupted fasted motility in the antrum, but not in the duodenum[7] (Figire 4A). The frequencies of fasted motility in the antrum were decreased to 58.9% and 54.5% by des-acyl ghrelin injcted icv and iv, respectively[7]. However icv and iv injections of des-acyl ghrelin did not alter fed motor activity in both the antrum and duodenum[7] (Figure 4A). These data indicate that the dominant role of exogenous des-acyl ghrelin affects fasted motility in the antrum, but not in the duodenum. The results showed that capsaicin

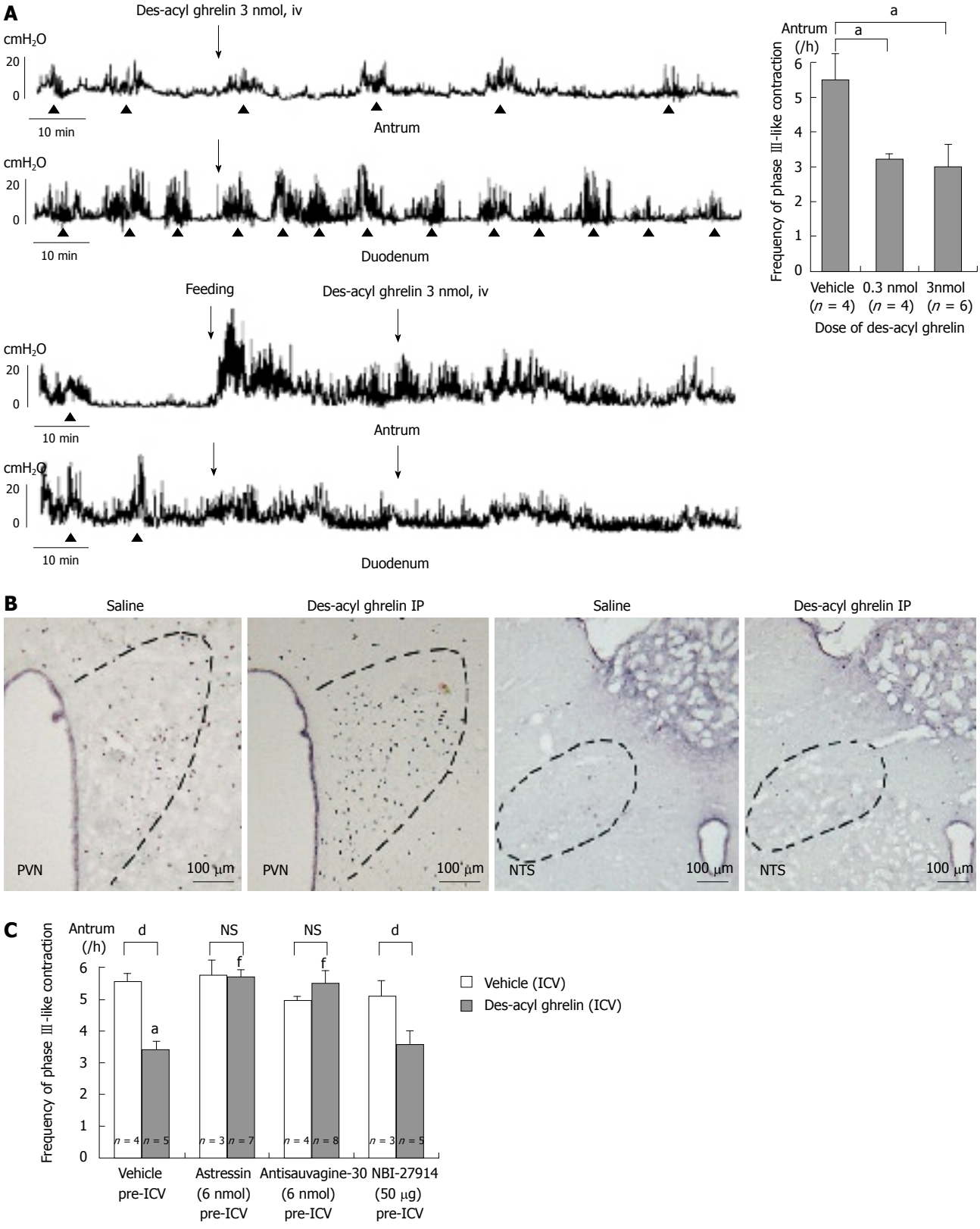


Figure 4 Des-acyl ghrelin and gastroduodenal motility. A: Effects of iv injection of des-acyl ghrelin on the fasted and fed motor activities of the antrum and duodenum. Iv injection of des-acyl ghrelin decreases the frequency of phase Ⅲ-like contractions in the antrum, but not in the duodenum. Iv injection of des-acyl ghrelin does not affect fed motor activity in both antrum and duodenum. [a]$P < 0.05$; B: The density of c-Fos-positive cells in the paraventricular nucleus (PVN) is increased by ip injection of des-acyl ghrelin compared to saline-injected control, whereas that in the NTS is not altered; C: The decreased frequency of phase Ⅲ-like contractions induced by iv injection of des-acyl ghrelin is restored to normal in pretreatment of nonselective CRF receptor antagonist astressin and the selective CRF type 2 receptor antagonist antisauvagine-30, but not CRF type 1 receptor antagonist NBI-27914. [d]$P < 0.01$, [f]$P < 0.001$ compared with a.

treatment did not alter the disruptive effect of iv injection of des-acyl ghrelin on fasted motility in the antrum[7]. These results were consistent with electrophysiological studies, which showed that peripheral administration of

ghrelin suppressed firing of the vagal afferent pathways, whereas des-acyl ghrelin had no effect on vagal afferent pathways[24]. Difference in the involvement of vagal afferent pathways in the action of ghrelin and des-acy ghrelin was confirmed by c-*Fos* expression in the NTS. Ip injection of ghrelin significantly increased the density of c-*Fos*-positive cells in the NTS (Figure 2D), while ip injection of des-acyl ghrelin induced no change in the density of c-*Fos*-positive cells in the NTS compared with vehicle-injected controls[7] (Figure 4B). Taken together, these results suggest that peripherally administered des-acyl ghrelin may cross the blood-brain barrier (BBB) and act directly on the brain receptor and disrupt the fasted motility in the antrum (Figure 3).

The results showed that the centrally administered CRF type 2 receptor antagonist, but not the CRF type 1 receptor antagonist, blocked the effects of centrally and peripherally administered des-acyl ghrelin on gastric motility[7] (Figure 4C). Among two CRF receptor subtypes, CRF type 1 receptor is highly involved in anxiety-related behavior and CRF type 2 receptor is involved in regulating food intake and peripheral functions such as gastric acid secretion or gastric emptying. CRF is a relatively selective ligand for CRF type 1 receptor, whereas urocortin is a ligand more selective for CRF type 2 receptor[25,26]. The density of c-*Fos*-positive cells in the paraventricular nucleus (PVN) was significantly increased by ip injection of des-acyl ghrelin compared to vehicle-injected controls[7] (Figure 4B). These data suggest that peripherally administered des-acyl ghrelin may activate neurons in the PVN by crossing the BBB, and exert inhibitory effects on the antral motility *via* CRF type 2 receptor in the brain (Figure 3).

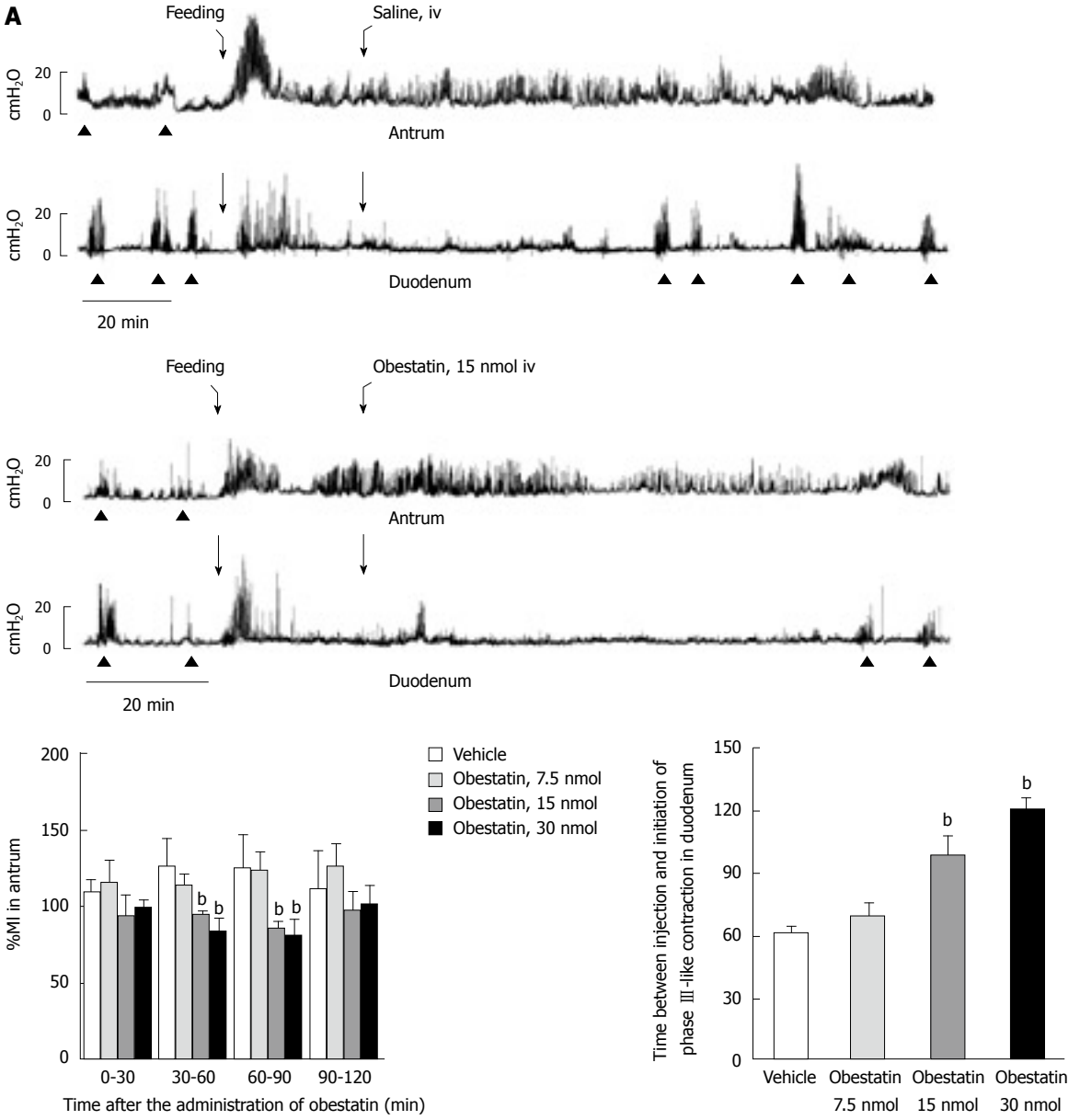
OBESTATIN AND GASTRODUODENAL MOTILITY

Zhang *et al*[2] first reported that ip injection of obestatin suppressed cumulative food intake, decreased body weight gain, and inhibited gastric emptying and jejunal muscle contraction in mice. Since then, however, the inhibitory effects of obestatin on food intake and gastrointestinal motility have remained controversial[8-13]. Most of the previous studies which showed the negative effects of obestatin on the gastrointestinal motility have only measured the gastric emptying or MMC cycle time as indices for motor activity. In our previous study, for more precise analysis, motor activity in both fed and fasted states was quantified by the %MI, and we measured the time taken to the initiation of phase Ⅲ-like contractions in the antrum and duodenum of conscious rats[14].

Results showed that motor activity in the antrum and duodenum was inhibited when obestatin was given iv to conscious rats in the fed state, but not when it was given in the fasted state[14]. Iv injection of obestatin decreased the %MI of fed motility in the antrum and prolonged the time before the return of fasted motility in the duodenum[14] (Figure 5A). Such inhibitory actions were the opposite of those obtained with ghrelin[16]. The results showed that the inhibitory action of obestatin appeared 30-90 min after iv injection[14] (Figure 5A), which is consistent with the timing of the effects of iv injection of ghrelin (approximately 30 min) on gastroduodenal motility[16] (Figure 2A). Iv injection of obestatin induced a significant increase in the number of c-*Fos*-positive cells in the PVN compared to saline-injected controls[14] (Figure 5B). Immunofluorescence overlap staining showed that the PVN neurons activated by iv injection of obestatin contain CRF or urocortin 2[14] (Figure 5B). The involvement of CRF type 1 and type 2 receptors in the action of obestatin on the gastroduodenal motility was examined[14]. Results showed that the inhibitory action of iv injection of obestatin on the motor activities in the antrum and duodenum were blocked by icv injection of CRF type 1 and type 2 receptor antagonists, suggesting that both types of CRF receptors in the brain may mediate the action of peripherally injected obestatin on gastroduodenal motility[14] (Figure 5C). The results showed that vagal afferent nerve blockade by capsaicin reverses the inhibitory effects of obestatin on duodenal motility, but does not alter the inhibitory effects of obestatin on antral motility[14]. These results suggest that vagal afferent pathways might be involved partially, but not entirely, in the action of obestatin. Involvement of vagal afferent pathways was confirmed by the finding that the number of c-*Fos*-positive neurons in the NTS was increased by iv injection of obestatin[14]. In addition to vagal afferent pathways, it is possible that circulating obestatin acts on brain targets directly by crossing the BBB, because a previous study has shown that there is a rapid influx of iv-injected ^{125}I-labeled obestatin from the blood to the brain[27]. Therefore, the lack of effects of obestatin on antral motility during capsaicin treatment might be explained by direct action of peripherally injected obestatin on brain targets by crossing the BBB, similar to what has been observed for des-acyl ghrelin. We further examined whether obestatin can antagonize the stimulatory effects of ghrelin on gastroduodenal motility[14]. We found that obestatin failed to antagonize the ability of ghrelin either to stimulate the %MI in the antrum or to accelerate the initiation of fasted motility in the duodenum when administrated in the fed state[14]. These results were consistent with previous studies in which obestatin failed to antagonize the ability of ghrelin to stimulate gastric emptying or to shorten the MMC cycle time[8].

GPR39 was initially proposed as the receptor for obestatin[2], and GPR39 expression has been detected in peripheral organs such as the duodenum and kidney, but not in the pituitary or hypothalamus[4]. However recent publications indicate that obestatin is unlikely to be the endogenous ligand for GPR39 on the basis of a lack of specific binding of obestatin to GPR39 receptor-expressing cells[2,4,5,28]. Nevertheless, although binding of obestatin to the receptor GPR39 remains controversial, the functional effect of obestatin on gastrointestinal motility has been clearly demonstrated in our study.

In conclusion, our study indicates that obestatin inhibits gastroduodenal motility in the fed state, but not in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200
150
100
50
0
Vehicle
Obestatin, 7.5 nmol
Obestatin, 15 nmol
Obestatin, 30 nmol
b
0-30
30-60
60-90
90-120
Time after the administration of obestatin (min)
Time between injection and initiation of phase Ⅲ-like contraction in duodenum
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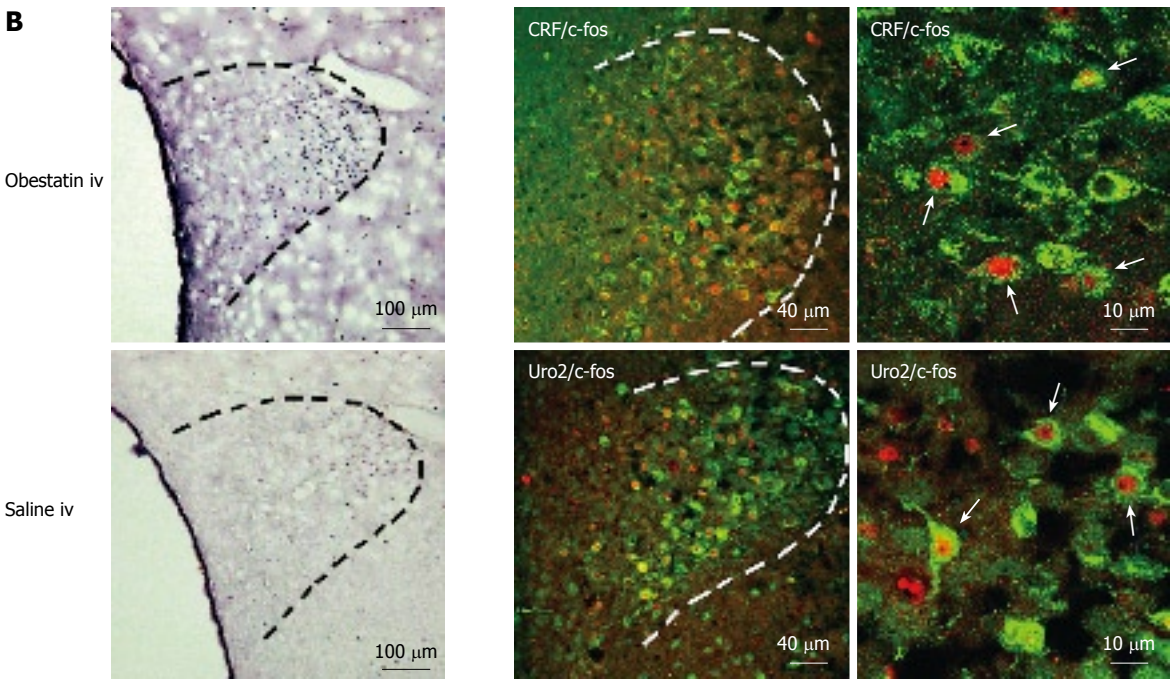
Obestatin 7.5 nmol
Obestatin 15 nmol
Obestatin 30 nmol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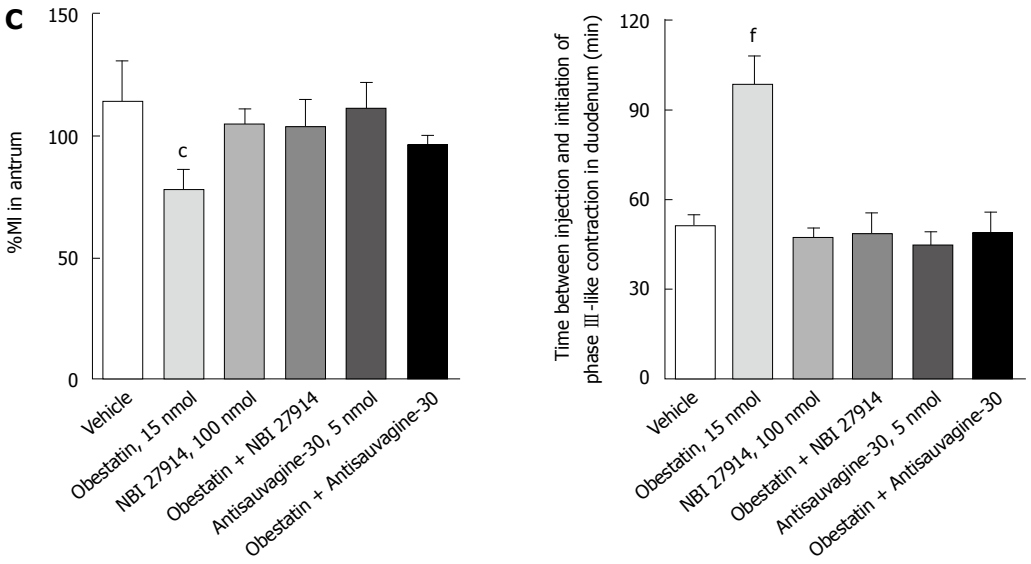

Figure 5 Obestatin and gastroduodenal motility. A: Effects of iv injection of obestatin on the fed motor activity of the antrum and duodenum. Iv injection of obestatin dose dependently decreases the %MI during the 30-90-min period after injection of obestatin in the antrum, and prolongs the time between the initiation of phase Ⅲ-like contractions and injection of obestatin in the duodenum. $^{b}P < 0.01$, compared with vehicle-injected controls; B: The density of c-Fos-positive cells in the PVN is increased by iv injection of obestatin compared to saline-injected control. CRF-positive or urocortin 2-positive neurons are overlapped with c-Fos-positive neurons in the PVN; C: The decrease in %MI that is observed 30-60 min after iv injection of obestatin is reversed by icv injection of the CRF type 1 antagonist NBI-27914 and the CRF type 2 receptor antagonist antisauvagine-30. The elongation of the time between injection of obestatin and initiation of phase Ⅲ-like contractions in the duodenum induced by iv injection of obestatin is also reversed by icv injection of NBI-27914 and antisauvagine-30. $^{c}P < 0.05$, $^{f}P < 0.01$, compared with vehicle-injected controls.

the fasted state of conscious rats. In the brain, CRF- and urocortin 2-containing neurons might be activated by iv injection of obestatin, and at the level, CRF type1 and type 2 receptors might be involved in the inhibitory action of obestatin on antral and duodenal motility (Figure 3). Vagal afferent pathways might be involved partially, but not entirely, in these actions of obestatin (Figure 3).

REFERENCES

1 **Kojima M**, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature* 1999; **402**: 656-660
2 **Zhang JV**, Ren PG, Avsian-Kretchmer O, Luo CW, Rauch R, Klein C, Hsueh AJ. Obestatin, a peptide encoded by the ghrelin gene, opposes ghrelin's effects on food intake. *Science* 2005; **310**: 996-999
3 **Chartrel N**, Alvear-Perez R, Leprince J, Iturrioz X, Reaux-Le Goazigo A, Audinot V, Chomarat P, Coge F, Nosjean O, Rodriguez M, Galizzi JP, Boutin JA, Vaudry H, Llorens-Cortes C. Comment on "Obestatin, a peptide encoded by the ghrelin gene, opposes ghrelin's effects on food intake". *Science* 2007; **315**: 766; author reply 766
4 **Holst B**, Egerod KL, Schild E, Vickers SP, Cheetham S, Gerlach LO, Storjohann L, Stidsen CE, Jones R, Beck-Sickinger AG, Schwartz TW. GPR39 signaling is stimulated by zinc ions but not by obestatin. *Endocrinology* 2007; **148**: 13-20
5 **Tremblay F**, Perreault M, Klaman LD, Tobin JF, Smith E, Gimeno RE. Normal food intake and body weight in mice lacking the G protein-coupled receptor GPR39. *Endocrinology* 2007; **148**: 501-506
6 **Asakawa A**, Inui A, Fujimiya M, Sakamaki R, Shinfuku N, Ueta Y, Meguid MM, Kasuga M. Stomach regulates energy balance via acylated ghrelin and desacyl ghrelin. *Gut* 2005; **54**: 18-24
7 **Chen CY**, Inui A, Asakawa A, Fujino K, Kato I, Chen CC, Ueno N, Fujimiya M. Des-acyl ghrelin acts by CRF type 2 receptors to disrupt fasted stomach motility in conscious rats. *Gastroenterology* 2005; **129**: 8-25
8 **Bassil AK**, Haglund Y, Brown J, Rudholm T, Hellstrom PM, Naslund E, Lee K, Sanger GJ. Little or no ability of obestatin to interact with ghrelin or modify motility in the rat gastrointestinal tract. *Br J Pharmacol* 2007; **150**: 58-64
9 **Bresciani E**, Rapetti D, Dona F, Bulgarelli I, Tamiazzo L, Locatelli V, Torsello A. Obestatin inhibits feeding but does not modulate GH and corticosterone secretion in the rat. *J Endocrinol Invest* 2006; **29**: RC16-RC18
10 **De Smet B**, Thijs T, Peeters TL, Depoortere I. Effect of peripheral obestatin on gastric emptying and intestinal contractility in rodents. *Neurogastroenterol Motil* 2007; **19**: 211-217
11 **Gourcerol G**, Million M, Adelson DW, Wang Y, Wang L, Rivier J, St-Pierre DH, Tache Y. Lack of interaction between peripheral injection of CCK and obestatin in the regulation of gastric satiety signaling in rodents. *Peptides* 2006; **27**: 2811-2819
12 **Lagaud GJ**, Young A, Acena A, Morton MF, Barrett TD, Shankley NP. Obestatin reduces food intake and suppresses body weight gain in rodents. *Biochem Biophys Res Commun* 2007; **357**: 264-269
13 **Nogueiras R**, Pfluger P, Tovar S, Arnold M, Mitchell S, Morris A, Perez-Tilve D, Vazquez MJ, Wiedmer P, Castaneda TR, DiMarchi R, Tschop M, Schurmann A, Joost HG, Williams LM, Langhans W, Dieguez C. Effects of obestatin on energy balance and growth hormone secretion in rodents. *Endocrinology* 2007; **148**: 21-26
14 **Ataka K**, Inui A, Asakawa A, Kato I, Fujimiya M. Obestatin inhibits motor activity in the antrum and duodenum in the fed state of conscious rats. *Am J Physiol Gastrointest Liver Physiol* 2008; **294**: G1210-G1218
15 **Fujimiya M**, Itoh E, Kihara N, Yamamoto I, Fujimura M, Inui A. Neuropeptide Y induces fasted pattern of duodenal motility via Y(2) receptors in conscious fed rats. *Am J Physiol Gastrointest Liver Physiol* 2000; **278**: G32-G38
16 **Fujino K**, Inui A, Asakawa A, Kihara N, Fujimura M, Fujimiya M. Ghrelin induces fasted motor activity of the gastrointestinal tract in conscious fed rats. *J Physiol* 2003; **550**: 227-240
17 **Kihara N**, Fujimura M, Yamamoto I, Itoh E, Inui A, Fujimiya M. Effects of central and peripheral urocortin on fed and

fasted gastroduodenal motor activity in conscious rats. *Am J Physiol Gastrointest Liver Physiol* 2001; **280**: G406-G419

18 **Asakawa A**, Inui A, Kaga T, Yuzuriha H, Nagata T, Ueno N, Makino S, Fujimiya M, Niijima A, Fujino MA, Kasuga M. Ghrelin is an appetite-stimulatory signal from stomach with structural resemblance to motilin. *Gastroenterology* 2001; **120**: 337-345

19 **Itoh Z**. Motilin and clinical application. *Peptides* 1997; **18**: 593-608

20 **Sarna SK**, Gonzalez A, Ryan RP. Enteric locus of action of prokinetics: ABT-229, motilin, and erythromycin. *Am J Physiol Gastrointest Liver Physiol* 2000; **278**: G744-G752

21 **Date Y**, Kojima M, Hosoda H, Sawaguchi A, Mondal MS, Suganuma T, Matsukura S, Kangawa K, Nakazato M. Ghrelin, a novel growth hormone-releasing acylated peptide, is synthesized in a distinct endocrine cell type in the gastrointestinal tracts of rats and humans. *Endocrinology* 2000; **141**: 4255-4261

22 **Hashmonai M**, Go VL, Yaksh T, Szurszewski JH. Effect of central administration of motilin on migrating complexes in the dog. *Am J Physiol* 1987; **252**: G195-G199

23 **Yamamoto O**, Matsunaga Y, Haga N, Mizumoto A, Itoh Z. Inhibition of phase III activity by acidifying stomach in vagally denervated and innervated dogs with gastric pouches. *Gastroenterology* 1994; **106**: 1533-1541

24 **Date Y**, Murakami N, Toshinai K, Matsukura S, Niijima A, Matsuo H, Kangawa K, Nakazato M. The role of the gastric afferent vagal nerve in ghrelin-induced feeding and growth hormone secretion in rats. *Gastroenterology* 2002; **123**: 1120-1128

25 **Chang CP**, Pearse RV 2nd, O'Connell S, Rosenfeld MG. Identification of a seven transmembrane helix receptor for corticotropin-releasing factor and sauvagine in mammalian brain. *Neuron* 1993; **11**: 1187-1195

26 **Coskun T**, Bozkurt A, Alican I, Ozkutlu U, Kurtel H, Yegen BC. Pathways mediating CRF-induced inhibition of gastric emptying in rats. *Regul Pept* 1997; **69**: 113-120

27 **Pan W**, Tu H, Kastin AJ. Differential BBB interactions of three ingestive peptides: obestatin, ghrelin, and adiponectin. *Peptides* 2006; **27**: 911-916

28 **Zhang JV**, Klein C, Ren PG, Kass S, Donck LV, Moechars D, Hsueh AJ. Response to Comment on "Obestatin, a Peptide Encoded by the Ghrelin Gene, Opposes Ghrelin's Effects on Food Intake". *Science* 2007; **315**: 766

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TOPIC HIGHLIGHT

Akio Inui, MD, PhD, Professor, *Series Editor*

Ghrelin and *Helicobacter pylori* infection

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Abstract

Ghrelin is primarily secreted from the stomach and has been implicated in the coordination of eating behavior and weight regulation. Ghrelin also plays an essential role in the mechanism of gastric mucosal defense. Thus, it is important to clarify which diseases primarily influence changes in plasma ghrelin concentrations. *Helicobacter pylori* (*H pylori*) infection is involved in the pathogenesis of gastritis, gastric and duodenal ulcer, gastric carcinoma, and mucosa-associated lymphoid tissue lymphoma. *H pylori* eradication is related to body weight change. Compared, *H pylori* infected and negative subjects with normal body mass index, plasma ghrelin concentration, gastric ghrelin mRNA, and the number of ghrelin producing cells in gastric mucosa are significantly lower in *H pylori* infected subjects than in *H pylori*-negative controls. Plasma ghrelin concentration decreases with the progression of gastric atrophy. Impaired gastric ghrelin production in association with atrophic gastritis induced by *H pylori* infection accounts for the decrease in plasma ghrelin concentration. However, the ratio of plasma acylated ghrelin to total ghrelin levels is higher in patients with chronic atrophic gastritis than in healthy subjects. This may result from the compensatory increase in plasma active ghrelin concentration in response to gastric atrophy. After *H pylori* eradication, gastric preproghrelin mRNA expression is increased nearly 4-fold in most cases. However, changes in plasma ghrelin concentrations before and after *H pylori* cure are not associated with the gastric ghrelin production. Plasma ghrelin changes are inversely correlated with both body weight change and initial plasma ghrelin levels.

Key words: Ghrelin; *Helicobacter pylori*; Eradication; Body weight; Leptin

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INTRODUCTION

Ghrelin, a 28 amino acid peptide isolated from rat and human stomach, possesses strong growth hormone-releasing activity and plays central as well as peripheral roles in food intake, gastric motility, and acid secretion[1-3]. Ghrelin has been shown to evoke weight gain by actions in the hypothalamus[3]. Plasma ghrelin concentrations rise before meals and fall after meals. This peptide also contributes to the regulation of both somatic growth and adipose tissue mass, and is therefore, a short-term, meal-related orexigen as well as a long-term regulator of body weight[4,5]. Circulating ghrelin concentrations in newborns are not associated with gender, body weight, or hormonal parameter[6]. In children and adults, however, plasma ghrelin concentrations are lower in obese subjects compared with those with normal body weight and lean subjects[7]. The decrease of plasma ghrelin concentrations appears to compensate for the positive energy balance in obese individuals[7].

SOURCES OF GHRELIN AND *HELICOBACTER PYLORI* (*H PYLORI*) INFECTION

Ghrelin is predominantly produced by the stomach[8], whereas substantially lower amounts are derived from bowel[9], pituitary, kidney, placenta, hypothalamus[8], lung, kidney, and A-cells of the pancreatic islet. Thus, it is important to clarify which organ primarily influences changes in plasma ghrelin concentrations in various diseases. Although the majority of circulating ghrelin is produced in the stomach, other sources may increase or decrease ghrelin secretion in a compensatory manner. After gastrectomy, for example, plasma ghrelin level is

surprisingly reduced only by 65%[5].

The gastric ghrelin is produced in X/A like-cells of enteroendocrine cells/oxyntic glands in the mammalian gastric mucosa[9]. Thus, there exists the possibility that chronic persistent damage of the gastric mucosa, such as chronic gastritis, might affect ghrelin production, leading to changes in food intake and body weight. *H pylori* is a gram-negative bacterium that colonizes the stomach. *H pylori* infection is involved in the pathogenesis of gastritis, gastric and duodenal ulcer, gastric carcinoma, and mucosa-associated lymphoid tissue lymphoma[10-12]. More than 50% of the adult population is infected with *H pylori* worldwide. *H pylori* infection first leads to atrophic gastritis and intestinal metaplasia, which may further lead to dysplasia and gastric carcinoma. Thus, it is an intriguing question whether *H pylori* infection affects gastric ghrelin production and consequently alters plasma ghrelin concentration.

RELATIONSHIP BETWEEN PLASMA GHRELIN LEVELS AND BODY MASS INDEX IN *H PYLORI* INFECTED PATIENTS

In determining whether ghrelin is involved in long-term energy homeostasis, several studies have found that circulating ghrelin is elevated in individuals with anorexia nervosa[7], reduced in obesity[7,13,14] and normalized with weight gain[13] or weight loss. Circulating ghrelin levels are negatively correlated with the percentage of body fat, fat mass, body mass index (BMI), body weight, insulin, leptin, and T3 in cross-sectional and longitudinal studies examining anorexia nervosa and obesity. In *H pylori*-infected subjects, however, the correlation between BMI and circulating ghrelin levels was weak[15,16]. This suggested that *H pylori* infection could affect plasma ghrelin levels strongly.

EFFECT OF *H PYLORI* INFECTION ON PLASMA GHRELIN LEVELS

Several investigators reported the relationship between plasma ghrelin levels and *H pylori* infection. Nwokolo *et al*[17] reported that plasma ghrelin concentrations increased after the eradication of *H pylori*. On the contrary, Gokcel *et al*[18] reported that *H pylori* infection has no effect on plasma ghrelin levels. Although the relationship between *H pylori* infection and plasma ghrelin concentrations had been still controversial in Western countries, Japanese investigators revealed the effects of *H pylori* infection on plasma ghrelin concentrations[17,18]. The direct relationship between *H pylori* infection and gastric ghrelin production, which could influence plasma ghrelin concentrations, have been demonstrated. Osawa *et al*[19] and Tatsuguchi *et al*[16] investigated the association of *H pylori* infection with gastric ghrelin production in the human stomach concomitantly examining plasma ghrelin concentrations.

PLASMA GHRELIN CONCENTRATIONS ARE LOWER IN *H PYLORI*-POSITIVE SUBJECTS

Several investigators clarified the effect of *H pylori* infection on plasma ghrelin levels. Plasma ghrelin concentrations were significantly lower in *H pylori*-positive patients than in *H pylori*-negative controls[15,16,19]. Its level is obviously independent of sex and BMI and varied among *H pylori* infected subjects even with same BMI[15]. Mean plasma ghrelin levels in *H pylori*-positive subjects remain two-third of those of *H pylori*-negative subjects[19]. In addition to several clinical factors including BMI, food intake, and serum insulin levels[7,20], *H pylori* infection is also another determinant of plasma ghrelin levels as well as body mass index.

EXPRESSION LEVELS OF GASTRIC GHRELIN ARE LOWER IN *H PYLORI*-POSITIVE SUBJECTS

It is important to focus on the gastric mucosa in order to better understand the effects of *H pylori* infection on the alteration of ghrelin expression. Gastric ghrelin mRNA levels were much lower in *H pylori*-positive patients than in *H pylori*-negative controls using real-time quantitative RT-PCR[16,19]. The median of gastric ghrelin mRNA expression levels in *H pylori*-positive subjects was less than one 45th of that in *H pylori*-negative controls[19]. Moreover, plasma ghrelin concentrations were in parallel with the gastric ghrelin mRNA expression levels in *H pylori*-positive patients. Therefore, the attenuation of the ghrelin production in the gastric mucosa accounts for the decrease in the plasma ghrelin concentrations in *H pylori*-positive individuals[19].

GHRELIN-PRODUCING CELLS IN THE GASTRIC MUCOSA ARE FEWER IN *H PYLORI*-POSITIVE SUBJECTS

Ghrelin immuno-reactive cells are seen in the lower half of fundic epithelial glands[9]. Immunoreactivity is concentrated in the basal cytoplasm of the positive cells. The number of ghrelin-positive cells in the gastric mucosa of *H pylori*-positive individuals was significantly lower than those of *H pylori*-negative individuals[19]. Furthermore, the numbers of ghrelin-positive cells in the gastric mucosa fell significantly in accompaniment to the decrease in plasma ghrelin concentrations in *H pylori*-positive subjects[16,19]. These results reinforce the fact that the attenuation of the gastric ghrelin production caused by *H pylori* infection accounts for the decrease in the plasma ghrelin concentrations in *H pylori*-positive individuals.

PLASMA GHRELIN CONCENTRATIONS ARE ASSOCIATED WITH THE DEGREE OF GASTRIC ATROPHY IN *H PYLORI*-POSITIVE SUBJECTS

Since *H pylori* infection first induces gastric atrophy in its pathological course, it is important to clarify the association between plasma ghrelin concentration and degree of gastric atrophy in *H pylori*-positive patients. Several reports revealed that groups of *H pylori*-positive subjects with higher degrees of gastric atrophy tended to have lower plasma ghrelin concentrations, leading to a negative association between plasma ghrelin concentration and gastric atrophy grade[15,16,19]. Moreover, activity and topography of gastritis affects circulating ghrelin levels[21]. Histological severity of mononuclear cell infiltration and glandular atrophy of the corpus significantly influenced the expression levels of ghrelin mRNA, its peptide contents and the density of immunoreactive cells, indicating that gastric ghrelin biosynthesis seems to be affected by chronic mucosal inflammation and/or atrophy in association with *H pylori* infection. In addition, plasma ghrelin concentrations in *H pylori*-positive patients correlated with serum pepsinogen I concentration as well as pepsinogen I / Ⅱ ratio. Pepsinogen I and pepsinogen Ⅱ differ in their location in the stomach. Both are located in the chief and mucous neck cells of the oxyntic gland mucosa in the gastric corpus but only pepsinogen Ⅱ is present in the gastric antrum. A pepsinogen I / Ⅱ ratio < 3 is considered to be a reliable marker for severe atrophic gastritis[22]. Serum levels of pepsinogen I as well as the ratio of pepsinogen I / Ⅱ fell significantly as plasma ghrelin concentrations decreased, indicating the positive association between plasma ghrelin and pepsinogen I concentrations as well as pepsinogen I / Ⅱ ratios in *H pylori*-positive patients[19]. Collectively, these results reveal that plasma ghrelin concentrations are associated with the progression of gastric atrophy. Although geographical differences in the prevalence of atrophic gastritis in Asians and Westerners would require additional consideration, these findings strongly suggest that the reduction of ghrelin-producing cells in the gastric mucosa by *H pylori* infection results in the lower plasma ghrelin concentration in *H pylori*-positive patients.

Checchi *et al*[23] reported that serum ghrelin levels are negatively affected by autoimmune gastritis as well as by *H pylori* associated gastritis, and represent the most sensitive and specific noninvasive markers for selecting those patients at high risk for having gastric damage. Of particular interest is the fact that the measurement of serum ghrelin levels is superior to that of pepsinogen I / Ⅱ ratio and serum gastrin to predict gastric damage.

STOMACH REGULATES ENERGY BALANCE *VIA* ACYLATED GHRELIN AND DESACYLATED GHRELIN

It is known that ghrelin circulates in two different forms: the so-called acylated ghrelin, octanoylated, in serine 3, and the so-called desacylated, without the octanoyl group[24]. This latter form is dramatically less potent on the GHS-receptor than the acylated form[25]. Acylated ghrelin is involved in the regulation of GH secretion, energy balance, gastrointestinal motility, cardiac performance, and anxiety[8]. Administered acylated ghrelin induces body weight gain and adiposity by promoting food intake and decreasing fat use or energy expenditure[26]. In contrast to acylated ghrelin, desacylated ghrelin induces a negative energy balance by decreasing food intake and delaying gastric emptying[27]. The effect is mediated *via* the hypothalamus. Although derived from the same precursor, the inverse effects of these two peptides suggest that the stomach might be involved as an endocrine organ in the regulation of the energy balance.

PLASMA ACYLATED GHRELIN LEVELS ARE HIGHER IN PATIENTS WITH CHRONIC ATROPHIC GASTRITIS

Total plasma ghrelin concentrations decrease in patients with gastric atrophy secondary to *H pylori* infection, and the levels are related to the degree of atrophy. These finding might be explained by the loss of ghrelin-producing cells caused by inflammatory and/or atrophic changes. Campana *et al*[28] reported that plasma acylated ghrelin levels were higher in patients with chronic atrophic gastritis than in healthy subjects. This opposite tendency compared to total plasma ghrelin concentration may result from the compensatory increase in plasma active ghrelin concentration in response to gastric atrophy. This hypothesis seems to be supported by a recent report showing that a significant decrease in gastric pH was found after injection of exogenous ghrelin. Gastric atrophy causes an increase gastric pH, leading to an increase in serum gastrin levels. Both the increase in acylated ghrelin and gastrin could represent a compensatory mechanism to stimulate gastric acid production.

GHRELIN HAS A PROTECTIVE EFFECT AGAINST MUCOSAL INJURY OF STOMACH

H pylori infection induces gastric mucosal damage including gastric ulcer and chronic gastritis. It is an intriguing question whether lower plasma ghrelin level in *H pylori*-infected patients affects gastric mucosa. Sibilia *et al*[29] reported that ghrelin protects against ethanol-induced gastric ulcers in rats. Similarly, Konturek *et al*[30] reported that ghrelin expression of gastric mucosa is enhanced after exposure to ethanol, and ghrelin exhibits a strong gastroprotection due to its anti-inflammatory action mediated by prostaglandins. The gastroprotective effect of ghrelin is accompanied by a significant rise in the gastric blood flow, which is known to play an essential role in the mechanism of gastric mucosal defense. This ghrelin-induced hyperemia could be prob-

ably attributed to the direct vasodilatory effect of this peptide.

PLASMA GHRELIN LEVEL AND BODY WEIGHT

Eradication of *H pylori* has been a standard therapy for peptic ulcer disease[10,11] and improves gastritis[10]. Much attention has recently been directed to the relationship between obesity and *H pylori* infection. Several studies showed that *H pylori* infection is inversely related to obesity. For example, Wu *et al*[31] reported that the seropositivity of *H pylori* infection was significantly lower in morbid obesity patients. Furuta *et al*[32] showed the body weight gain after *H pylori* cure. As ghrelin is mainly synthesized and secreted by gastric mucosa, it has been assumed that the inverse effect of *H pylori* infection on body weight may attribute to the difference of plasma ghrelin concentrations in patients with or without *H pylori* infection[33]. This hypothesis states that an increase of gastric ghrelin production after *H pylori* cure may elevate plasma ghrelin concentration resulting in body weight gain.

PLASMA GHRELIN LEVELS AND BODY WEIGHT GAIN AFTER *H PYLORI* ERADICATION

Do plasma ghrelin levels affect body weight gain after *H pylori* eradication, or body weight gain affect plasma ghrelin levels? Gastric ghrelin production is decreased by *H pylori* infection and increased by eradication therapy[19]. As ghrelin is a body weight regulating peptide, much attention has been paid to the nutritional status and the dynamics of gastric and plasma ghrelin in response to *H pylori* infection[31,32]. In this respect, Nwokolo *et al*[17] reported that plasma ghrelin levels increased at 6 wk after *H pylori* cure in 10 patients in UK. Because plasma ghrelin levels increased significantly by 75%, they proposed that increased ghrelin following *H pylori* eradication may play a role in obesity. This could lead to increased appetite and weight gain, and contribute to the increasing obesity seen in Western populations where *H pylori* prevalence is low. Also, Czesnikiewicz-Guzik *et al*[34] reported that plasma ghrelin levels increased significantly to two-fold levels at 4 wk after *H pylori* cure in 41 patients in Poland. After Nwokolo's report, it has been believed that plasma ghrelin concentrations will increase after *H pylori* cure due to the increase of gastric ghrelin production, leading to body weight gain[16]. However, their reports suggested that plasma ghrelin levels increased, but did not reveal a changes of plasma ghrelin levels in patients with body weight gain after *H pylori* cure. Plasma ghrelin levels decrease as a compensatory effect in obesity patients who has positive energy balance. Therefore, it is questionable whether plasma ghrelin levels increase in a condition of body weight gain after eradication. Another study found that plasma ghrelin levels were unaffected[35]. In fact, plasma ghrelin concentration is not simply regulated by the levels of gastric ghrelin production. Even in healthy humans, plasma ghrelin concentration is tightly correlated with body weight[7]. Therefore, it has been proposed[36] that it should be re-examined whether the rise in plasma ghrelin following *H pylori* eradication exists and whether it can be an important determinant of body weight increase. It is possible that only a subpopulation of infected patients may show a rise in ghrelin following eradication.

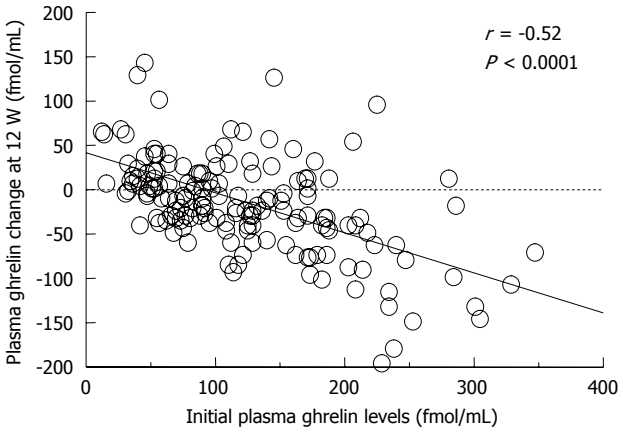

Figure 1 The relationship between the initial plasma ghrelin levels and the change in plasma ghrelin levels at 12 wk after *H pylori* cure. The change was obtained by subtracting the levels before the treatment from the levels at 12 wk after. The change in 12 wk correlated inversely with initial plasma ghrelin levels. This figure is cited from *J Gastroenterol* 2006; 41: 954.

DISPARATE CHANGES IN PLASMA GHRELIN AFTER *H PYLORI* CURE

The effect of *H pylori* eradication on plasma ghrelin concentration was reported in 134 Japanese patients[37]. Interestingly, mean plasma ghrelin concentrations decreased significantly from 120 fmol/mL before *H pylori* eradication to 103 fmol/mL at 12 wk after *H pylori* eradication. However, its levels after treatment changed diversely among enrolled patients. In fact, levels increased in 50 patients and decreased in 84 patients. There are some potential mechanisms leading to disparate changes in plasma ghrelin levels after *H pylori* eradication. The relationship between the initial plasma ghrelin levels and their changes were analyzed after *H pylori* cure. Figure 1 shows the relationship between the initial plasma ghrelin levels, and the changes in plasma ghrelin concentration at 12 wk after *H pylori* cure. Interestingly, higher initial plasma ghrelin levels decreased after the cure, but lower initial plasma ghrelin levels did not change significantly. The change of plasma ghrelin concentration after 12 wk was inversely correlated with the initial plasma ghrelin levels.

EXPRESSION LEVELS OF GASTRIC GHRELIN INCREASES AFTER *H PYLORI* CURE

The effect of *H pylori* eradication on the ghrelin pro-

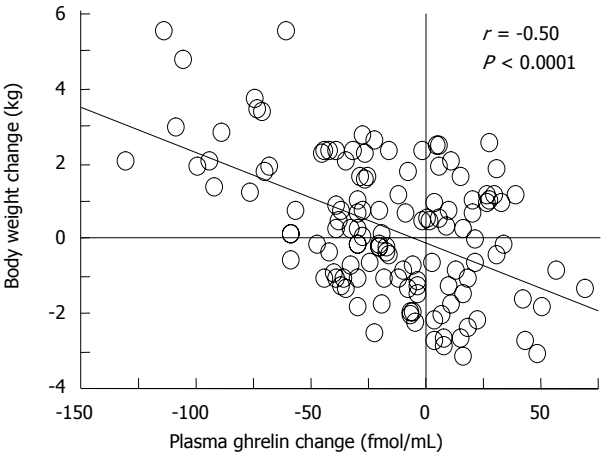

Figure 2 The relationship between the plasma ghrelin change and body weight change at 12 wk after *H pylori* cure. The alteration of plasma ghrelin levels correlated inversely with body weight change after *H pylori* cure. This figure is cited from *J Gastroenterol* 2006; 41: 954.

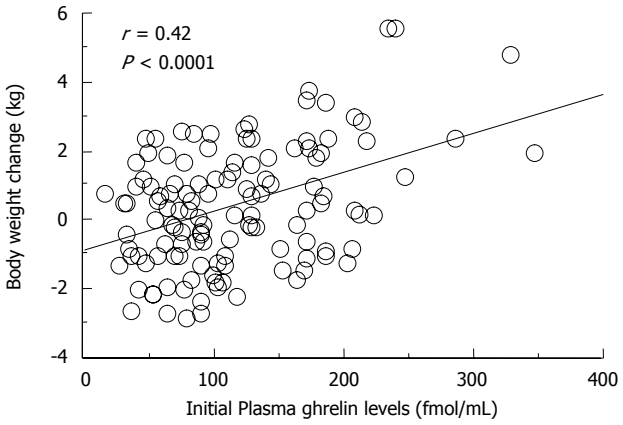

Figure 3 The relationship between the initial plasma ghrelin levels and body weight change at 12 wk after *H pylori* cure. Initial plasma ghrelin levels correlated positively with body weight changes. This figure is cited from *J Gastroenterol* 2006; 41: 954.

duction in the gastric mucosa was reported in several studies. Tatsuguchi *et al*[16] reported that ghrelin immunoreactive cells increase in the gastric mucosa after *H pylori* eradication irrespective of the recovery of glandular atrophy. Osawa *et al*[37] also reported that the number of ghrelin positive cells per oxyntic gland was increased in 77 patients and was unchanged in 57 patients after *H pylori* eradication. The number of ghrelin producing cells tend to increase despite the change of plasma ghrelin levels before and after *H pylori* cure. In recent reports, gastric glandular atrophy recovers gradually over the long term after *H pylori* eradication. Arkkila *et al*[38] reported that atrophy can diminish or even disappear, especially in the antrum, during a 1-year follow-up after eradication of infection. If glandular atrophy recovers by *H pylori* cure, the number of ghrelin producing cell may increase more and more. Osawa *et al*[37] compared gastric preproghrelin mRNA expression levels before and 12 wk after treatment using the corpus mucosa. Median preproghrelin mRNA expression was increased nearly 4-fold after *H pylori* cure. Preproghrelin mRNA expression was also increased in the antral mucosa. No correlation was observed between the changes in plasma ghrelin and those of gastric preproghrelin mRNA or ghrelin positive cells after *H pylori* cure. Similarly, Isomoto *et al*[21] reported that preproghrelin mRNA expression was increased in the corpus mucosa at 4 wk after *H pylori* cure. Therefore, gastric ghrelin production is enhanced after *H pylori* eradication even in patients with decreased plasma ghrelin concentrations.

BODY WEIGHT CHANGES CORRELATE INVERSELY WITH CHANGES IN PLASMA GHRELIN CONCENTRATION

Body weight gain is a well-known effect of *H pylori* eradication and plasma ghrelin concentration is influenced by body weight change[38,39]. The question as to whether ghrelin is involved in weight gain after *H pylori* cure has been discussed[19]. Figure 2 showed clearly that the change in plasma ghrelin is inversely correlated with body weight change after *H pylori* cure[37]. Plasma ghrelin decreased in 23 of 28 patients (82%) with more than 2 kg of weight gain, and in all 7 patients with more than 3 kg of weight gain. These data suggest that plasma ghrelin concentration after *H pylori* cure is more strongly influenced by body weight change than the increase of gastric preproghrelin mRNA and ghrelin producing cells.

In contrast, patients with less than 2 kg of body weight gain or with body weight loss had minor changes of plasma ghrelin levels. Increased plasma ghrelin levels after the cure in European studies can be associated with patients having minor change of body weight. The racial difference of enrolled subjects may account for the discrepancy. In this respect, Asians including Japanese are more prone to central adiposity than are white individuals[40]. As body fat storage is closely associated with plasma ghrelin levels, the racial difference of body fat distribution may account for the discrepancy.

INITIAL PLASMA GHRELIN LEVELS CAN BE A PREDICTIVE FACTOR OF BODY WEIGHT GAIN AFTER *H PYLORI* ERADICATION

Initial plasma ghrelin levels before eradication therapy were significantly higher in those whose plasma ghrelin decreased after treatment[37]. In addition, these subjects had a significantly greater increase in body weight than those with increased plasma ghrelin after treatment. Figure 3 shows the positive correlation between the initial plasma ghrelin levels and body weight changes. In particular, 12 of 14 patients (86%) with more than 200 fmol/mL of initial ghrelin levels had an increase in body weight, suggesting high levels of initial plasma ghrelin can be a predictive factor of body weight gain after *H pylori* eradication. The correlation between initial

plasma ghrelin levels and weight changes suggests the participation of ghrelin in the weight gain after *H pylori* eradication. The weight gain after *H pylori* eradication does not simply result from an increase in plasma ghrelin by the recovery of gastric ghrelin production.

However, additional research is needed to clarify the relationship between the body weight gain and the plasma ghrelin levels after *H pylori* cure in Western population.

CONCLUSION

Plasma ghrelin concentrations are influenced by the presence of chronic gastritis in association with *H pylori* infection. The decrease in gastric ghrelin production accounts for lower concentrations of plasma ghrelin in *H pylori*-positive individuals. Gastric ghrelin production increases after *H pylori* cure. Plasma ghrelin concentrations decrease in subjects with body weight gain after *H pylori* cure. Initial plasma ghrelin levels before eradication can be a predictive factor for body weight gain after *H pylori* cure.

REFERENCES

1 **Date Y**, Nakazato M, Murakami N, Kojima M, Kangawa K, Matsukura S. Ghrelin acts in the central nervous system to stimulate gastric acid secretion. *Biochem Biophys Res Commun* 2001; **280**: 904-907

2 **Masuda Y**, Tanaka T, Inomata N, Ohnuma N, Tanaka S, Itoh Z, Hosoda H, Kojima M, Kangawa K. Ghrelin stimulates gastric acid secretion and motility in rats. *Biochem Biophys Res Commun* 2000; **276**: 905-908

3 **Nakazato M**, Murakami N, Date Y, Kojima M, Matsuo H, Kangawa K, Matsukura S. A role for ghrelin in the central regulation of feeding. *Nature* 2001; **409**: 194-198

4 **Asakawa A**, Inui A, Kaga T, Yuzuriha H, Nagata T, Ueno N, Makino S, Fujimiya M, Niijima A, Fujino MA, Kasuga M. Ghrelin is an appetite-stimulatory signal from stomach with structural resemblance to motilin. *Gastroenterology* 2001; **120**: 337-345

5 **Cummings DE**, Weigle DS, Frayo RS, Breen PA, Ma MK, Dellinger EP, Purnell JQ. Plasma ghrelin levels after diet-induced weight loss or gastric bypass surgery. *N Engl J Med* 2002; **346**: 1623-1630

6 **Bellone S**, Rapa A, Vivenza D, Vercellotti A, Petri A, Radetti G, Bellone J, Broglio F, Ghigo E, Bona G. Circulating ghrelin levels in newborns are not associated to gender, body weight and hormonal parameters but depend on the type of delivery. *J Endocrinol Invest* 2003; **26**: RC9-RC11

7 **Shiiya T**, Nakazato M, Mizuta M, Date Y, Mondal MS, Tanaka M, Nozoe S, Hosoda H, Kangawa K, Matsukura S. Plasma ghrelin levels in lean and obese humans and the effect of glucose on ghrelin secretion. *J Clin Endocrinol Metab* 2002; **87**: 240-244

8 **Kojima M**, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature* 1999; **402**: 656-660

9 **Date Y**, Kojima M, Hosoda H, Sawaguchi A, Mondal MS, Suganuma T, Matsukura S, Kangawa K, Nakazato M. Ghrelin, a novel growth hormone-releasing acylated peptide, is synthesized in a distinct endocrine cell type in the gastrointestinal tracts of rats and humans. *Endocrinology* 2000; **141**: 4255-4261

10 **Marshall BJ**, Goodwin CS, Warren JR, Murray R, Blincow ED, Blackbourn SJ, Phillips M, Waters TE, Sanderson CR. Prospective double-blind trial of duodenal ulcer relapse after eradication of Campylobacter pylori. *Lancet* 1988; **2**: 1437-1442

11 **Uemura N**, Okamoto S, Yamamoto S, Matsumura N, Yamaguchi S, Yamakido M, Taniyama K, Sasaki N, Schlemper RJ. Helicobacter pylori infection and the development of gastric cancer. *N Engl J Med* 2001; **345**: 784-789

12 **Wotherspoon AC**, Doglioni C, Diss TC, Pan L, Moschini A, de Boni M, Isaacson PG. Regression of primary low-grade B-cell gastric lymphoma of mucosa-associated lymphoid tissue type after eradication of Helicobacter pylori. *Lancet* 1993; **342**: 575-577

13 **Tolle V**, Kadem M, Bluet-Pajot MT, Frere D, Foulon C, Bossu C, Dardennes R, Mounier C, Zizzari P, Lang F, Epelbaum J, Estour B. Balance in ghrelin and leptin plasma levels in anorexia nervosa patients and constitutionally thin women. *J Clin Endocrinol Metab* 2003; **88**: 109-116

14 **Otto B**, Cuntz U, Fruehauf E, Wawarta R, Folwaczny C, Riepl RL, Heiman ML, Lehnert P, Fichter M, Tschop M. Weight gain decreases elevated plasma ghrelin concentrations of patients with anorexia nervosa. *Eur J Endocrinol* 2001; **145**: 669-673

15 **Shiotani A**, Miyanishi T, Uedo N, Iishi H. Helicobacter pylori infection is associated with reduced circulating ghrelin levels independent of body mass index. *Helicobacter* 2005; **10**: 373-378

16 **Tatsuguchi A**, Miyake K, Gudis K, Futagami S, Tsukui T, Wada K, Kishida T, Fukuda Y, Sugisaki Y, Sakamoto C. Effect of Helicobacter pylori infection on ghrelin expression in human gastric mucosa. *Am J Gastroenterol* 2004; **99**: 2121-2127

17 **Nwokolo CU**, Freshwater DA, O'Hare P, Randeva HS. Plasma ghrelin following cure of Helicobacter pylori. *Gut* 2003; **52**: 637-640

18 **Gokcel A**, Gumurdulu Y, Kayaselcuk F, Serin E, Ozer B, Ozsahin AK, Guvener N. Helicobacter pylori has no effect on plasma ghrelin levels. *Eur J Endocrinol* 2003; **148**: 423-426

19 **Osawa H**, Nakazato M, Date Y, Kita H, Ohnishi H, Ueno H, Shiiya T, Satoh K, Ishino Y, Sugano K. Impaired production of gastric ghrelin in chronic gastritis associated with Helicobacter pylori. *J Clin Endocrinol Metab* 2005; **90**: 10-16

20 **Date Y**, Nakazato M, Hashiguchi S, Dezaki K, Mondal MS, Hosoda H, Kojima M, Kangawa K, Arima T, Matsuo H, Yada T, Matsukura S. Ghrelin is present in pancreatic alpha-cells of humans and rats and stimulates insulin secretion. *Diabetes* 2002; **51**: 124-129

21 **Isomoto H**, Nishi Y, Ohnita K, Mizuta Y, Kohno S, Ueno H, Nakazato M. The Relationship between Plasma and Gastric Ghrelin Levels and Strain Diversity in Helicobacter pylori Virulence. *Am J Gastroenterol* 2005; **100**: 1425-1427

22 **Samloff IM**, Varis K, Ihamaki T, Siurala M, Rotter JI. Relationships among serum pepsinogen I, serum pepsinogen II, and gastric mucosal histology. A study in relatives of patients with pernicious anemia. *Gastroenterology* 1982; **83**: 204-209

23 **Checchi S**, Montanaro A, Pasqui L, Ciuoli C, Cevenini G, Sestini F, Fioravanti C, Pacini F. Serum ghrelin as a marker of atrophic body gastritis in patients with parietal cell antibodies. *J Clin Endocrinol Metab* 2007; **92**: 4346-4351

24 **Hosoda H**, Kojima M, Matsuo H, Kangawa K. Ghrelin and des-acyl ghrelin: two major forms of rat ghrelin peptide in gastrointestinal tissue. *Biochem Biophys Res Commun* 2000; **279**: 909-913

25 **Thompson NM**, Gill DA, Davies R, Loveridge N, Houston PA, Robinson IC, Wells T. Ghrelin and des-octanoyl ghrelin promote adipogenesis directly in vivo by a mechanism independent of the type 1a growth hormone secretagogue receptor. *Endocrinology* 2004; **145**: 234-242

26 **Inui A**. Ghrelin: an orexigenic and somatotrophic signal from the stomach. *Nat Rev Neurosci* 2001; **2**: 551-560

27 **Asakawa A**, Inui A, Fujimiya M, Sakamaki R, Shinfuku N, Ueta Y, Meguid MM, Kasuga M. Stomach regulates energy balance via acylated ghrelin and desacyl ghrelin. *Gut* 2005;

54: 18-24
28 **Campana D**, Nori F, Pagotto U, De Iasio R, Morselli-Labate AM, Pasquali R, Corinaldesi R, Tomassetti P. Plasma acylated ghrelin levels are higher in patients with chronic atrophic gastritis. *Clin Endocrinol* (Oxf) 2007; **67**: 761-766
29 **Sibilia V**, Rindi G, Pagani F, Rapetti D, Locatelli V, Torsello A, Campanini N, Deghenghi R, Netti C. Ghrelin protects against ethanol-induced gastric ulcers in rats: studies on the mechanisms of action. *Endocrinology* 2003; **144**: 353-359
30 **Konturek PC**, Brzozowski T, Pajdo R, Nikiforuk A, Kwiecien S, Harsch I, Drozdowicz D, Hahn EG, Konturek SJ. Ghrelin-a new gastroprotective factor in gastric mucosa. *J Physiol Pharmacol* 2004; **55**: 325-336
31 **Wu MS**, Lee WJ, Wang HH, Huang SP, Lin JT. A case-control study of association of Helicobacter pylori infection with morbid obesity in Taiwan. *Arch Intern Med* 2005; **165**: 1552-1555
32 **Furuta T**, Shirai N, Xiao F, Takashima M, Hanai H. Effect of Helicobacter pylori infection and its eradication on nutrition. *Aliment Pharmacol Ther* 2002; **16**: 799-806
33 **Blaser MJ**, Atherton JC. Helicobacter pylori persistence: biology and disease. *J Clin Invest* 2004; **113**: 321-333
34 **Czesnikiewicz-Guzik M**, Loster B, Bielanski W, Guzik TJ, Konturek PC, Zapala J, Konturek SJ. Implications of oral Helicobacter pylori for the outcome of its gastric eradication therapy. *J Clin Gastroenterol* 2007; **41**: 145-151
35 **Isomoto H**, Nakazato M, Ueno H, Date Y, Nishi Y, Mukae H, Mizuta Y, Ohtsuru A, Yamashita S, Kohno S. Low plasma ghrelin levels in patients with Helicobacter pylori-associated gastritis. *Am J Med* 2004; **117**: 429-432
36 **Peeters TL**. Ghrelin: a new player in the control of gastrointestinal functions. *Gut* 2005; **54**: 1638-1649
37 **Osawa H**, Kita H, Ohnishi H, Nakazato M, Date Y, Bowlus CL, Ishino Y, Watanabe E, Shiiya T, Ueno H, Hoshino H, Satoh K, Sugano K. Changes in plasma ghrelin levels, gastric ghrelin production, and body weight after Helicobacter pylori cure. *J Gastroenterol* 2006; **41**: 954-961
38 **Arkkila PE**, Seppala K, Farkkila MA, Veijola L, Sipponen P. Helicobacter pylori eradication in the healing of atrophic gastritis: a one-year prospective study. *Scand J Gastroenterol* 2006; **41**: 782-790
39 **Leidy HJ**, Gardner JK, Frye BR, Snook ML, Schuchert MK, Richard EL, Williams NI. Circulating ghrelin is sensitive to changes in body weight during a diet and exercise program in normal-weight young women. *J Clin Endocrinol Metab* 2004; **89**: 2659-2664
40 **McNeely MJ**, Boyko EJ, Shofer JB, Newell-Morris L, Leonetti DL, Fujimoto WY. Standard definitions of overweight and central adiposity for determining diabetes risk in Japanese Americans. *Am J Clin Nutr* 2001; **74**: 101-107

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TOPIC HIGHLIGHT

Akio Inui, MD, PhD, Professor, *Series Editor*

Ghrelin and gastric acid secretion

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Abstract

Ghrelin, a novel growth hormone-releasing peptide, was originally isolated from rat and human stomach. Ghrelin has been known to increase the secretion of growth hormone (GH), food intake, and body weight gain when administered peripherally or centrally. Ghrelin is also known to stimulate the gastric motility and the secretion of gastric acid. In the previous studies, the action of ghrelin on acid secretion was shown to be as strong as that of histamine and gastrin in *in-vivo* experiment. In the studies, the mechanism for the action of ghrelin was also investigated. It was shown that vagotomy completely inhibited the action of ghrelin on the secretion of gastric acid suggesting that vagal nerve is involved in the mechanism for the action of ghrelin on acid secretion. As famotidine did not inhibit ghrelin-induced acid secretion in the study by Masuda *et al*, they concluded that histamine was not involved in the action of ghrelin on acid secretion. However, we have shown that famotidine completely inhibited ghrelin-induced acid secretion and histidine decarboxylase (HDC) mRNA was increased in gastric mucosa by ghrelin injection which is inhibited by vagotomy Our results indicate that histamine is involved in the action of ghrelin on acid secretion. Furthermore synergistic action of gastrin and ghrelin on gastric acid secretion was shown. Although gastrin has important roles in postprandial secretion of gastric acid, ghrelin may be related to acid secretion during fasting period or at night. However, further studies are needed to elucidate the physiological role of ghrelin in acid secretion.

Key words: Ghrelin; Acid secretion; Vagal nerve; Vagotomy; Histamine; Histidine decarboxylase

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GHRELIN AND THE REGULATION OF GASTRIC ACID SECRETION

Ghrelin, a novel growth-hormone-releasing peptide, was originally isolated from rat and human stomachs[1] and was demonstrated to localize in the endocrine cells of the stomach and hypothalamus[1,2]. Ghrelin has been known as a multifunctional hormone. Ghrelin increases secretion of growth hormone (GH), food intake, and body weight gain when administered peripherally or centrally[1,3-9]. Ghrelin has also positive cardiovascular effects. In humans, infusion of ghrelin decreases systemic vascular resistance and increases cardiac output in patients with heart failure. On the functions of stomach, ghrelin was also known to stimulate gastric motility and the secretion of gastric acid when administered peripherally or centrally[10,11]. As ghrelin has such multiple actions on many organs, physiological roles of ghrelin *in vivo* might be important even if many of them are not elucidated.

The mechanism for the action of ghrelin on feeding, growth hormone secretion and the secretion of gastric acid was studied and demonstrated that the vagal nerve was involved in the action of ghrelin[10-12]. When ghrelin was administered peripherally, ghrelin induced c-fos expression in the neurons of the arcuate nucleus of rats[12]. However, ghrelin did not induce c-fos expression in the neuron in both capsaicin-treated rats and vagotomized rats[12]. The effects of ghrelin on feeding were also abolished with capsaisin treatment and vagotomy[12]. These results suggest that gastric vagal afferent is the major pathway conveying ghrelin's signals for starvation and GH secretion to the brain. On the action of ghrelin on the secretion of gastric acid, vagal nerve was indicated to be involved in the action of ghrelin[10]. Masuda and coworkers indicated that the action of ghrelin on the secretion of gastric acid was abolished by the pretreatment

with atropine or bilateral cervical vagotomy[10]. Date and co-workers demonstrated that intracerebroventricular (ICV) administration of ghrelin induced the increase in the secretion of gastric acid and vagotomy and the pretreatment with atropine abolished the action of ghrelin[11]. They also demonstrated that ICV administration of ghrelin induced c-fos expression in the neurons of the nucleus of the solitary tract (NTS) and the dorsal motor nucleus of the vagus nerve (DMX)[11]. Taken together, these results suggest that vagal nerve plays important roles in the action of ghrelin on the secretion of gastric acid.

GHRELIN AND HISTAMINE RELEASE

As dictated above, ghrelin stimulates the secretion of gastric acid[10,11]. The mechanism for the action of ghrelin on acid secretion was also demonstrated that the vagal nerve was involved in the action of ghrelin[10,11]. However, the details of the mechanism after the activation of vagal nerve by ghrelin administration remains to be elucidated.

In the mechanism for acid secretion, vagal nerve has been known to play an important role, especially in the regulation by central nervous system[13] and in the stimulation by the distenstion of stomach[14]. Vagal nerve has stimulatory and inhibitory actions on acid secretion[15,16] and it contains and releases several neurotransmitters such as acetylcholine[17], gastrin-releaseing peptide (GRP)[18], substance P[19]. Calcitonin gene-related peptide[19] and pituitary adenylate cyclase activating peptide (PACAP)[20]. Among these, GRP has a stimulatory action on gastrin release from G cells[21]. Gastrin released by GRP stimulation is the most important physiological secretagogue and it has a primary role in postprandial acid secretion[22]. Gastrin stimulates acid secretion *via* enterochromaffin-like cells (ECL cell)[23]. Another transmitter, PACA, was also found to increase histamine release from ECL cell[24]. PACAP-immunoreactive nerve fibers are abundant in the gastric mucosa of both rat and humans[25] and gastric ECL cells possesses PACAP receptors(PAC1)[26]. Gastrin is now known as major stimulant of histamine release from gastric ECL cells[27]. Histamine released from ECL cells acts on parietal cells through H_2 receptor[28]. It was indicated that the activation of vagal nerve induced an increase in histamine release from gastric mucosa[29]. Accordingly it is plausible that histamine release may be involved in the increased acid secretion induced by administration of ghrelin.

In a previous report, Masuda *et al*[10] demonstrated that vagal nerve is involved in the action of ghrelin on acid secretion. However, they concluded that histamine was not involved in the action of ghrelin on acid secretion, as famotidine did not inhibited ghrelin-induced acid secretion[10]. Tthe mechanism that was demonstrated by Masuda seemed to require further investigation**,** because the vagal stimulation[29], as well as transmitter PACAP, is known to increase release of histamine from ECL cells[24]. Therefore, if ghrelin administration stimulates the vagal nerve, an increase in histamine release would consequently occur.

Previously we attempted to clarify whether histamine

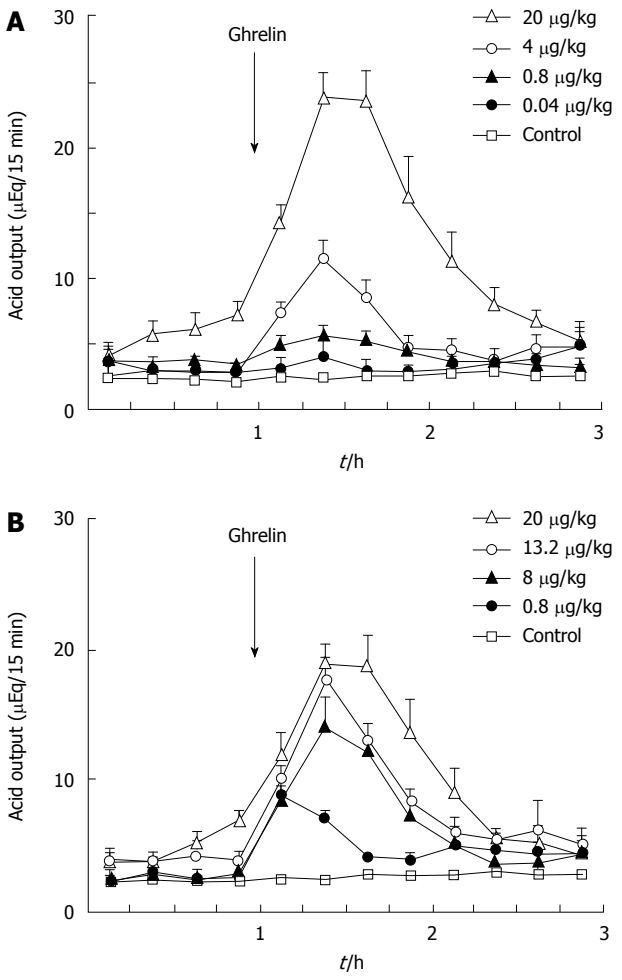

Figure 1 Effects of rat ghrelin (A) and gastrin-17 (B) on acid output in gastric lumen-perfused rats. A: Time course of acid output by Ⅳ administration of ghrelin (0.04, 0.8, 4, 20 μg/kg) in gastric lumen perfused rats; B: Time course of acid output by Ⅳ administration of gastrin (0.8, 8, 13.2 and 20 μg/kg) in gastric lumen perfused rats. Saline was administered in control rats. Each value represents the mean ± SE of acid output at 15 min intervals. *n* = 4, 5, 6, 7. Reprinted with permission[30].

is involved in the action of ghrelin[30]. In our study, ghrelin (0.8-20 μg/kg) dose-dependently increased gastric acid secretion and the action of ghrelin (20 μg/kg, 6×10^{-9} mol/kg) on acid secretion was almost as efficient as that of gastrin (20 μg/kg, 9.2×10^{-9} mol/kg) (Figure 1) [30]. The study demonstrated that vagotomy abolished the increase in acid secretion by ghrelin administration (Figure 2)[30] and also that famotidine completely inhibited the stimulatory action of ghrelin [30]. Furthermore, administration of ghrelin significantly increased HDC mRNA concentration of gastric mucosa (Figure 3) [30]. Vagotomy also abolished the increase in HDC mRNA by ghrelin administration (Figure 4) [30]. Furthermore, in the study, isolated vascularly-perfused stomachs that lacked vagal innervation were prepared in order to examine the effect of ghrelin on histamine release. Although the infusion of gastrin (2.1 μg/10 min) increased histamine release, ghrelin (5 μg/10 min) did not induce histamine release from isolated rat stomachs. Taken together, the results demonstrate the mechanism of action of ghrelin involves the vagal nerve as well as increases in release and synthesis of histamine,

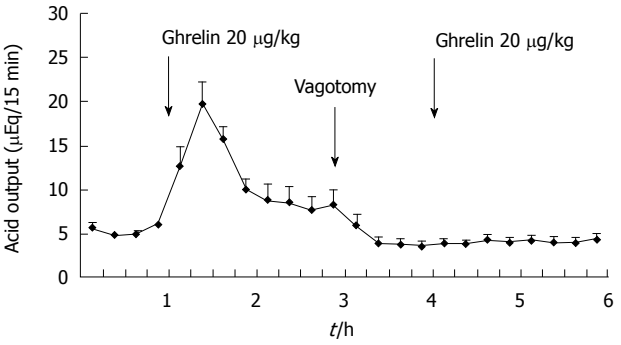

Figure 2 Effect of famotidine on ghrelin-stimulated acid output in gastric lumen-perfused rats. Time course of acid output by the Ⅳ administration of famotidine (0.33 mg/kg) and ghrelin (20 μg/kg) in gastric lumen-perfused rats. Famotidine was administered intravenously 15 min before ghrelin injection. Each value represents the mean ± SE of acid output at 15 min intervals. n = 4. Reprinted with permission[30].

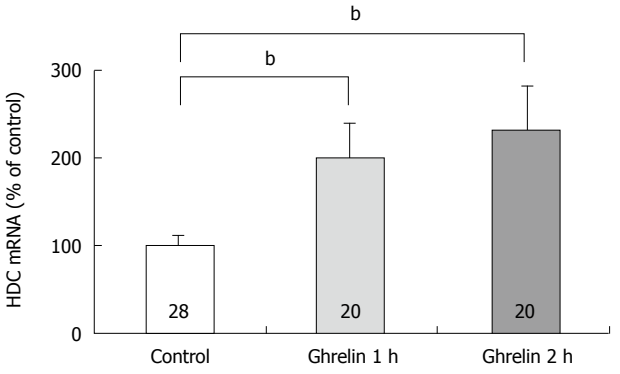

Figure 3 Effect of ghrelin on the concentration of HDC mRNA in rat gatsric mucosa. Ghrelin (20 μg/kg) was administered intravenously. The concentration of HDC mRNA was measured by real time RT-PCR using LightCycler. Control: The concentration of HDC mRNA in gastric mucosa of rats with saline administration. 1 h; the concentration of HDC mRNA in gastric mucosa of rats with ghrelin administration, the stomachs were excised 1 h after the administration. 2 h; the concentration of HDC mRNA in gastric mucosa of rats with ghrelin administration, the stomachs were excised 2 h after the administration. Each value represents the mean ± SE of HDC mRNA demonstrated as % of control. n = 28, 20, 20. ^bP < 0.01. Reprinted with permission[30].

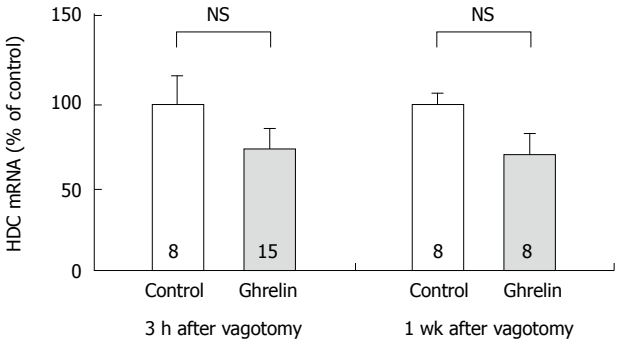

Figure 4 Effect of vagotomy on the concentration of HDC mRNA in rat gastric mucosa stimulated by ghrelin administration. Subdiaphragmatic vagotomy was performed in rats 3 h or 7 d before the experiments. Ghrelin (20 μg/kg) was administered Ⅳ. The concentration of HDC mRNA was measured by real time RT-PCR using Light Cycler. Control: The concentration of HDC mRNA in gastric mucosa of rats with saline administration. Ghrelin: The concentration of HDC mRNA in gastric mucosa of rats with ghrelin administration, the stomachs were excised 2 h after the administration. 3 h after vagotomy, the experiments were performed 3 h after vagotomy. 1 wk after vagotomy, the experiments were performed 7 d after vagotomy. Each value represents the mean ± SE of HDC mRNA demonstrated as % of control. n = 8 or 15. NS: Not significant. Reprinted with permission[30].

consequently induces the stimulation of parietal cells.

The reason that Masuda *et al* did not observe the inhibitory effect of famotidine on the action of ghrelin is unclear. However, there is a difference between the administration methods in the study by Matsuda *et al* and those used in our study. We administered ghrelin intravenously, while Matsuda *et al* administered ghrelin subcutaneously. The difference in the results may thus be due to the difference in the administration methods.

GHRELIN AND GASTRIN IN THE MECHANISM FOR THE REGULATION OF GASTRIC ACID SECRETION

Gastrin is known to be released into the circulation in response to food[31], and to play an important roles in increasing postprandial acid secretion[22], and to exert activity on ECL cells[23]. It was shown that vagotomy did not affect the maximal response of acid secretion to gastrin administration thus indicating that the action of gastrin was not primarily dependent on vagal nerve[32]. On the other hand, ghrelin is released during fasting period, and food intake suppresses its release[33]. As vagotomy completely abolished ghrelin-induced acid secretion and HDC mRNA production, ghrelin is thought to induce histamine release *via* vagal nerve activation, consequently resulting in increased acid secretion by parietal cells. Accordingly the mechanism for the secretion of ghrelin appears to differ from that of gastrin.

Recently, however, synergistic action of ghrelin and ghrelin on gastric acid secretion was reported from two groups[34,35]. Fukumoto *et al* have shown that Ⅳ administration of gastrin induced transient increases of ghrelin levels within 10 minutes and that simultaneous administration of both gastrin and ghrelin resulted in a synergistic increase of gastric acid secretion in rat[35]. They supposed that gastrin may directly stimulate ghrelin release and both hormones may increase gastric acid secretion synergistically[35]. We also presented the data that shows synergistic effects of gastrin and ghrelin on gastric acid secretion and histamine production by gastric mucosa which involves the vagal nerve[34]. Circulating ghrelin decreases soon after the initiation of feeding, gastrin oppositely increase with food intake. Generally simultaneous increases of gastrin and ghrelin do not occur. Therefore physiological role of this synergistic action of these hormones remain to be clarified. We are supposing this synergistic action may occurs in subjects with Hp infection that induces hypergastrinemia in fasting when ghrelin level rises.

CENTRAL REGULATION OF GASTRIC ACID SECRETION BY GHRELIN

The peripheral vagal nerve and the nuclei of central nerves are thought to be involved in the mechanism of

action of ghrelin[6,12]. Ghrelin administered Ⅳ induced Fos expression only in the neurons of the arcuate nucleus of the hypothalamus in rats[12]. On the other hand, ICV injection induced Fos expression in the neurons of NTS and DMX of the medulla oblongata and other nuclei such as several hypothalamic nuclei, the dentate gyrus, the hippocampus, and the cerebral cortex[6,11]. The hypothalamus is known to be the center for hunger and satiety[36]. In particular, the arcuate nucleus of the hypothalamus is activated by ghrelin administration and this region is known to have important role in controlling food intake relating to the action of leptin[37]. Date *et al*[12] indicated that Ⅳ injection of ghrelin induces Fos expression in neuropeptide Y (NPY)-producing and growth hormone-releasing hormone (GHRH)-producing neurons in the arcuate neucleus. It is known that injection of NPY into cerebral ventricles or directly into the hypothalamus of rats potently stimulates food intake[38]. Therefore, the activation of NPY neuron in the arcuate nucleus of the hypothalamus by ghrelin is thought to relate to the stimulatory effect of ghrelin on food intake. However, it is still unclear whether activation of the arcuate nucleus induces increase in the secretion of gastric acid. It has been shown that ghrelin receptors are synthesized in vagal afferent neurons and transported to the afferent terminals[12]. Date *et al*[12] suggested that the gastric vagal afferent nerve, which is capsaicin sensitive, is the major pathway conveying ghrelin signals for starvation and growth hormone secretion to the brain. It is possible that the same pathway mediates the mechanism for the action on the secretion of gastric acid when ghrelin is administered Ⅳ. On the other hand, as described above, DMX, NTS and several nuclei of the brain are apparently activated by ICV administration of ghrelin[6,11]. As DMX has been demonstrated to relate to the secretion of gastric acid, the activation of DMX may be related to the central nervous system mechanism for the action of ghrelin on acid secretion. Furthermore, vagotomy also abolished the stimulatory effect of ICV administration of ghrelin on acid secretion, thus the vagal efferent may also be involved in the action of cerebral ghrelin[11].

The neuronal pathway mediating the peripheral ghrelin appears to be different from that mediating central ghrelin. Although the vagal afferent nerve was demonstrated to be involved in the action of peripheral ghrelin[10,12], the role of vagal efferent nerve remained to be elucidated. However, as the action of cerebral ghrelin is thought to involve vagal nerve, possibly vagal efferent nerve, and the results of our previous study demonstrated that the actions of ghrelin on acid secretion and HDC mRNA production require the vagal nerve to be intact, it is possible that vagal efferent nerve is also involved in the action of peripheral ghrelin. A likely hypothesis based on the results of our study is that peripheral ghrelin stimulation of gastric acid secretion initiates the activation of central regulatory system that induces the activation of vagal nerve, resulting in possibly release of neurotransmitters and ECL cells stimulation.

Recently, as to the action of ghrelin, involvement of NO synthesis has been indicated[40]. Bilgin *et al* reported that ghrelin stimulates the secretion of gastric acid through NO as a mediator, because acid secretion induced by ghrelin administration was inhibited by applying L-NAME in rats. Previously the gastroprotective effect of ghrelin was reported and this can be attenuated by pretreatment of L-NAME suggesting involvement of NO pathways in the effect of ghrelin[40]. As NO increases mucosal blood flow, the effect of ghrelin on the secretion of gastric acid may partially dependent of an increase of mucosal blood flow.

THE PHYSIOLOGICAL ROLE OF GHRELIN IN THE REGULATION OF ACID SECRETION

The physiological role of ghrelin in the regulation of acid secretion still remains unclear. Comparing the secretion of ghrelin with that of gastrin, the secretion of gastrin is induced by food intake[26], while the secretion of ghrelin is increased during fasting period[33]. As described above, there are many differences between gastrin and ghrelin in the actions, their roles may be different. Gastrin is known to play important roles in the postprandial secretion of gastric acid, while ghrelin may be related to acid secretion during fasting period or at night. To elucidate the physiological role of ghrelin in acid secretion, further studies are required.

REFERENCES

1 **Kojima M**, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature* 1999; **402**: 656-660

2 **Date Y**, Kojima M, Hosoda H, Sawaguchi A, Mondal MS, Suganuma T, Matsukura S, Kangawa K, Nakazato M. Ghrelin, a novel growth hormone-releasing acylated peptide, is synthesized in a distinct endocrine cell type in the gastrointestinal tracts of rats and humans. *Endocrinology* 2000; **141**: 4255-4261

3 **Tschop M**, Smiley DL, Heiman ML. Ghrelin induces adiposity in rodents. *Nature* 2000; **407**: 908-913

4 **Wren AM**, Small CJ, Ward HL, Murphy KG, Dakin CL, Taheri S, Kennedy AR, Roberts GH, Morgan DG, Ghatei MA, Bloom SR. The novel hypothalamic peptide ghrelin stimulates food intake and growth hormone secretion. *Endocrinology* 2000; **141**: 4325-4328

5 **Kamegai J**, Tamura H, Shimizu T, Ishii S, Sugihara H, Wakabayashi I. Central effect of ghrelin, an endogenous growth hormone secretagogue, on hypothalamic peptide gene expression. *Endocrinology* 2000; **141**: 4797-4800

6 **Nakazato M**, Murakami N, Date Y, Kojima M, Matsuo H, Kangawa K, Matsukura S. A role for ghrelin in the central regulation of feeding. *Nature* 2001; **409**: 194-198

7 **Asakawa A**, Inui A, Kaga T, Yuzuriha H, Nagata T, Ueno N, Makino S, Fujimiya M, Niijima A, Fujino MA, Kasuga M. Ghrelin is an appetite-stimulatory signal from stomach with structural resemblance to motilin. *Gastroenterology* 2001; **120**: 337-345

8 **Shintani M**, Ogawa Y, Ebihara K, Aizawa-Abe M, Miyanaga F, Takaya K, Hayashi T, Inoue G, Hosoda K, Kojima M, Kangawa K, Nakao K. Ghrelin, an endogenous growth hormone secretagogue, is a novel orexigenic peptide that antagonizes leptin action through the activation of hypothalamic neuropeptide Y/Y1 receptor pathway.

Diabetes 2001; **50**: 227-232
9 **Wren AM**, Seal LJ, Cohen MA, Brynes AE, Frost GS, Murphy KG, Dhillo WS, Ghatei MA, Bloom SR. Ghrelin enhances appetite and increases food intake in humans. *J Clin Endocrinol Metab* 2001; **86**: 5992
10 **Masuda Y**, Tanaka T, Inomata N, Ohnuma N, Tanaka S, Itoh Z, Hosoda H, Kojima M, Kangawa K. Ghrelin stimulates gastric acid secretion and motility in rats. *Biochem Biophys Res Commun* 2000; **276**: 905-908
11 **Date Y**, Nakazato M, Murakami N, Kojima M, Kangawa K, Matsukura S. Ghrelin acts in the central nervous system to stimulate gastric acid secretion. *Biochem Biophys Res Commun* 2001; **280**: 904-907
12 **Date Y**, Murakami N, Toshinai K, Matsukura S, Niijima A, Matsuo H, Kangawa K, Nakazato M. The role of the gastric afferent vagal nerve in ghrelin-induced feeding and growth hormone secretion in rats. *Gastroenterology* 2002; **123**: 1120-1128
13 **Tache Y**, Goto Y, Hamel D, Pekary A, Novin D. Mechanisms underlying intracisternal TRH-induced stimulation of gastric acid secretion in rats. *Regul Pept* 1985; **13**: 21-30
14 **Grossman MI**. Secretion of acid and pepsin in response to distention of vagally innervated fundic gland area in dogs. *Gastroenterology* 1962; **42**: 718-721
15 **Antia F**, Rosiere CE, Robertson C, Grossman MI. Effect of vagotomy on gastric secretion and emptying time in dogs. *Am J Physiol* 1951; **166**: 470-479
16 **Debas HT**, Konturek SJ, Walsh JH, Grossman MI. Proof of a pyloro-oxyntic reflex for stimulation of acid secretion. *Gastroenterology* 1974; **66**: 526-532
17 **Ternaux JP**, Falempin M, Palouzier B, Chamoin MC, Portalier P. Presence of cholinergic neurons in the vagal afferent system: biochemical and immunohistochemical approaches. *J Auton Nerv Syst* 1989; **28**: 233-242
18 **Dockray GJ**, Vaillant C, Walsh JH. The neuronal origin of bombesin-like immunoreactivity in the rat gastrointestinal tract. *Neuroscience* 1979; **4**: 1561-1568
19 **Sternini C**. Vagal afferent innervation of the enteric nervous system. In: Ritter S, Ritter RC, Barnes CD. editors. Neuroanatomy and physiology of abdominal vagal afferents. Boca Raton, FL: CRC Press, 1992: 135-156
20 **Tornoe K**, Hannibal J, Georg B, Schmidt PT, Hilsted L, Fahrenkrug J, Holst JJ. PACAP 1-38 as neurotransmitter in the porcine antrum. *Regul Pept* 2001; **101**: 109-121
21 **Sugano K**, Park J, Soll AH, Yamada T. Stimulation of gastrin release by bombesin and canine gastrin-releasing peptides. Studies with isolated canine G cells in primary culture. *J Clin Invest* 1987; **79**: 935-942
22 **Richardson CT**, Walsh JH, Hicks MI, Fordtran JS. Studies on the mechanisms of food-stimulated gastric acid secretion in normal human subjects. *J Clin Invest* 1976; **58**: 623-631
23 **Chen D**, Monstein HJ, Nylander AG, Zhao CM, Sundler F, Hakanson R. Acute responses of rat stomach enterochromaffinlike cells to gastrin: secretory activation and adaptation. *Gastroenterology* 1994; **107**: 18-27
24 **Lindstrom E**, Bjorkqvist M, Boketoft A, Chen D, Zhao CM, Kimura K, Hakanson R. Neurohormonal regulation of histamine and pancreastatin secretion from isolated rat stomach ECL cells. *Regul Pept* 1997; **71**: 73-86
25 **Kivipelto L**, Absood A, Arimura A, Sundler F, Hakanson R, Panula P. The distribution of pituitary adenylate cyclase-activating polypeptide-like immunoreactivity is distinct from helodermin- and helospectin-like immunoreactivities in the rat brain. *J Chem Neuroanat* 1992; **5**: 85-94
26 **Zeng N**, Kang T, Lyu RM, Wong H, Wen Y, Walsh JH, Sachs G, Pisegna JR. The pituitary adenylate cyclase activating polypeptide type 1 receptor (PAC1-R) is expressed on gastric ECL cells: evidence by immunocytochemistry and RT-PCR. *Ann N Y Acad Sci* 1998; **865**: 147-156
27 **Prinz C**, Zanner R, Gratzl M. Physiology of gastric enterochromaffin-like cells. *Annu Rev Physiol* 2003; **65**: 371-382
28 **Chew CS**, Hersey SJ, Sachs G, Berglindh T. Histamine responsiveness of isolated gastric glands. *Am J Physiol* 1980; **238**: G312-G320
29 Uvnas B. The part played by the pyloric region in the cephalic phase of gastric secretion. *Acta Physiol Scan* 1942; **4** (Suppl 13): 1-7
30 **Yakabi K**, Ro S, Onouhi T, Tanaka T, Ohno S, Miura S, Johno Y, Takayama K. Histamine mediates the stimulatory action of ghrelin on acid secretion in rat stomach. *Dig Dis Sci* 2006; **51**: 1313-1321
31 **Feldman M**, Walsh JH, Wong HC, Richardson CT. Role of gastrin heptadecapeptide in the acid secretory response to amino acids in man. *J Clin Invest* 1978; **61**: 308-313
32 **Emas S**, Grossman MI. Effect of truncal vagotomy on acid and pepsin responses to histamine and gastrin in dogs. *Am J Physiol* 1967; **212**: 1007-1012
33 **Cummings DE**, Purnell JQ, Frayo RS, Schmidova K, Wisse BE, Weigle DS. A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. *Diabetes* 2001; **50**: 1714-1719
34 **Ro S**, Tanaka T, Ochiai M, Yakabi K. The interaction between gastrin and ghrelin on acid secfretion in rat stomsch. *Gastroentwrology* 2004; **26**: A147
35 **Fukumoto K**, Nakahara K, Katayama T, Miyazatao M, Kangawa K, Murakami N. Synergistic action of gastrin and ghrelin on gastric acid secretion in rats. *Biochem Biophys Res Commun* 2008; **374**: 60-63
36 **Bray GA**, Fisler J, York DA: Neuroendocrine control of the development of obesity: understanding gained from studies of experimental animal models. *Front Neuroendocrinol* 1990; **11**: 128-181
37 **Satoh N**, Ogawa Y, Katsuura G, Hayase M, Tsuji T, Imagawa K, Yoshimasa Y, Nishi S, Hosoda K, Nakao K. The arcuate nucleus as a primary site of satiety effect of leptin in rats. *Neurosci Lett* 1997; **224**: 149-152
38 **Stanley BG**, Kyrkouli SE, Lampert S, Leibowitz SF. Neuropeptide Y chronically injected into the hypothalamus: a powerful neurochemical inducer of hyperphagia and obesity. *Peptides* 1986; **7**: 1189-1192
39 **Wyrwicka W**, Garcia R. Effect of electrical stimulation of the dorsal nucleus of the vagus nerve on gastric acid secretion in cats. *Exp Neurol* 1979; **65**: 315-325
40 **Bilgin HM**, Tumer C, Diken H, Kelle M, Sermet A. Role of ghrelin in the regulation of gastric acid secretion involving nitrergic mechanisms in rats. *Physiol Res* 2008; **57**: 563-568

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c-Fos overexpression increases the proliferation of human hepatocytes by stabilizing nuclear Cyclin D1

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Abstract

AIM: To investigate the effect of stable c-Fos overexpression on immortalized human hepatocyte (IHH) proliferation.

METHODS: IHHs stably transfected with c-Fos (IHH-Fos) or an empty vector (IHH-C) were grown in medium supplemented with 1% serum or stimulated with 10% serum. Cell proliferation was assessed by cell counts, 3H-thymidine uptake and flow cytometry analyses. The levels of cell cycle regulatory proteins (Cyclin D1, E, A) cyclin dependent kinases (cdk) cdk2, cdk4, cdk6, and their inhibitors p15, p16, p21, p27, total and phosphorylated GSK-3β and epidermal growth factor receptor (EGF-R) were assayed by Western blotting. Analysis of *Cyclin D1* mRNA levels was performed by reverse transcription-polymerase chain reaction and real-time polymerase chain reaction (PCR) analysis. Stability of Cyclin D1 was studied by cycloheximide blockade experiments.

RESULTS: Stable c-Fos overexpression increased cell proliferation under low serum conditions and resulted in a two-fold increase in [^{3}H]-thymidine incorporation following serum addition. Cell cycle analysis by flow cytometry showed that c-Fos accelerated the cell cycle kinetics. Following serum stimulation, Cyclin D1 was more abundantly expressed in c-Fos overexpressing cells. Cyclin D1 accumulation did not result from increased transcriptional activation, but from nuclear stabilization. Overexpression of c-Fos correlated with higher nuclear levels of inactive phosphorylated GSK-3β, a kinase involved in Cyclin D1 degradation and higher levels of *EGF-R* mRNA, and EGF-R protein compared to IHH-C both in serum starved, and in serum stimulated cells. Abrogation of EGF-R signalling in IHH-Fos by treatment with AG1478, a specific EGF-R tyrosine kinase inhibitor, prevented the phosphorylation of GSK-3β induced by serum stimulation and decreased Cyclin D1 stability in the nucleus.

CONCLUSION: Our results clearly indicate a positive role for c-Fos in cell cycle regulation in hepatocytes. Importantly, we delineate a new mechanism by which c-Fos could contribute to hepatocarcinogenesis through stabilization of Cyclin D1 within the nucleus, evoking a new feature to c-Fos implication in hepatocellular carcinoma.

Key words: c-Fos; Cyclin D1; GSK-3; Cell growth; Cell cycle; Hepatoma; Epidermal growth factor

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common cancer in the world, with an increasing number of new cases emerging each year. Etiologically it is linked to chronic viral infections (hepatitis B and C viruses), alcohol-related cirrhosis or aflatoxin B1 exposure, which

all cause disruptions in signal transduction cascades leading to abnormalities in gene expression.

The proto-oncogene *c-fos* is an important member of the activating protein 1 (AP-1) transcription factor involved in major cellular functions such as transformation, proliferation, differentiation and apoptosis[1,2]. Such a large variety of functions is achieved by the combination of different Jun (c-Jun, JunB or JunD) and Fos (c-Fos, FosB, ΔfosB, Fra-1, Fra-2) family members forming various AP-1 homo and heterodimers. *c-fos* is an immediate early gene whose expression is rapidly and transiently induced after mitogenic stimulation[3]. The role of c-Fos in cell proliferation and transformation remains controversial. c-Fos is required during all phases of the cell cycle in exponentially growing cells and is a potent inducer of cell proliferation[4]. However, some studies have suggested that c-Fos poorly contributes to proliferation[5], was totally dispensable for[6], or even down-regulated, cell growth[7,8]. Overexpression of c-Fos leads to morphological transformation of fibroblasts[9,10], and to osteosarcoma formation in transgenic mice[11,12]. Apart from one study describing a negative role for c-Fos in hepatocellular tumorigenesis[8], several reports rather support a potential positive role for c-Fos in this process. High expression levels of c-Fos were determined in tumour tissue compared to the adjacent non-tumour liver in human HCC[13-15], as well as in several models of HCC in rodents[16-18]. A recent study in humans identified a subtype of HCC sharing gene expression patterns with foetal hepatoblasts which can be distinguished from another HCC subtype closer to adult hepatocytes[19]. Interestingly, c-Fos, but not c-Jun expression was higher in the foetal subtype which displayed a poorer prognosis and a greater tendency to invasion than the adult subtype. In addition, the expression of DNA 5-methylcytosine transferase, a c-Fos target gene involved in DNA methylation[20] is increased in human tumour cells and in HCCs[21]. Despite these studies showing that c-Fos overexpression might be an important step towards the development of liver cancer, its precise role in hepatocarcinogenesis remains ill-defined.

In order to clarify c-Fos implication in hepatocarcinogenesis, we examined the effect on proliferation of stable c-Fos overexpression in immortalized human hepatocytes (IHH). We show, for the first time, that a positive role for c-Fos on hepatocyte proliferation can be attained by stabilization of Cyclin D1 in the nuclear compartment, a mechanism which has not been described as a c-Fos related process in any cell type to date.

MATERIALS AND METHODS

Cell culture and reagents

IHH were cultured in Williams' medium E (Invitrogen, Cergy Pontoise, France) supplemented with 100 mL/L fetal calf serum (FCS) (Biochrom AG, Cambridge, UK), 1% penicillin-streptomycin, 1% Glutamax and 1% DMEM sodium pyruvate (Invitrogen). Specific reagents were AG1478 (Calbiochem, San Diego, CA) and cycloheximide (CHX) (Euromedex, Souffelweyersheim, France).

Generation of stably transfected cells

The human *c-fos* cDNA was inserted into the cytomegalovirus driven pCIneo expression vector (Promega, Charbonnières, France) containing a neomycin resistance gene to obtain the pCIneo-*cfos* vector. Cells were stably transfected by electroporation (230V, 960 μF) in PBS-Hepes Buffer 10 mmol/L, pH 7.4 with the empty vector (pCIneo) or with pCIneo-*cfos*. Two days post-transfection, stable clones were selected in media containing 500 μg/mL of G418 (Invitrogen). The resistant clones were pooled after 3 wk of selection, and maintained with G418. c-Fos overexpression was verified by Western blot analysis as shown in Figure 1A.

Growth curve analysis

Cells were plated in triplicate at a density of 1.0×10^5 per well in six-well plates, and cultured in low serum (1% FCS) conditions for 5 d. Triplicate cultures were trypsinized and diluted in an equal volume of trypan blue solution (Invitrogen). Viable cells were counted daily in a haemocytometer counting chamber.

[^{3}H]-thymidine incorporation

DNA synthesis was determined by measuring [^{3}H]-thymidine incorporation. Cells were plated onto 24-well plates at a density of 1.0×10^5 cells/well in quadruplets. Cells were serum deprived for 24 h, and serum stimulated in culture media containing 1.5 μCi/mL tritiated thymidine ([^{3}H]dT) (specific activity of 740 GBq/mmol) (Perkin-Elmer, Waltham, Ma) for 4 h. Cells were fixed and washed in ice-cold 10% trichloroacetic acid. DNA was solubilized in 0.1 mol/L NaOH for 1 h at 37℃. [^{3}H]dT incorporated into the DNA was measured using liquid scintillation counting.

Flow cytometry

DNA cell cycle analysis was measured by 5-bromodeoxyuridine (BrdU) incorporation and propidium iodide staining of the nuclei by flow cytometry (FACScalibur, BD Biosciences, Mansfield, MA) and analyzed with the ProCellQuest software provided by the manufacturer. Cell cycle progression was measured by pulse/chase experiments. Cells plated at a density of 5×10^5 per 6-cm dish were serum starved for 24 h, serum stimulated for 12 h and stained with BrdU (30 μg/mL) for 1 h. Cells were then chased with BrdU free medium for 0, 3, 6, 9, 12 h, stained with propidium iodide and harvested in 70% ethanol. Cells were then treated with 2 N HCl and pepsin (0.2 mg/mL) for 30 min. BrdU content was analyzed using a FITC-labeled monoclonal antibody to BrdU (BD Pharmingen, Le-Pont-de-Claix, France). Labeled cells were washed and resuspended in PBS containing propidium iodide (10 μg/mL) for 30 min prior to flow cytometric analysis.

Western blot analysis

Nuclear proteins were extracted as described[22]. Total proteins were extracted with lysis buffer [1% (v/v) SDS, 1 mmol/L Na_3VO_4, 10 mmol/L Tris pH 7.4, 1% benzonase) for 10 min at room temperature and heated for

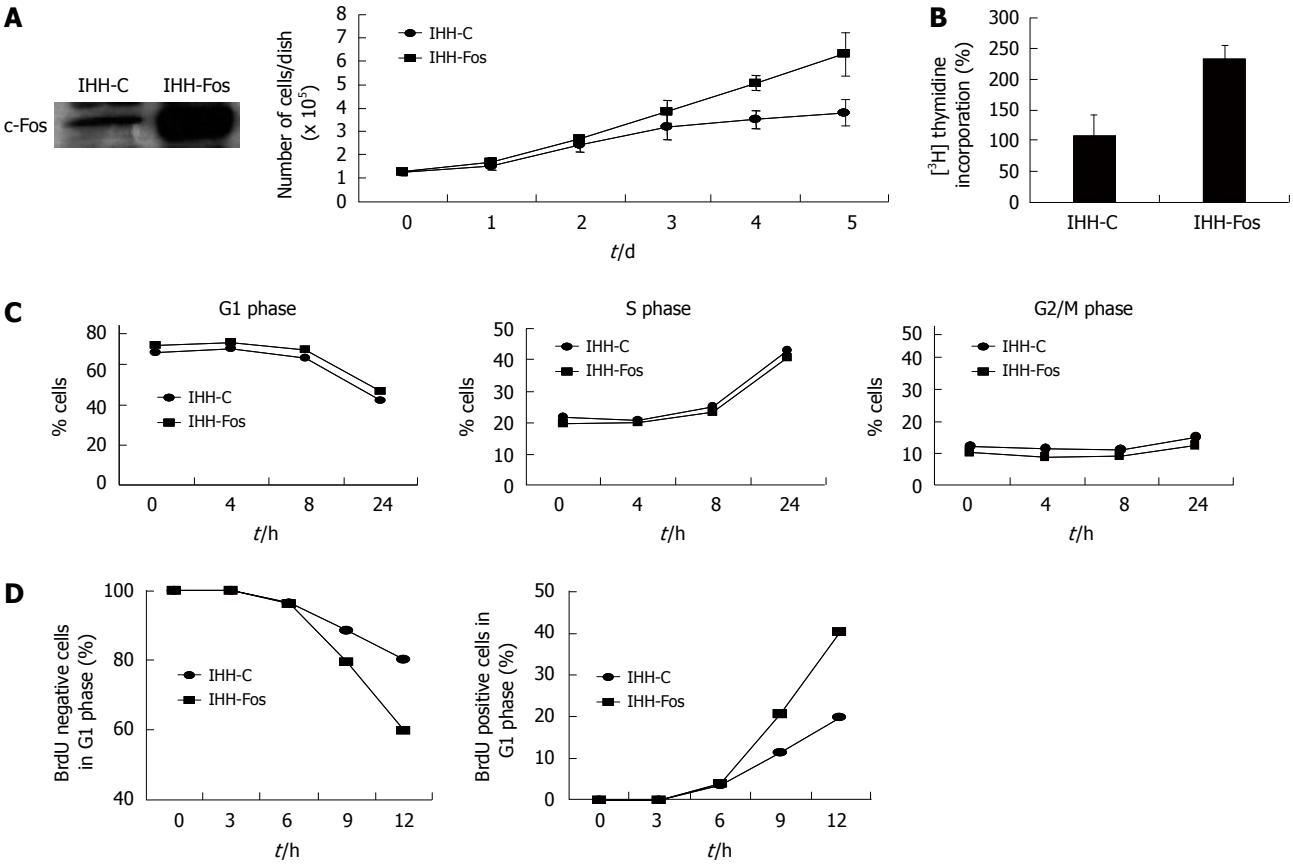

Figure 1 Overexpression of c-Fos accelerates the cell cycle. A: IHH-C and IHH-Fos were grown in 1% FCS, cultured for 5 d and counted daily. Cell growth was determined by counting the number of attached cells every day. Results are the mean ± SE of three independent experiments; B: [^{3}H] thymidine incorporation into DNA. Non-synchronized IHH-C or IHH-Fos serum starved for 24 h then serum stimulated for 4 h were incubated with [^{3}H] thymidine for 4 h. DNA was extracted as described in materials and methods, and [^{3}H] thymidine incorporation into DNA was assessed by scintillation counting. Results are expressed as percentage of increase of [^{3}H] thymidine incorporation in serum-stimulated cells over that of quiescent cells for each cell population. Results are the mean ± SE of six independent experiments; C: Flow cytometry analysis for quantification of cell cycle phase distribution and progression through cell cycle. IHH-C or IHH-Fos serum starved for 24 h were incubated with BrdU for 1 h and stained with propidium iodide 0, 4, 8 and 24 h after serum stimulation. The percentage of cells in each phase is plotted against time. Results of a representative experiment are shown (out of 3); D: IHH-C or IHH-Fos serum starved for 24 h were serum stimulated for 12 h, BrdU pulsed for 1 h, chased with fresh medium for 0, 3, 6, 9, 12 h, and then stained with propidium iodide. The percentage of BrdU-negative cells in the G1 phase (G1 exit) (left panel) and of the BrdU-positive cells in the G1 phase (G1 entry) (right panel) of the cell cycle is plotted against time. Results are representative of four independent experiments.

5 min at 100℃. Equal quantities of nuclear proteins were fractionated on a SDS-polyacrylamide gel and transferred onto nitrocellulose membranes by electroblotting. The antibodies used in this study were as follows: c-Fos, Cyclin D1, Cyclin E, Cyclin A, cdk2, cdk4, cdk6, p15, p16, p21, p27 and EGF-R (Santa Cruz Biotechnology, Santa Cruz, CA), GSK3β (Affinity BioReagents, Golden, CO), Phospho-GSK3β (Serine9) (Abcam, Paris, France). Immunoreactive bands were visualized using the ECL kit (Amersham BioSciences, Saclay, France) according to the manufacturer's instructions.

Reverse transcription-polymerase chain reaction (RT-PCR) and real-time quantitative PCR analysis

mRNA was isolated from cells by Nucleospin RNA II kit (Macherey-Nagel, Hoerdt, France) following the manufacturer's instructions. RNA (1 μg) was reverse transcribed using ThermoScript™ RT-PCR System (Invitrogen, Cergy-Pontoise, France). Real-time quantitative PCR was performed using the following primers: *Cyclin D1*: forward 5'-GCATGTTCGTGGCCTCTAAGA-3'; reverse 5'-CGGTGTAGATGCACAGCTTCTC-3', *EGF-R*: forward 5'-GCGTCTCTTGCCGGAATGT-3' and reverse 5'-GGCTCACCCTCCAGAAGGTT-3'. Real-time quantitative PCR was performed with an ABI PRISM 7700 instrument (Applied Biosystems, Foster City, CA)) using SYBRGreen PCR core reagents (Applied Biosystems). Fold changes in mRNA were calculated by the $\Delta\Delta C_t$ method using *cyclophilin A* (forward: 5'-CAAATGCTGGACCCAACACA-3'; reverse: 5'-TGCCATCCAACCACTCAGTCT-3') as a standard. All PCR reactions were done in triplicate.

Statistical analysis

Data were expressed as means ± SE. Student's *t*-test was performed and statistical significance was considered as $P < 0.05$.

RESULTS

Growth rate and cell cycle regulation by c-Fos overexpression

To determine whether c-Fos could modulate hepatocyte growth, we carried out growth curve assays. While

cell growth was similar in the presence of 10% FCS in IHH-C and IHH-Fos (data not shown), we observed that the growth pattern of the two cell lines differed in low serum conditions (1% FCS). While the number of IHH-Fos increased exponentially over 5 d in culture, IHH-C number increased slowly during the first 3 d of culture, and then reached a plateau, due to the induction of cell death by serum deprivation (Figure 1A and data not shown). Thus, c-Fos overexpression correlated with a more rapid growth in low serum conditions. The effect of c-Fos overexpression on cell proliferation was further established by measuring [^{3}H]dT incorporation following 4 h serum stimulation of cells deprived of serum for 24 h. The increase of [^{3}H]dT incorporation induced by serum was 2.2 times higher in IHH-Fos than in IHH-C (231% and 107%, respectively), and the difference was statistically significant ($P < 0.001$) (Figure 1B). To further analyze the role of c-Fos on the cell cycle, cell cycle phase distribution and cell cycle kinetics were analyzed by flow cytometry. Following serum stimulation, the percentage of cells in the G1 phase decreased, while the percentage of cells in the S-phase increased 24 h after serum stimulation. However, the percentage of cells in the different stages of the cell cycle was comparable in IHH-Fos and IHH-C (Figure 1C). Cell cycle progression was measured by BrdU pulse/chase experiments. The rate at which BrdU positive cells progress into G1 indicates the rate of transit through S, G2 and M phases. Similarly, the rate at which BrdU negative cells become depleted from the G1 pool indicates the transit rate through G1. We show that IHH-Fos quit (Figure 1D, left panel) and enter (Figure 1D, right panel) G1 faster than IHH-C, which reflects a global increase in cell cycle kinetics. The fact that the cell cycle profile was not altered by c-Fos indicates that the acceleration is proportional in all phases of the cycle. These data taken together indicate that c-Fos overexpression increases the growth of exponentially growing cells cultured in low serum medium as well as the proliferation response induced by serum refeeding.

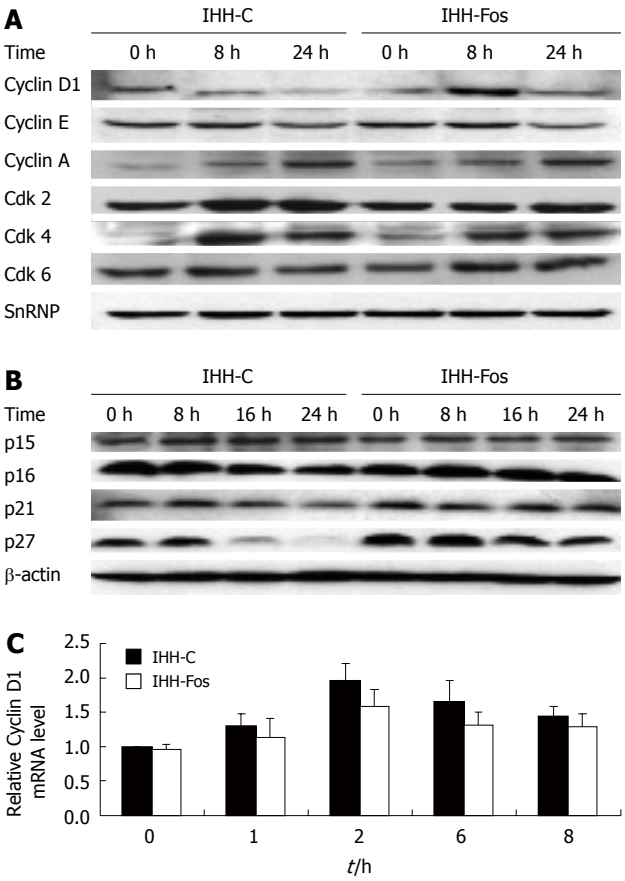

Figure 2 Induction of cell cycle regulatory proteins after serum refeeding. IHH-C or IHH-Fos were serum starved for 24 h. Nuclear (A) or total (B) extracts prepared before or after serum stimulation for 8 h or 24 h were immunoblotted with antibodies, as indicated. Loading of nuclear or total extracts was normalized using a SnRNP or a β-actin antibody, respectively. Results of a representative experiment are shown (out of 3); C: Quantitative real time PCR of Cyclin D1 mRNA levels in quiescent IHH-C or IHH-Fos serum stimulated for the indicated times. Bars indicate mean ± SE of three independent experiments each performed in triplicate.

Induction of cell cycle regulatory proteins by c-Fos

The levels of various cell cycle regulatory proteins before and after serum stimulation of IHH-C and IHH-Fos were analyzed by Western blotting experiments. In both cell lines, serum addition induced an increase in the nuclear levels of Cyclin A, cdk2 and cdk4, but no change in Cyclin E. Of interest, the nuclear levels of Cyclin D1 were increased after 8 h of stimulation in IHH-Fos, but not in IHH-C (Figure 2A). In addition, the levels of p27 were higher in the absence of serum stimulation or following serum stimulation in IHH-Fos than in IHH-C (Figure 2B).

Quantitative RT-PCR analysis was performed to determine whether the increase of Cyclin D1 at 8 h of serum stimulation in IHH-Fos was controlled transcriptionally. Interestingly, a similar 2-fold increase in *Cyclin D1* mRNA 2 h following serum stimulation was observed in IHH-Fos and IHH-C, without any significant differences at any of the time points (Figure 2C), indicating that the higher levels of Cyclin D1 in the nucleus in IHH-Fos are not due to transcriptional mechanisms.

Cyclin D1 stabilization in the nucleus

We next determined whether post-translational regulations could explain the increase of nuclear Cyclin D1 in serum-stimulated IHH-Fos. CHX, a translational inhibitor, was used to block protein synthesis. While in IHH-C, nuclear Cyclin D1 protein levels started to decline as from 1 h, and decreased by 85% after 2 h of CHX treatment, Cyclin D1 nuclear levels were decreased by only 20% upon 2 h of CHX treatment in IHH-Fos (Figure 3), indicating that c-Fos overexpression correlates with increased stability of nuclear Cyclin D1.

Inactivation of GSK-3β in IHH-Fos contributes to Cyclin D1 stabilization

Previous studies have indicated that Cyclin D1 degradation is triggered by GSK-3β-induced phosphorylation on a single threonine residue (Thr-286)[23]. Of note, phosphorylated GSK-3β is the inactive form of the protein[24]. We, therefore, compared the level of

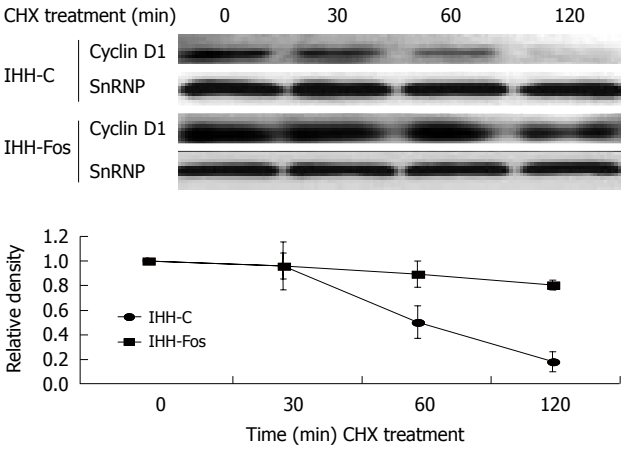

Figure 3 Nuclear Cyclin D1 stability is increased by c-Fos overexpression. IHH-C or IHH-Fos serum starved for 24 h were serum stimulated for 6 h and then treated with CHX (30 μg/mL) for 30 min, 1 h or 2 h. Nuclear extracts were immunoblotted with an antibody against Cyclin D1, and normalized with a SnRNP antibody, as indicated (Upper panels). The Cyclin D1 over SnRNP ratios were quantified by densitometric analysis of the immunoreactive bands (Lower panel). The results are the mean ± SE of three independent experiments.

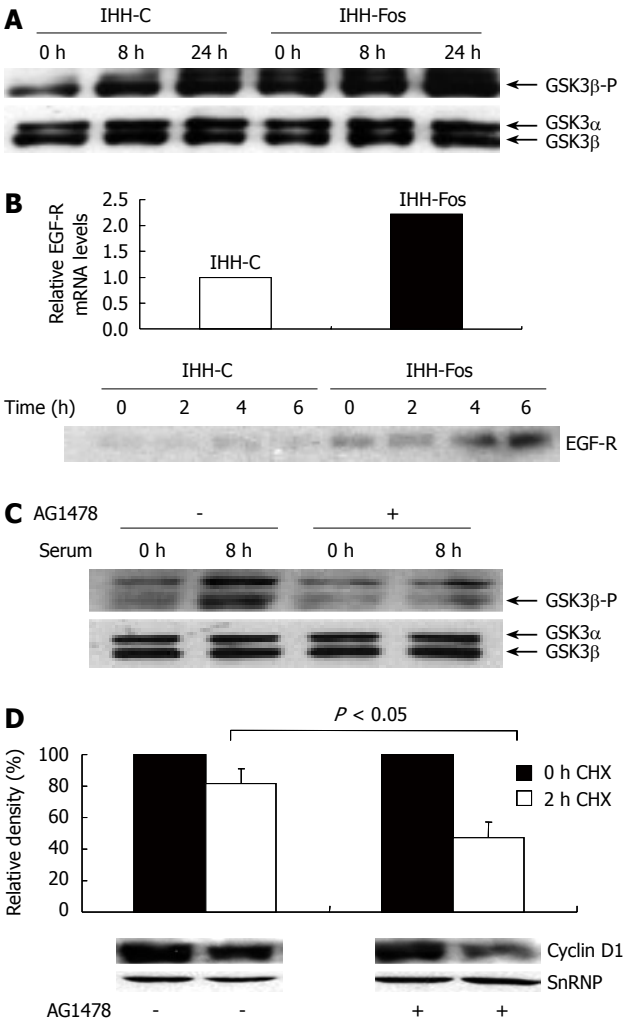

Figure 4 Stimulation of EGF-R signaling by c-Fos overexpression. A: Total and phosphorylated levels of nuclear GSK-β. IHH-C or IHH-Fos were serum starved for 24 h. Nuclear extracts prepared from unstimulated (0 h), 8 h or 24 h serum stimulated IHH-C or IHH-Fos, were immunoblotted with an antibody against phosphorylated or total GSK3β. B: Upper panel, detection of EGF-R mRNA in IHH-C and IHH-Fos by quantitative real time PCR analysis of mRNA isolated from cells grown in the presence of serum. Lower panel, Western blot analysis of EGF-R in total cell extracts from IHH-C and IHH-Fos cells serum starved for 24 h (0) or stimulated with serum for the indicated times; C: Serum deprived IHH-Fos were pre-treated (+) or not (-) with AG1478 (10 μmol/L) for 1 h. Nuclear proteins were prepared from non stimulated and 8 h serum-stimulated cells. Phosphorylated and total GSK3β levels were detected by Western blot; D: Serum-deprived IHH-Fos were pretreated or not with AG1478 (10 μmol/L) for 1 h, then serum-stimulated for 6 h. Nuclear proteins were extracted before (0 h, filled columns) or after 2 h (empty columns) of CHX treatment (30 μg/mL). Cyclin D1 levels were quantified by Western blotting. The immunoreactive bands were quantified by densitometric analysis after loading normalization of the blot using a SnRNP antibody. The results are expressed as the % of Cyclin D1/SnRNP expression and are the mean ± SE of 3 independent experiments. The lower panel illustrates one representative experiment.

phosphorylated GSK-3β between the two cell lines by Western blot analysis. As shown in Figure 4A, the levels of GSK3β phosphorylation were much higher in the nucleus of IHH-Fos than in IHH-C, both in unstimulated and in serum stimulated conditions, indicating higher basal, and induced levels of inactive GSK-3β in IHH-Fos (Figure 4A). Therefore, a decrease in active GSK-3β in IHH-Fos could be responsible for the increased stability of nuclear Cyclin D1 after serum stimulation.

Several signaling pathways are able to induce GSK-3β phosphorylation, including the two main cascades targeted by tyrosine kinase receptors: the phosphatidyl inositol 3-kinase (PI3K) and the Ras/mitogen activated protein kinase pathways[24]. Since the epidermal growth factor receptor (EGF-R) is a known transcriptional target of AP-1[25-27], we tested the hypothesis that overexpression of EGF-R might contribute to high levels of GSK-3β phosphorylation in IHH-Fos. Quantitative RT-PCR analysis revealed a 2.2-fold increase in the basal level of *EGF-R* mRNA in IHH-Fos compared to IHH-C (Figure 4B). Higher levels of EGF-R protein were also observed in serum starved and serum-stimulated IHH-Fos compared to IHH-C (Figure 4B), strongly indicative of increased EGF-R signaling in c-Fos-overexpressing cells. Altogether, these data suggest that increased EGF-R signaling might contribute, at least partly, to increased levels of GSK-3β phosphorylation in IHH-Fos cells.

To demonstrate the implication of EGF-R in GSK-3β-mediated Cyclin D1 stabilization, IHH-Fos cells were treated with AG1478, a specific inhibitor of the EGF-R tyrosine kinase before serum stimulation. Western blot analysis indicated that AG1478 treatment did block the phosphorylation of GSK-3β induced by serum (Figure 4C). Interestingly, the decrease in the nuclear level of Cyclin D1 protein observed after a 2 h-CHX treatment of IHH-Fos cells stimulated by serum was more important in cells treated with AG1478 (50% decrease) than in untreated cells (10% decrease), and the difference was statistically significant ($P < 0.05$, Figure 4D), confirming that blockade of EGF-R induced signaling in IHH-Fos leads to a more rapid nuclear Cyclin D1 degradation.

DISCUSSION

Our results indicate that c-Fos overexpression accelerates

cell growth under reduced serum concentration suggesting that hepatocytes overexpressing c-Fos become relatively independent of the presence of growth factors. We show that c-Fos enhances DNA synthesis after serum stimulation, and accelerates hepatocyte cell cycle progression without altering the overall distribution of cells in each phase due to a proportional acceleration of cell cycle kinetics in all phases.

Our results are at variance with those obtained in immortalized murine hepatocytes[8]. In this model, c-Fos conditional expression for 48 h was shown to decrease cell growth and [^{3}H]dT incorporation of cells grown in serum-supplemented medium. Besides species differences (murine *vs* human cells), the discrepancy in results can be explained by the use of very different cellular models which cannot be compared. The human hepatocytes used in our study were immortalized by SV40 T antigen while murine hepatocytes were immortalized using truncated c-Met[28]. Overexpression of c-Fos in our model was permanently established as the result of stable transfection, while in Mikula's study a c-Fos-estrogen receptor fusion protein was expressed for a limited period (1-3 d) following estradiol treatment of the cultures[8]. Furthermore, the function of the conditionally expressed c-Fos protein may have been modified, since gene fusion has been shown to alter the function of Fos family proteins[29].

We aimed to determine whether the positive role of c-Fos on hepatocyte proliferation depicted in our study was mediated through changes in cell cycle regulation. Different studies have reported an effect of *c-fos* gene deletion or c-Fos protein overexpression on Cyclin D1[9,30,31], Cyclin E[31] or Cyclin A[32] expression, depending on the cell type studied. In our study, while the levels of Cyclin E and A and their associated kinases varied with a similar pattern in both cell types following serum stimulation, nuclear Cyclin D1 levels were higher in IHH-Fos compared to IHH-C 8 h after serum re-feeding. Contrary to previous reports describing c-Fos as a transcriptional activator of Cyclin D1[30], the higher levels of nuclear Cyclin D1 in IHH-Fos than in IHH-C were not due to differences in transcriptional regulation, but to increased protein stability in the nucleus. A similar lack of correlation between Cyclin D1 mRNA and protein expression has been previously described in an *in vivo* experimental model of HCC[33]. Our results strongly suggest a mechanism whereby c-Fos induces nuclear accumulation of Cyclin D1 without affecting the total cellular amount of the protein.

The Cyclin D1 protein is quite unstable, with a half-life of less than 30 min[34]. It accumulates in the nucleus during the G1 phase and exits into the cytoplasm during the S phase. Nuclear export of Cyclin D1, and its subsequent ubiquitination and proteolysis, are dependent on phosphorylation on a single threonine residue (Thr-286) performed mainly by GSK-3β[23], a protein kinase active only when dephosphorylated. In contrast to Cyclin D1, GSK-3β is predominantly cytoplasmic during G1 phase, but a considerable amount becomes nuclear during S phase[23]. We show herein that phosphorylated levels of nuclear GSK-3β are higher in IHH-Fos than in IHH-C. Lower levels of active GSK-3β would consequently lead to a decrease in Cyclin D1 phosphorylation, resulting in its nuclear accumulation in IHH-Fos. Since EGF-R is a known transcriptional target of AP-1[25-27], we tested the possibility that c-Fos overexpression increases the activation of the pathways downstream to EGF signaling. EGF-R activates both the PI3K and the mitogen-activated protein kinase cascades[35], two upstream activators of GSK-3β phosphorylation[24,36]. In support of an involvement of EGF-R signaling in GSK-3β inactivation and nuclear cylin D1 stabilization, we show that IHH-Fos display increased levels of expression of *EGF-R* mRNA and protein than IHH-C. Furthermore, blocking the activation of the EGF-R tyrosine kinase significantly accelerates the rate of Cyclin D1 degradation assessed in CHX experiments. Upregulated expression of EGF-R is a frequent finding in HCC[37-39], and increased EGF-R signaling has been associated with a poorer prognosis[40]. c-Fos is also frequently overexpressed in HCC tumoral tissues[13-15,41]. Our data, therefore, suggest that a causal relationship could exist between c-Fos and EGF-R overexpression in HCC.

Our finding of high levels of nuclear Cyclin D1 associated with c-Fos overexpression adds further support for a contributing effect of c-Fos on HCC development. Indeed, Cyclin D1 exit from the nucleus during S phase is essential for regulated cell division, and its retention in the nucleus is a cancer promoting or predisposing event[42]. Thus, expression of a Cyclin D1 mutant that cannot be phosphorylated by GSK-3β, and remains nuclear throughout the cell cycle is highly transforming and induces tumour growth in nude mice[43].

In accordance with previous reports[32,44], we also found that p27 protein levels were higher in c-Fos overexpressing cells. It is now well recognized that the family of p21/p27 proteins plays a dual role in cell cycle regulation. On one hand, they bind to cdk2 complexes and inhibit their kinase activities. On the other hand, they are able to promote the activation of Cyclin D1/cdk4-6 by complex stabilization, and by facilitating the nuclear import of these complexes, without inhibiting Cyclin D-associated kinase activity[45-48]. In our study, higher levels of p27 in IHH-Fos could, therefore, represent another mechanism contributing to the increase in nuclear levels of Cyclin D1, although the precise mechanisms linking c-Fos and p27 overexpression are currently unknown. Nevertheless, the mechanism is not at the level of transcription, as indicated by our quantitative PCR analysis (data not shown).

To conclude, our results clearly indicate a positive role for c-Fos in cell cycle regulation in hepatocytes. Importantly, we delineate a new mechanism by which c-Fos could contribute to hepatocarcinogenesis through stabilization of Cyclin D1 within the nucleus, evoking a new feature to c-Fos implication in HCC.

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COMMENTS

Background

Human hepatocellular carcinoma (HCC) is the fifth most common cancer in the world. Among the numerous genes potentially implicated in hepatocarcinogenesis, the proto-oncogene c-Fos, a member of activating protein 1 (AP-1) transcription factor is a good candidate. Apart from one study reporting a negative role for c-Fos in hepatocellular tumorigenesis, several papers rather support a positive role in this process. High expression levels of c-Fos were determined in tumor tissue compared to the adjacent non-tumor liver in human HCC. However, in different cell types or tissues, the role of c-Fos in cell proliferation and/or transformation remains controversial. This study was designed to determine whether c-Fos could contribute to hepatocarcinogenesis by increasing cell proliferation.

Research frontiers

The role of c-Fos on hepatocyte proliferation has never been studied in human cells, but only in murine hepatocytes. These cells had been immortalized and stably transfected by c-Fos using different techniques than those reported in the present study. The authors showed that c-Fos overexpression led to decreased hepatocyte proliferation. However, these results did not appear consistent with most studies suggesting a positive role for c-Fos in hepatocarcinogenesis.

Innovations and breakthroughs

This study shows for the first time that c-Fos deregulates hepatocyte proliferation by stabilizing Cyclin D1 in the nucleus which is a cancer promoting or predisposing event.

Applications

Strategies designed to suppress c-Fos expression in HCC could contribute reducing hepatocyte proliferation and thereby cancer development.

Terminology

Human immortalized hepatocytes are hepatocytes which have been transfected by SV40 T antigen, allowing them to proliferate when cultured contrary to normal hepatocytes. However these immortalized cells are not tumorigenic *in vitro* and *in vivo*.

Peer review

This is an interesting study. Authors investigated the effect of stable c-Fos overexpression on IHH proliferation.

REFERENCES

1 **Shaulian E**, Karin M. AP-1 as a regulator of cell life and death. *Nat Cell Biol* 2002; **4**: E131-E136
2 **Eferl R**, Wagner EF. AP-1: a double-edged sword in tumorigenesis. *Nat Rev Cancer* 2003; **3**: 859-868
3 **Angel P**, Karin M. The role of Jun, Fos and the AP-1 complex in cell-proliferation and transformation. *Biochim Biophys Acta* 1991; **1072**: 129-157
4 **Pai SR**, Bird RC. c-fos expression is required during all phases of the cell cycle during exponential cell proliferation. *Anticancer Res* 1994; **14**: 985-994
5 **Kovary K**, Bravo R. The jun and fos protein families are both required for cell cycle progression in fibroblasts. *Mol Cell Biol* 1991; **11**: 4466-4472
6 **Brusselbach S**, Mohle-Steinlein U, Wang ZQ, Schreiber M, Lucibello FC, Muller R, Wagner EF. Cell proliferation and cell cycle progression are not impaired in fibroblasts and ES cells lacking c-Fos. *Oncogene* 1995; **10**: 79-86
7 **Balsalobre A**, Jolicoeur P. Fos proteins can act as negative regulators of cell growth independently of the fos transforming pathway. *Oncogene* 1995; **11**: 455-465
8 **Mikula M**, Gotzmann J, Fischer AN, Wolschek MF, Thallinger C, Schulte-Hermann R, Beug H, Mikulits W. The proto-oncoprotein c-Fos negatively regulates hepatocellular tumorigenesis. *Oncogene* 2003; **22**: 6725-6738
9 **Miao GG**, Curran T. Cell transformation by c-fos requires an extended period of expression and is independent of the cell cycle. *Mol Cell Biol* 1994; **14**: 4295-4310
10 **Hennigan RF**, Hawker KL, Ozanne BW. Fos-transformation activates genes associated with invasion. *Oncogene* 1994; **9**: 3591-3600
11 **Grigoriadis AE**, Schellander K, Wang ZQ, Wagner EF. Osteoblasts are target cells for transformation in c-fos transgenic mice. *J Cell Biol* 1993; **122**: 685-701
12 **Wang ZQ**, Grigoriadis AE, Mohle-Steinlein U, Wagner EF. A novel target cell for c-fos-induced oncogenesis: development of chondrogenic tumours in embryonic stem cell chimeras. *EMBO J* 1991; **10**: 2437-2450
13 **Feng DY**, Zheng H, Tan Y, Cheng RX. Effect of phosphorylation of MAPK and Stat3 and expression of c-fos and c-jun proteins on hepatocarcinogenesis and their clinical significance. *World J Gastroenterol* 2001; **7**: 33-36
14 **Yuen MF**, Wu PC, Lai VC, Lau JY, Lai CL. Expression of c-Myc, c-Fos, and c-jun in hepatocellular carcinoma. *Cancer* 2001; **91**: 106-112
15 **Tabor E**. Tumor suppressor genes, growth factor genes, and oncogenes in hepatitis B virus-associated hepatocellular carcinoma. *J Med Virol* 1994; **42**: 357-365
16 **Masui T**, Nakanishi H, Inada K, Imai T, Mizoguchi Y, Yada H, Futakuchi M, Shirai T, Tatematsu M. Highly metastatic hepatocellular carcinomas induced in male F344 rats treated with N-nitrosomorpholine in combination with other hepatocarcinogens show a high incidence of p53 gene mutations along with altered mRNA expression of tumor-related genes. *Cancer Lett* 1997; **112**: 33-45
17 **Yao X**, Hu JF, Daniels M, Yien H, Lu H, Sharan H, Zhou X, Zeng Z, Li T, Yang Y, Hoffman AR. A novel orthotopic tumor model to study growth factors and oncogenes in hepatocarcinogenesis. *Clin Cancer Res* 2003; **9**: 2719-2726
18 **Borlak J**, Meier T, Halter R, Spanel R, Spanel-Borowski K. Epidermal growth factor-induced hepatocellular carcinoma: gene expression profiles in precursor lesions, early stage and solitary tumours. *Oncogene* 2005; **24**: 1809-1819
19 **Lee JS**, Heo J, Libbrecht L, Chu IS, Kaposi-Novak P, Calvisi DF, Mikaelyan A, Roberts LR, Demetris AJ, Sun Z, Nevens F, Roskams T, Thorgeirsson SS. A novel prognostic subtype of human hepatocellular carcinoma derived from hepatic progenitor cells. *Nat Med* 2006; **12**: 410-416
20 **Bakin AV**, Curran T. Role of DNA 5-methylcytosine transferase in cell transformation by fos. *Science* 1999; **283**: 387-390
21 **Saito Y**, Kanai Y, Nakagawa T, Sakamoto M, Saito H, Ishii H, Hirohashi S. Increased protein expression of DNA methyltransferase (DNMT) 1 is significantly correlated with the malignant potential and poor prognosis of human hepatocellular carcinomas. *Int J Cancer* 2003; **105**: 527-532
22 **Sadowski HB**, Gilman MZ. Cell-free activation of a DNA-binding protein by epidermal growth factor. *Nature* 1993; **362**: 79-83
23 **Diehl JA**, Cheng M, Roussel MF, Sherr CJ. Glycogen synthase kinase-3beta regulates cyclin D1 proteolysis and subcellular localization. *Genes Dev* 1998; **12**: 3499-3511
24 **Cohen P**, Frame S. The renaissance of GSK3. *Nat Rev Mol Cell Biol* 2001; **2**: 769-776
25 **Johnson AC**, Murphy BA, Matelis CM, Rubinstein Y, Piebenga EC, Akers LM, Neta G, Vinson C, Birrer M. Activator protein-1 mediates induced but not basal epidermal growth factor receptor gene expression. *Mol Med* 2000; **6**: 17-27
26 **Zenz R**, Scheuch H, Martin P, Frank C, Eferl R, Kenner L, Sibilia M, Wagner EF. c-Jun regulates eyelid closure and skin tumor development through EGFR signaling. *Dev Cell* 2003; **4**: 879-889
27 **Mialon A**, Sankinen M, Soderstrom H, Junttila TT, Holmstrom T, Koivusalo R, Papageorgiou AC, Johnson RS, Hietanen S, Elenius K, Westermarck J. DNA topoisomerase I

is a cofactor for c-Jun in the regulation of epidermal growth factor receptor expression and cancer cell proliferation. *Mol Cell Biol* 2005; **25**: 5040-5051

28 **Amicone L**, Spagnoli FM, Spath G, Giordano S, Tommasini C, Bernardini S, De Luca V, Della Rocca C, Weiss MC, Comoglio PM, Tripodi M. Transgenic expression in the liver of truncated Met blocks apoptosis and permits immortalization of hepatocytes. *EMBO J* 1997; **16**: 495-503

29 **Schuermann M**, Hennig G, Muller R. Transcriptional activation and transformation by chimaeric Fos-estrogen receptor proteins: altered properties as a consequence of gene fusion. *Oncogene* 1993; **8**: 2781-2790

30 **Brown JR**, Nigh E, Lee RJ, Ye H, Thompson MA, Saudou F, Pestell RG, Greenberg ME. Fos family members induce cell cycle entry by activating cyclin D1. *Mol Cell Biol* 1998; **18**: 5609-5619

31 **Sunters A**, McCluskey J, Grigoriadis AE. Control of cell cycle gene expression in bone development and during c-Fos-induced osteosarcoma formation. *Dev Genet* 1998; **22**: 386-397

32 **Sunters A**, Thomas DP, Yeudall WA, Grigoriadis AE. Accelerated cell cycle progression in osteoblasts overexpressing the c-fos proto-oncogene: induction of cyclin A and enhanced CDK2 activity. *J Biol Chem* 2004; **279**: 9882-9891

33 **Ramljak D**, Calvert RJ, Wiesenfeld PW, Diwan BA, Catipovic B, Marasas WF, Victor TC, Anderson LM, Gelderblom WC. A potential mechanism for fumonisin B(1)-mediated hepatocarcinogenesis: cyclin D1 stabilization associated with activation of Akt and inhibition of GSK-3beta activity. *Carcinogenesis* 2000; **21**: 1537-1546

34 **Diehl JA**, Zindy F, Sherr CJ. Inhibition of cyclin D1 phosphorylation on threonine-286 prevents its rapid degradation via the ubiquitin-proteasome pathway. *Genes Dev* 1997; **11**: 957-972

35 **Normanno N**, De Luca A, Bianco C, Strizzi L, Mancino M, Maiello MR, Carotenuto A, De Feo G, Caponigro F, Salomon DS. Epidermal growth factor receptor (EGFR) signaling in cancer. *Gene* 2006; **366**: 2-16

36 **Roux PP**, Shahbazian D, Vu H, Holz MK, Cohen MS, Taunton J, Sonenberg N, Blenis J. RAS/ERK signaling promotes site-specific ribosomal protein S6 phosphorylation via RSK and stimulates cap-dependent translation. *J Biol Chem* 2007; **282**: 14056-14064

37 **Nicholson RI**, Gee JM, Harper ME. EGFR and cancer prognosis. *Eur J Cancer* 2001; **37** Suppl 4: S9-S15

38 **Ito Y**, Takeda T, Sakon M, Tsujimoto M, Higashiyama S, Noda K, Miyoshi E, Monden M, Matsuura N. Expression and clinical significance of erb-B receptor family in hepatocellular carcinoma. *Br J Cancer* 2001; **84**: 1377-1383

39 **Breuhahn K**, Longerich T, Schirmacher P. Dysregulation of growth factor signaling in human hepatocellular carcinoma. *Oncogene* 2006; **25**: 3787-3800

40 **Daveau M**, Scotte M, Francois A, Coulouarn C, Ros G, Tallet Y, Hiron M, Hellot MF, Salier JP. Hepatocyte growth factor, transforming growth factor alpha, and their receptors as combined markers of prognosis in hepatocellular carcinoma. *Mol Carcinog* 2003; **36**: 130-141

41 **Moghaddam SJ**, Haghighi EN, Samiee S, Shahid N, Keramati AR, Dadgar S, Zali MR. Immunohistochemical analysis of p53, cyclinD1, RB1, c-fos and N-ras gene expression in hepatocellular carcinoma in Iran. *World J Gastroenterol* 2007; **13**: 588-593

42 **Gladden AB**, Diehl JA. Location, location, location: the role of cyclin D1 nuclear localization in cancer. *J Cell Biochem* 2005; **96**: 906-913

43 **Alt JR**, Cleveland JL, Hannink M, Diehl JA. Phosphorylation-dependent regulation of cyclin D1 nuclear export and cyclin D1-dependent cellular transformation. *Genes Dev* 2000; **14**: 3102-3114

44 **Kobayashi K**, Phuchareon J, Inada K, Tomita Y, Koizumi T, Hatano M, Miyatake S, Tokuhisa T. Overexpression of c-fos inhibits down-regulation of a cyclin-dependent kinase-2 inhibitor p27Kip1 in splenic B cells activated by surface Ig cross-linking. *J Immunol* 1997; **158**: 2050-2056

45 **LaBaer J**, Garrett MD, Stevenson LF, Slingerland JM, Sandhu C, Chou HS, Fattaey A, Harlow E. New functional activities for the p21 family of CDK inhibitors. *Genes Dev* 1997; **11**: 847-862

46 **Soos TJ**, Kiyokawa H, Yan JS, Rubin MS, Giordano A, DeBlasio A, Bottega S, Wong B, Mendelsohn J, Koff A. Formation of p27-CDK complexes during the human mitotic cell cycle. *Cell Growth Differ* 1996; **7**: 135-146

47 **Cheng M**, Olivier P, Diehl JA, Fero M, Roussel MF, Roberts JM, Sherr CJ. The p21(Cip1) and p27(Kip1) CDK 'inhibitors' are essential activators of cyclin D-dependent kinases in murine fibroblasts. *EMBO J* 1999; **18**: 1571-1583

48 **Sherr CJ**, Roberts JM. CDK inhibitors: positive and negative regulators of G1-phase progression. *Genes Dev* 1999; **13**: 1501-1512

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BASIC RESEARCH

Leptin transiently antagonizes ghrelin and long-lastingly orexin in regulation of Ca^{2+} signaling in neuropeptide Y neurons of the arcuate nucleus

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Abstract

AIM: To explore the mechanism for interactions of leptin with ghrelin and orexin in the arcuate nucleus (ARC) activating neuropeptide Y (NPY) neurons during physiological regulation of feeding.

METHODS: Single neurons from ARC of adult rats with matured feeding function were isolated. $[Ca^{2+}]_i$ was measured to monitore their activities. The time course of leptin effects on ghrelin-induced *versus* orexin-induced $[Ca^{2+}]_i$ increases in NPY neurons was studied.

RESULTS: Administration of ghrelin or orexin-A at 10^{-10} mol/L increased cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) in NPY neurons isolated from the ARC of adult rats. Upon administration of leptin at 10^{-14}-10^{-12} mol/L, ghrelin-induced $[Ca^{2+}]_i$ increases were initially (< 10 min) inhibited but later restored, exhibiting a transient pattern of inhibition. In contrast, orexin-induced $[Ca^{2+}]_i$ increases were inhibited by leptin in a long-lasting manner. Furthermore, a prior administration of leptin inhibited orexin action but not ghrelin action to increase $[Ca^{2+}]_i$.

CONCLUSION: Leptin counteracted ghrelin effects transiently and orexin effects long-lastingly in NPY neurons. The transient property with which leptin counteracts ghrelin action in NPY neurons may allow the fasting-associated increase in ghrelin levels to activate NPY neurons in the presence of physiological leptin and to stimulate feeding.

Key words: Leptin; Ghrelin; Orexin; Arcuate nucleus; Neuropeptide Y; Ca^{2+}; Feeding; Phosphatidylinositol 3-kinase; Phosphodiesterase 3; Signal transducer and activator of transcription 3

Kohno D, Suyama S, Yada T. Leptin transiently antagonizes ghrelin and long-lastingly orexin in regulation of Ca^{2+} signaling in neuropeptide Y neurons of the arcuate nucleus. *World J Gastroenterol* 2008; 14(41): 6347-6354 Available from: URL: http://www.wjgnet.com/1007-9327/14/6347.asp DOI: http://dx.doi.org/10.3748/wjg.14.6347

INTRODUCTION

Food intake is controlled by the feeding regulatory centers, in which the arcuate nucleus (ARC) in the hypothalamus is considered the first order center that senses and integrates a variety of central and peripheral factors[1]. In the ARC, neuropeptide Y (NPY) neurons that coexpress agouti-related peptide (AgRP) are mandatory for feeding[2,3], while proopiomelanocortin (POMC) neurons are essential for satiety[1]. Orexin-A and -B (hypocretin-1 and -2) are orexigenic peptides[4] localized in neurons in the lateral hypothalamus (LH)[5], an area implicated in feeding behavior[6]. Fasting and lowering glucose concentrations increase prepro-orexin mRNA level[4] and cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) in orexin neurons[7], suggesting a possible physiological role of orexins in feeding. Ghrelin is an orexigenic peptide released predominantly from the stomach[8-10] and also from the intestine and pancreas[11-13]. Ghrelin is the only one orexigenic peptide of the peripheral origin known. The plasma concentration of ghrelin increases before meals and rapidly declines upon food intake[14]. Orexin levels might also change during a day,

since this peptide is implicated in regulation of sleep/wakefulness[15]. These rhythmic changes in ghrelin and orexin levels may alter their inputs on the feeding center and thereby regulate feeding. Both ghrelin[9,16-17] and orexin[18] stimulate food intake primarily by activating NPY neurons in the ARC.

Leptin, a powerful anorectic hormone produced and released from the adipocytes, is present constantly in the plasma at the nanomolar range[14] and is considered to enter the brain through the blood-brain barrier[19]. Therefore, leptin is likely to act continuously on the feeding center and thereby modulate the efficacy of orexigenic substances. The primary action of leptin in the feeding center is inhibition of NPY neurons as well as activation of POMC neurons in the ARC. The obesity syndrome in ob/ob mice resulting from lack of functional leptin is attenuated by the loss of neuropeptide Y[20]. Therefore, elucidation of the interaction of leptin with ghrelin and with orexin in the ARC NPY neurons may provide a clue to understand the neuronal mechanisms for physiological regulation of feeding. It has recently been shown that leptin counteracts ghrelin action to increase cytosolic free $[Ca^{2+}]_i$ in NPY neurons in the ARC, and that the PI3-kinase (phosphatidylinositol 3-kinase)-PDE3 (phosphodiesterase 3) signaling plays a key role in the leptin action[17]. In the present study, we isolated single neurons from ARC of adult rats with matured feeding function and monitored their activities by measuring $[Ca^{2+}]_i$. We studied the time course of leptin effects on ghrelin-induced *vs* orexin-induced $[Ca^{2+}]_i$ increases in NPY neurons.

MATERIALS AND METHODS

Animals and preparation of single neurons from the ARC of adult rats

Adult male Sprague-Dawley (SD) rats were maintained on a 12-h light/dark cycle and given conventional food and water ad libitum. The ARC was isolated from the brain of 5-8-wk-old SD rats and single neurons were prepared according to the procedures reported previously[16,21] with slight modifications. Briefly, rats were anaesthetized with an intraperitoneal injection of carbamic acid ethyl ester (900 mg/kg) and decapitated, and their brains were removed. Brain slices containing the ARC were prepared and the whole ARC of the left and right sides was cut out. The dissected tissues were washed with 10 mmol/L HEPES-buffered Krebs-Ringer bicarbonate buffer (HKRB) containing 10 mmol/L glucose. Then they were incubated in HKRB supplemented with 20 U/mL papain (Sigma Chemical Co., St. Louis, MO), 0.015 mg/mL deoxyribonuclease, 0.75 mg/mL bovine serum albumin and 1mmol/L cysteine for 15 min at 36℃ in a shaking water bath, followed by gentle mechanical trituration for 5-10 min. After trituration, the cell suspension was centrifuged at 100 × *g* for 5 min. The pellet was resuspended in HKRB and distributed on coverslips. The cells were kept at 20℃ in moisture-saturated dishes for up to 10 h. The animal protocols were approved by the Jichi Medical School Institute of Animal Care and Use Committee.

Measurements of $[Ca^{2+}]_i$ in single neurons of the ARC

At 2 to 10 h after cell preparation, $[Ca^{2+}]_i$ was measured by ratiometric fura-2 microfluorometry in combination with digital imaging as previously reported[16,21]. Briefly, following incubating with 2 μmol/L fura-2-AM for 30 min at room temperature, the cells were mounted in a chamber and superfused with HKRB at 1 mL/min at 34℃. Fluorescence images due to excitation at 340 nm and 380 nm were detected every 8.0 s with an intensified charge-coupled device (ICCD) camera, and the ratio image was produced by an Argus-50 system (Hamamatsu Photonics Co., Hamamatsu, Japan). Ratio values were converted to $[Ca^{2+}]_i$ according to calibration curves. Data were taken from the cells identified as neurons by the procedures reported previously[16,21].

Post-$[Ca^{2+}]_i$ imaging immunocytochemistry and identification of NPY neurons

Neurochemical identification of the neurons that exhibited $[Ca^{2+}]_i$ responses were performed according to the original method[22] with slight modification[16]. Briefly, the cells were fixed with 4% paraformaldehyde over night. They were blocked in 10% normal goat serum (NGS) and in 0.1 mol/L PBS for 1 h at room temperature. Primary antiserum against NPY (DiaSorin, Stillwater, MN) was diluted 1:1000 in PBS containing 1.5% NGS and was incubated 24 h at 4℃. The antiserum were then rinsed and incubated with biotinylated secondary antibody raised against rabbit IgG (Vector Laboratories Inc., Burlingame, CA; diluted at 1:400) for 1 h at room temperature. The secondary antibody was rinsed, and the sections were labeled with avidin-peroxidase complex (ABC kit, Vector) for 1 h and color-developed with 3,3'-diaminobenzidine (DAB). Control experiments were carried out by omitting the primary antiserum.

To correlate $[Ca^{2+}]_i$ and immunocytochemical data, photographs of all the cells in the microscopic field subjected to $[Ca^{2+}]_i$ measurements were taken at the end of $[Ca^{2+}]_i$ imaging. Based on these photographs, the cells in which $[Ca^{2+}]_i$ was recorded were correlated with their corresponding immunocytochemical results.

Criteria for $[Ca^{2+}]_i$ responses and determination of response amplitude

Ghrelin, orexin-A and leptin were administered to the superfusion solution. Amplitudes of $[Ca^{2+}]_i$ increases in response to agents were calculated by subtracting pre-stimulatory basal $[Ca^{2+}]_i$ levels from peak $[Ca^{2+}]_i$ levels. When increases in $[Ca^{2+}]_i$ took place within 5 min after addition of agents and their amplitudes were 150 nmol/L or larger, they were considered responses. Suppression by leptin was judged by the following criteria. In Figures 1 and 2, when the peak amplitude of ghrelin-induced $[Ca^{2+}]_i$ increase was decreased to a level of 40%

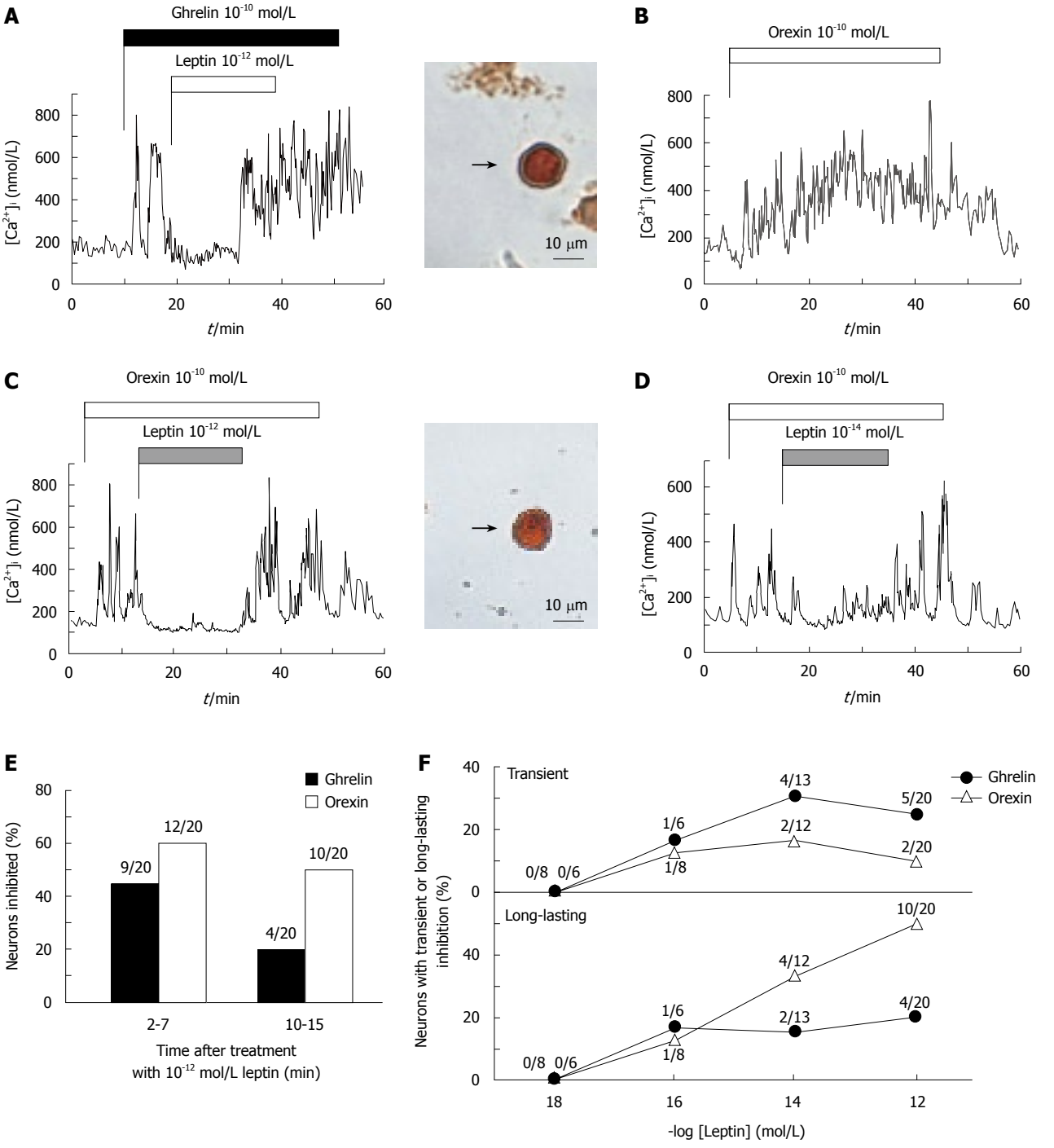

Figure 1 Ghrelin- and orexin-induced $[Ca^{2+}]_i$ increases were suppressed by leptin in a transient or long-lasting manner in NPY neurons. A-D: Ghrelin and orexin-A at 10^{-10} mol/L increased $[Ca^{2+}]_i$ in single neurons isolated from the ARC, and these $[Ca^{2+}]_i$ increases were suppressed by administration of leptin at 10^{-12} mol/L (A, C) and 10^{-14} mol/L (D). Following $[Ca^{2+}]_i$ measurements, the neurons in (A) and (C) were proved to contain NPY by immunocytochemistry using anti-NPY antibody (A, C; right panels). The bars indicate 10 μm. E: Leptin inhibited ghrelin-induced $[Ca^{2+}]_i$ increases in a greater number of neurons in 2-7 min after administration than in 10-15 min. In contrast, leptin inhibited orexin-A-induced $[Ca^{2+}]_i$ increases in the majority of neurons in 2-7 min and 10-15 min after administration. The numbers above the bars indicate the number of neurons inhibited by leptin over that responded to ghrelin or orexin-A. F: The number of neurons whose $[Ca^{2+}]_i$ responses to ghrelin or orexin-A were suppressed by leptin in either a transient or long-lasting manner is expressed by percentage. The numbers above the points indicate the number of neurons inhibited by leptin in the specified manner over that responded to ghrelin or orexin-A.

or smaller for at least 5 min and the recovery of $[Ca^{2+}]_i$ increase was observed after washing out leptin, it was considered inhibition. In Figure 3, repetitive additions of ghrelin or orexin-A twice induced repeated $[Ca^{2+}]_i$ increases, and the second challenge to ghrelin or orexin-A was performed in the presence of leptin. When the amplitude of the $[Ca^{2+}]_i$ response to the second addition was less than 150 nmol/L, it was considered inhibition.

Solutions and chemicals

The measurements were carried out in HKRB solution composed of 129 mmol/L NaCl, 5.0 mmol/L $NaHCO_3$,

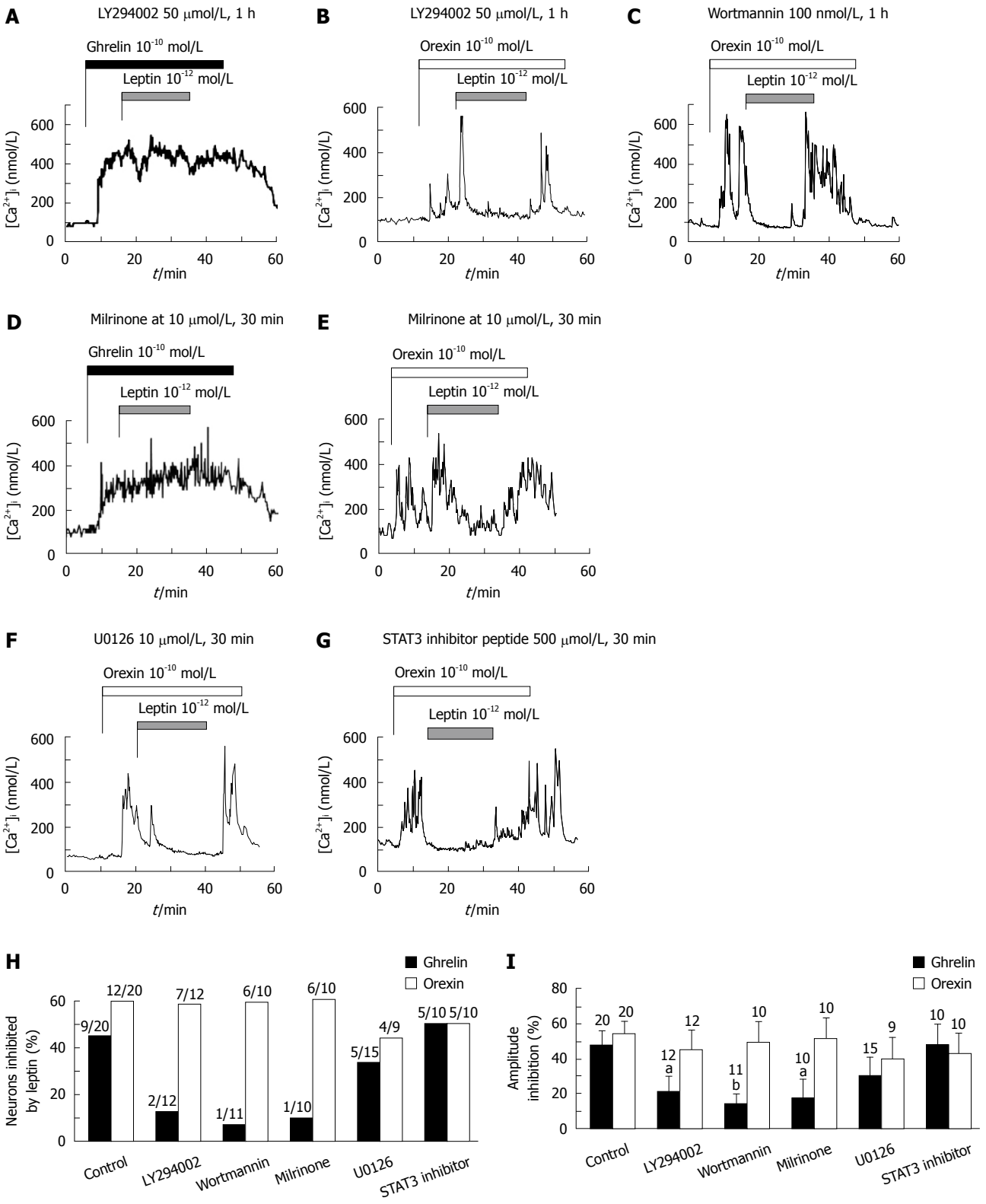

Figure 2 Leptin suppresses ghrelin-induced and orexin-induced $[Ca^{2+}]_i$ increases *via* different signaling pathways in NPY neurons. A-G: Effects of inhibitors for signaling molecules on the leptin action against ghrelin and orexin. Preincubation for 1 h with 50 μmol/L LY294002, an inhibitor for PI3-kinase, interfered with leptin action to suppress $[Ca^{2+}]_i$ responses to ghrelin (A), but not orexin (B). Preincubation for 1 h with 100 nmol/L wortmannin, another PI3-kinase inhibitor, little affected the leptin suppression of orexin-induced $[Ca^{2+}]_i$ increases (C). Preincubation for 30 min with 10 μmol/L milrinone, a PDE3 inhibitor, blocked the leptin action to suppress $[Ca^{2+}]_i$ responses to ghrelin (D), but not orexin (E). Preincubation for 30 min with 10 μmol/L U0126, an inhibitor for MAP-kinase, little affected the leptin action to suppress orexin-induced $[Ca^{2+}]_i$ increases (F). Preincubation for 30 min with a STAT3 inhibitor peptide at 500 μmol/L, did not significantly alter the leptin suppression of orexin-induced $[Ca^{2+}]_i$ increases (G). H: The number of neurons whose $[Ca^{2+}]_i$ responses to ghrelin or orexin were suppressed by leptin in the presence of inhibitors is expressed by percentage. The numbers above the bars indicate the number of neurons inhibited by leptin over that responded to ghrelin or orexin-A. I: Reduction of amplitudes of $[Ca^{2+}]_i$ responses to ghrelin or orexin-A by leptin is expressed by percentage. $^aP < 0.05$, $^bP < 0.01$ *vs* control. The data regarding ghrelin in H and I were in part taken from our previous paper[17].

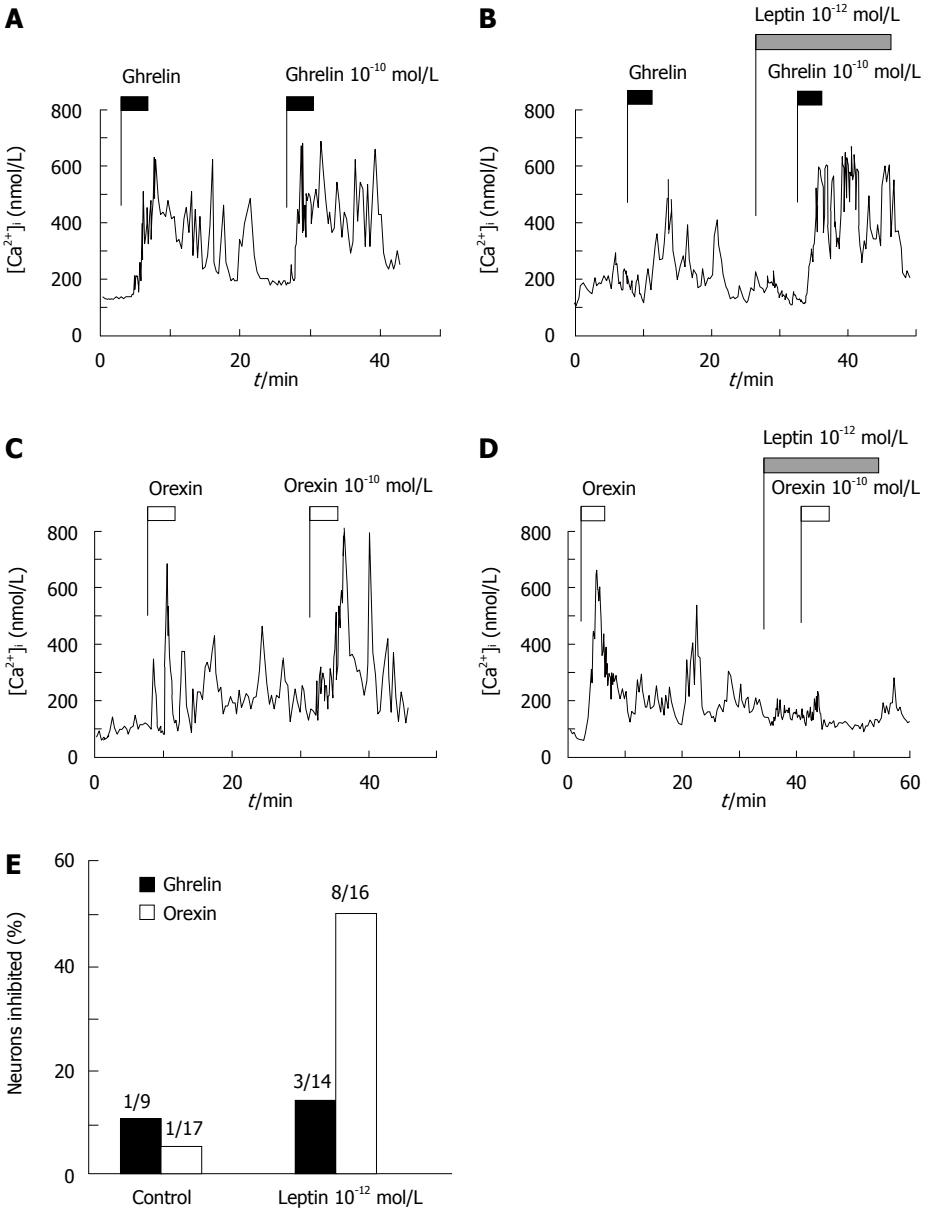

Figure 3 Prior treatment with leptin suppressed $[Ca^{2+}]_i$ responses to subsequent administration of orexin, but not ghrelin, in NPY neurons. A, C: Repeated administrations of ghrelin or orexin-A at 10^{-10} mol/L increased $[Ca^{2+}]_i$ twice in single ARC neurons; B, D: Prior administration of leptin failed to inhibit the effect of ghrelin added subsequently (B), but inhibited the effect of orexin-A (D); E: The number of neurons in which $[Ca^{2+}]_i$ responses to the second ghrelin or orexin addition were suppressed by prior administration of leptin or HKRB (Control) is expressed by percentage. The numbers above the bars indicate the number of neurons whose second responses were inhibited over that exhibited first responses to ghrelin or orexin-A.

4.7 mmol/L KCl, 1.2 mmol/L KH_2PO_4, 1.8 mmol/L $CaCl_2$, 1.2 mmol/L $MgSO_4$, and 10 mmol/L N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) at pH 7.4. Fura 2-acetoxymethylester was obtained from Dojin Chemical (Kumamoto, Japan). Ghrelin and orexin-A were obtained from Peptide Institute, Inc. (Osaka, Japan), Leptin was from R&D Systems (Mineapolis, MN).

Data presentation and statistical analysis

The data are presented as the mean ± SE (*n*: number of neurons). Each study was based on at least 7 neurons prepared from at least 3 rats. Student's paired or unpaired *t*-test was used to evaluate differences and values of $P < 0.05$ were considered to be significant.

RESULTS

Leptin inhibits ghrelin- and orexin-A-induced $[Ca^{2+}]_i$ increases in NPY neurons

Single neurons isolated from the ARC were superfused with HKRB and subjected to measurements of $[Ca^{2+}]_i$ with fura-2 fluorescence imaging. Administration of ghrelin or orexin-A at 10^{-10} mol/L for 40-50 min into superfusion solutions increased $[Ca^{2+}]_i$ in a continuous manner (Figure 1A and B) as reported previously[16-18]. The $[Ca^{2+}]_i$ increases in response to ghrelin and orexin took place in an oscillatory manner. The peaks of $[Ca^{2+}]_i$ responses to ghrelin [486 ± 49 nmol/L (n = 33)] and orexin-A [433 ± 47 nmol/L (n = 27)] were significantly ($P < 0.001$) higher than the corresponding basal $[Ca^{2+}]_i$ levels prior to administration of the peptides [107 ± 12 nmol/L (n = 33) for ghrelin; 110 ± 10 nmol/L (n = 27) for orexin].

Both ghrelin-induced (Figure 1A) and orexin-induced $[Ca^{2+}]_i$ increases (Figure 1C) were inhibited by administration of 10^{-12} mol/L leptin in the ARC neurons, which were subsequently proven to be immunoreactive to NPY (Figure 1A and C, right panels). The results that leptin counteracts ghrelin and orexin actions on $[Ca^{2+}]_i$ in the ARC NPY neurons confirm previous reports[16-18].

Leptin inhibits ghrelin-induced $[Ca^{2+}]_i$ increases transiently and orexin-A-induced $[Ca^{2+}]_i$ increases long-lastingly in NPY neurons

Typical results of the effects of leptin on $[Ca^{2+}]_i$ responses to ghrelin and orexin-A are shown in Figure 1; leptin at 10^{-12} mol/L inhibited ghrelin-induced $[Ca^{2+}]_i$ increases in a transient manner (Figure 1A) and orexin-induced $[Ca^{2+}]_i$ increases in a longer-lasting manner (Figure 1C) during the 20 min period of leptin administration. Among 20 neurons that exhibited $[Ca^{2+}]_i$ responses to ghrelin, administration of 10^{-12} mol/L leptin inhibited $[Ca^{2+}]_i$ increases in 9 neurons (45%) during the earlier 2-7 min of leptin treatment, but only in 4 neurons (20%) later in the 10-15 min period of treatment (Figure 1E), showing attenuation of the counteracting effect of leptin for ghrelin in the later period (Figure 1F). In contrast, among 20 neurons that exhibited $[Ca^{2+}]_i$ responses to orexin, administration of 10^{-12} mol/L leptin inhibited $[Ca^{2+}]_i$ increases in 12 neurons (60%) during the 2-7 min of leptin treatment, and in 10 neurons (50%) in the 10-15 min period of treatment (Figure 1E), showing a long-lasting counteracting effect of leptin (Figure 1F). The long-lasting effect was also evoked by leptin at a lower concentration of 10^{-14} mol/L (Figure 1D). Leptin at both 10^{-14} mol/L and 10^{-12} mol/L counteracted ghrelin-induced $[Ca^{2+}]_i$ increases predominantly in a transient manner and orexin-induced $[Ca^{2+}]_i$ increases mainly in a long-lasting manner (Figure 1F).

Pretreatment with leptin inhibited orexin-induced, but not ghrelin-induced, $[Ca^{2+}]_i$ increases in NPY neurons

The data that leptin inhibited ghrelin-induced $[Ca^{2+}]_i$ increases transiently prompted us to hypothesize that efficacy of leptin is attenuated by time. Therefore, we examined whether prior administration of leptin is less effective in counteracting ghrelin action. Repetitive additions of ghrelin or orexin-A twice induced repeated $[Ca^{2+}]_i$ increases twice in a similar manner (Figure 3A and C). Following infusion of leptin that had started 8 min in advance, the addition of ghrelin induced $[Ca^{2+}]_i$ increases with amplitudes comparable to those in the control without leptin (Figure 3B), and the similar result was observed in the majority of neurons (Figure 3E). This result indicates a marked attenuation of inhibitory ability of leptin by time. In contrast, infusion of leptin that started 8 min in advance inhibited $[Ca^{2+}]_i$ responses to the addition of orexin-A in 8 of 16 orexin-responsive neurons (50%) (Figure 3D and E). This incidence of the inhibition by leptin administered in prior to orexin was comparable to the inhibition by leptin administered after orexin (12 of 20 neurons, 60%) (Figure 1E). These data indicate that the ability of leptin to counteract orexin action is well preserved without appreciable attenuation.

We next examined whether the difference in the time dependence of leptin action on ghrelin-*vs* orexin-induced $[Ca^{2+}]_i$ increases could involve different leptin signaling mechanisms in NPY neurons. Leptin is linked to several signaling pathways, which include phosphatidylinositol 3 (PI3)-kinase and, its downstream effecter phosphodiesterase 3 (PDE3)[23], signal transducer and activator of transcription 3 (STAT3)[24], and mitogen-activated protein (MAP)-kinase[25]. We have previously shown that leptin suppresses ghrelin-induced $[Ca^{2+}]_i$ increases *via* PI3-kinase- and PDE3-, but not MAP-kinase- and STAT3-, mediated pathway[17]. Therefore, whether these signaling mechanisms could be involved in the leptin action to counteract orexin-induced $[Ca^{2+}]_i$ increases was examined. Pretreatment with inhibitors for PI3-kinase, LY294002 (Figure 2A) or wortmannin (data not shown), blocked the leptin action to suppress $[Ca^{2+}]_i$ responses to ghrelin in both the response incidence (Figure 2H) and response amplitude (Figure 2I). Likewise, pretreatment with an inhibitor for PDE3, milrinone, blocked the leptin action against ghrelin (Figure 2D, H and I). These results confirm previous report[17]. In contrast, LY294002 (Figure 2B), wortmannin (Figure 2C) and milrinone (Figure 2E) failed to significantly affect the leptin suppression of orexin-induced $[Ca^{2+}]_i$ increases in both the response incidence and amplitude (Figure 2H and I). Furthermore, pretreatment with a MAP kinase inhibitor U0126 (Figure 2F) or a STAT3 inhibitor peptide (Figure 2G) little altered the leptin ability to inhibit $[Ca^{2+}]_i$ responses to orexin in both the response incidence and amplitude (Figure 2H and I), the results similar to those reported for ghrelin-induced $[Ca^{2+}]_i$ increases[17].

DISCUSSION

The present data indicate that leptin inhibits ghrelin-induced $[Ca^{2+}]_i$ increases in a transient manner and orexin-induced $[Ca^{2+}]_i$ increases in a long-lasting manner. The transient action of leptin to inhibit ghrelin-induced $[Ca^{2+}]_i$ increases is not due to insufficient concentration of leptin, since leptin at a lower concentration of 10^{-14} mol/L is already maximal in counteracting the ghrelin effect[17] and more specifically in exhibiting the transient inhibitory property (Figure 1F). Furthermore, the transient property for leptin inhibition of ghrelin-induced $[Ca^{2+}]_i$ increases is neither due to excessive concentration of ghrelin, since the ghrelin concentration of 10^{-10} mol/L used in the present study is close to a maximal, but never super-maximal concentration in activating NPY neurons[16]. Therefore, the transient manner with which leptin counteracts ghrelin action reflects the intrinsic property of interaction between leptin and ghrelin.

The present study clearly indicated that the leptin signaling underlying the inhibition of $[Ca^{2+}]_i$ responses to orexin-A in NPY neurons is distinct from that to ghrelin. The transient action of leptin to inhibit ghrelin-induced $[Ca^{2+}]_i$ increases in NPY neurons may be mediated by the leptin signaling *via* PI3-kinase and PDE3, since the inhibitors for these enzymes block the leptin action (Figure 2A and D)[17]. The ghrelin signaling *via* the cAMP system could be the target for this leptin signaling[17]. On the other hand, the long-lasting action of leptin to counteract orexin-induced $[Ca^{2+}]_i$ increases

was not affected by inhibitors for PI3-kinase, PDE3, MAP-kinase and STAT3, well known leptin signaling molecules. This result suggests that the long-lasting counteracting action of leptin for orexin is mediated by a yet unidentified leptin signaling, which may long-lastingly inhibit the orexin-stimulated PLC-PKC-IP3 pathway reported previously[18], though further study is definitely needed to elucidate the signaling interaction between leptin and orexin.

The transient nature with which leptin counteracts the ghrelin action on NPY neurons may serve as the neuronal mechanism that allows fasting-associated increases in ghrelin levels to activate the ARC NPY neurons in the continuous presence of leptin[14] and thereby stimulate feeding.

Both ghrelin and orexin have been suggested to be concerned with obesity and type 2 diabetes[26,27]. Based on the present results, leptin resistance could alter the sensitivity to ghrelin and orexin in the ARC NPY neurons, which may alter the energy metabolism and thereby influence the pathogenesis of obesity and type 2 diabetes.

COMMENTS

Background

Ghrelin and orexins are potent orexigenic peptides working primarily via activating neuropeptide Y (NPY) neurons in the arcuate nucleus (ARC). NPY neurons in the ARC also serve as a major target for the anorexigenic leptin. Therefore, interactions of leptin with ghrelin and orexin in the ARC NPY neurons may play a key role in physiological regulation of feeding.

Research frontiers

The authors study the time course of leptin effects on ghrelin-induced *vs* orexin-induced $[Ca^{2+}]_i$ increases in NPY neurons.

Innovations and breakthroughs

The study clearly indicated that the leptin signaling underlying the inhibition of $[Ca^{2+}]_i$ responses to orexin-A in NPY neurons is distinct from that to ghrelin. The transient action of leptin to inhibit ghrelin-induced $[Ca^{2+}]_i$ increases in NPY neurons may be mediated by the leptin signaling *via* PI3-kinase and PDE3, The ghrelin signaling *via* the cAMP system could be the target for this leptin signaling.

Applications

The transient nature with which leptin counteracts the ghrelin action on NPY neurons may serve as the neuronal mechanism that allows fasting-associated increases in ghrelin levels to activate the ARC NPY neurons in the continuous presence of leptin[14] and thereby stimulate feeding.

Peer review

A well designed, in-depth paper about the time course of leptin effects on ghrelin-induced *vs* orexin-induced $[Ca^{2+}]_i$ increases in NPY neurons.

REFERENCES

1 **Schwartz MW**, Woods SC, Porte D Jr, Seeley RJ, Baskin DG. Central nervous system control of food intake. *Nature* 2000; **404**: 661-671

2 **Gropp E**, Shanabrough M, Borok E, Xu AW, Janoschek R, Buch T, Plum L, Balthasar N, Hampel B, Waisman A, Barsh GS, Horvath TL, Bruning JC. Agouti-related peptide-expressing neurons are mandatory for feeding. *Nat Neurosci* 2005; **8**: 1289-1291

3 **Luquet S**, Perez FA, Hnasko TS, Palmiter RD. NPY/AgRP neurons are essential for feeding in adult mice but can be ablated in neonates. *Science* 2005; **310**: 683-685

4 **Sakurai T**, Amemiya A, Ishii M, Matsuzaki I, Chemelli RM, Tanaka H, Williams SC, Richardson JA, Kozlowski GP, Wilson S, Arch JR, Buckingham RE, Haynes AC, Carr SA, Annan RS, McNulty DE, Liu WS, Terrett JA, Elshourbagy NA, Bergsma DJ, Yanagisawa M. Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. *Cell* 1998; **92**: 573-585

5 **Date Y**, Ueta Y, Yamashita H, Yamaguchi H, Matsukura S, Kangawa K, Sakurai T, Yanagisawa M, Nakazato M. Orexins, orexigenic hypothalamic peptides, interact with autonomic, neuroendocrine and neuroregulatory systems. *Proc Natl Acad Sci USA* 1999; **96**: 748-753

6 **Oomura Y**, Ooyama H, Sugimori M, Nakamura T, Yamada Y. Glucose inhibition of the glucose-sensitive neurone in the rat lateral hypothalamus. *Nature* 1974; **247**: 284-286

7 **Muroya S**, Uramura K, Sakurai T, Takigawa M, Yada T. Lowering glucose concentrations increases cytosolic Ca2+ in orexin neurons of the rat lateral hypothalamus. *Neurosci Lett* 2001; **309**: 165-168

8 **Kojima M**, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature* 1999; **402**: 656-660

9 **Nakazato M**, Murakami N, Date Y, Kojima M, Matsuo H, Kangawa K, Matsukura S. A role for ghrelin in the central regulation of feeding. *Nature* 2001; **409**: 194-198

10 **Asakawa A**, Inui A, Kaga T, Katsuura G, Fujimiya M, Fujino MA, Kasuga M. Antagonism of ghrelin receptor reduces food intake and body weight gain in mice. *Gut* 2003; **52**: 947-952

11 **Date Y**, Kojima M, Hosoda H, Sawaguchi A, Mondal MS, Suganuma T, Matsukura S, Kangawa K, Nakazato M. Ghrelin, a novel growth hormone-releasing acylated peptide, is synthesized in a distinct endocrine cell type in the gastrointestinal tracts of rats and humans. *Endocrinology* 2000; **141**: 4255-4261

12 **Date Y**, Nakazato M, Hashiguchi S, Dezaki K, Mondal MS, Hosoda H, Kojima M, Kangawa K, Arima T, Matsuo H, Yada T, Matsukura S. Ghrelin is present in pancreatic alpha-cells of humans and rats and stimulates insulin secretion. *Diabetes* 2002; **51**: 124-129

13 **Dezaki K**, Hosoda H, Kakei M, Hashiguchi S, Watanabe M, Kangawa K, Yada T. Endogenous ghrelin in pancreatic islets restricts insulin release by attenuating Ca2+ signaling in beta-cells: implication in the glycemic control in rodents. *Diabetes* 2004; **53**: 3142-3151

14 **Cummings DE**, Purnell JQ, Frayo RS, Schmidova K, Wisse BE, Weigle DS. A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. *Diabetes* 2001; **50**: 1714-1719

15 **Chemelli RM**, Willie JT, Sinton CM, Elmquist JK, Scammell T, Lee C, Richardson JA, Williams SC, Xiong Y, Kisanuki Y, Fitch TE, Nakazato M, Hammer RE, Saper CB, Yanagisawa M. Narcolepsy in orexin knockout mice: molecular genetics of sleep regulation. *Cell* 1999; **98**: 437-451

16 **Kohno D**, Gao HZ, Muroya S, Kikuyama S, Yada T. Ghrelin directly interacts with neuropeptide-Y-containing neurons in the rat arcuate nucleus: Ca2+ signaling via protein kinase A and N-type channel-dependent mechanisms and cross-talk with leptin and orexin. *Diabetes* 2003; **52**: 948-956

17 **Kohno D**, Nakata M, Maekawa F, Fujiwara K, Maejima Y, Kuramochi M, Shimazaki T, Okano H, Onaka T, Yada T. Leptin suppresses ghrelin-induced activation of neuropeptide Y neurons in the arcuate nucleus via phosphatidylinositol 3-kinase- and phosphodiesterase 3-mediated pathway. *Endocrinology* 2007; **148**: 2251-2263

18 **Muroya S**, Funahashi H, Yamanaka A, Kohno D, Uramura K, Nambu T, Shibahara M, Kuramochi M, Takigawa M, Yanagisawa M, Sakurai T, Shioda S, Yada T. Orexins (hypocretins) directly interact with neuropeptide Y, POMC and glucose-responsive neurons to regulate Ca 2+ signaling in a reciprocal manner to leptin: orexigenic neuronal pathways in the mediobasal hypothalamus. *Eur J Neurosci* 2004; **19**: 1524-1534

19 **Banks WA**, Kastin AJ, Huang W, Jaspan JB, Maness LM. Leptin enters the brain by a saturable system independent

of insulin. *Peptides* 1996; **17**: 305-311
20 **Erickson JC**, Hollopeter G, Palmiter RD. Attenuation of the obesity syndrome of ob/ob mice by the loss of neuropeptide Y. *Science* 1996; **274**: 1704-1707
21 **Muroya S**, Yada T, Shioda S, Takigawa M. Glucose-sensitive neurons in the rat arcuate nucleus contain neuropeptide Y. *Neurosci Lett* 1999; **264**: 113-116
22 **Yada T**, Vigh S, Arimura A. Pituitary adenylate cyclase activating polypeptide (PACAP) increases cytosolic-free calcium concentration in folliculo-stellate cells and somatotropes of rat pituitary. *Peptides* 1993; **14**: 235-239
23 **Zhao AZ**, Huan JN, Gupta S, Pal R, Sahu A. A phosphatidylinositol 3-kinase phosphodiesterase 3B-cyclic AMP pathway in hypothalamic action of leptin on feeding. *Nat Neurosci* 2002; **5**: 727-728
24 **Gao Q**, Wolfgang MJ, Neschen S, Morino K, Horvath TL, Shulman GI, Fu XY. Disruption of neural signal transducer and activator of transcription 3 causes obesity, diabetes, infertility, and thermal dysregulation. *Proc Natl Acad Sci USA* 2004; **101**: 4661-4666
25 **Benomar Y**, Roy AF, Aubourg A, Djiane J, Taouis M. Cross down-regulation of leptin and insulin receptor expression and signalling in a human neuronal cell line. *Biochem J* 2005; **388**: 929-939
26 **Yada T**, Dezaki K, Sone H, Koizumi M, Damdindorj B, Nakata M, Kakei M. Ghrelin regulates insulin release and glycemia: physiological role and therapeutic potential. *Curr Diabetes Rev* 2008; **4**: 18-23
27 **Tsuneki H**, Sugihara Y, Honda R, Wada T, Sasaoka T, Kimura I. Reduction of blood glucose level by orexins in fasting normal and streptozotocin-diabetic mice. *Eur J Pharmacol* 2002; **448**: 245-252

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BASIC RESEARCH

Metabolism for cyclosporin A during liver regeneration after partial hepatectomy in rats

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Abstract

AIM: To elucidate the metabolism and the effect of the cyclosporin A (CyA) as a representative immunosuppressive drug used in transplantation in a partially hepatectomized rat model.

METHODS: CyA was administered to rats that underwent a 70% hepatectomy. These rats were randomly assigned into three groups according to the dose of CyA administration as follows; (group 1) water, (group 2) 5 mg/kg CyA, (group 3) 10 mg/kg CyA. On postoperative days-1, 3, 7 and 14, the rats were killed to analyze the serum concentration of CyA, the liver regeneration ratio, biochemical or histological markers, and mRNA expression using reverse transcriptase-polymerase chain reaction method to determine albumin and cytochrome p450 expression.

RESULTS: The serum concentration of CyA in group 3 was significantly higher than group 2 during liver regeneration. CyA enhanced the liver regeneration in a dose dependent manner. The mRNA expression associated with CyA metabolism was significantly decreased on day 14, while preserving the albumin producing activity.

CONCLUSION: These data indicate that the p-450 activity required to metabolize the CyA may be reduced during regeneration of the remnant liver after a hepatectomy, which may, therefore, be linked to difficulty in controlling the optimal dose of CyA during early period of LDLT.

Key words: Cyclosporin A; Liver regeneration; Partial hepatectomy; Rat

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Nagayoshi S, Kawashita Y, Eguchi S, Kamohara Y, Takatsuki M, Miyamoto S, Mochizuki S, Soyama A, Tokai H, Hidaka M, Tajima Y, Kanematsu T. Metabolism for cyclosporin A during liver regeneration after partial hepatectomy in rats. *World J Gastroenterol* 2008; 14(41): 6355-6359 Available from: URL: http://www.wjgnet.com/1007-9327/14/6355.asp DOI: http://dx.doi.org/10.3748/wjg.14.6355

INTRODUCTION

Orthotopic liver transplantation is an established treatment for patients with end-stage liver disease. However, donor organ shortages remain extremely problematic. To address this issue, living donor liver transplantation (LDLT) was developed[1]. During transplantation, the liver graft is subjected to a variety of potential hepatic injuries including ischemic injury associated with organ harvesting and the obligate storage before revascularization, reperfusion injury following revascularization, immunological attack caused by the immune system of the recipient, toxicity of certain drugs used during the post-transplant period, and certain infections[2,3]. After transplantation the liver graft goes into a regeneration process, which may be important for the overall success of the transplant procedures. Notably, the liver graft must be capable of normal growth, repair, and regeneration in the presence of immunosuppressive drugs such as calcineurin inhibitors. The aim of the present study was, therefore, to investigate the pharmacokinetics of cyclosporin A (CyA)[4] and its effect on liver regeneration and metabolic

activity to elucidate the mechanism of metabolic activity and serum concentration of cyclosporin A as an example of calcineurin inhibitors administered during liver regeneration in a rat model.

MATERIALS AND METHODS

Animals and treatments

Adult male Sprague Dawley rats, weighting 250-320 g (CRJ Charles River Japan, Kanagawa, Japan), were provided with water, and a standard laboratory diet ad libitum. All of the studies were performed according to the rules and regulations of the University of Nagasaki Research Animal Resources Guidelines.

Surgical procedures

A 70% hepatectomy was carried out according to the method described by Higgins and Anderson[5] under light ether anesthesia. Surgery was performed between 9:00 and 12:00 a.m. to avoid diurnal variation in the regenerative responses. The rats were randomly assigned to three groups, and treated daily by gavage beginning immediately after the hepatectomy. Group 1 animals were given water. Group 2 animals received 5 mg/kg CyA (Neoral®, Novartis Pharma, Basel) and group 3 animals received 5 or 10 mg/kg CyA. These CyA doses were selected based on the results reported by Morii *et al*[6].

In each group, five rats were killed before and at day 1, 3, 7 and 14 after the hepatectomy. Immediately before they were sacrifices, blood samples were obtained from the inferior vena cava. The remnant liver was removed to investigate hepatic restoration. The experimental protocol is demonstrated in Figure 1.

Serum concentration of CyA

The serum concentration of CyA was measured in the whole blood by fluorescence polarization according to the manufacturer's protocols (AxSYM® analyzer, Abbott, Tokyo)[7].

Regeneration ratio

The liver regeneration ratio in each experiment was defined as the ratio of the remaining liver weight to the initial body weight.

Serum ALT and T-Bil level

To evaluate liver toxicity of CyA administration, plasma concentrations of alanine aminotransferase (ALT) and total bilirubin (T-Bil) were examined using an automated analyzing system according to the manufacturer's protocol.

RT-PCR analysis

Total hepatic RNA was prepared by the method as described previously[8] and used for the determination of the expression levels of albumin (ALB) and cytochrome-P 3A2 (CYP3A2). In addition, the level of gene expression of glyceral-dehyde-3-phosphate-dehydrogenase (GAPDH) was measured as an internal control. Complementary DNA (c-DNA) was prepared from total RNA by the method described previously[8]. The primers used in the present study are listed in Table 1.

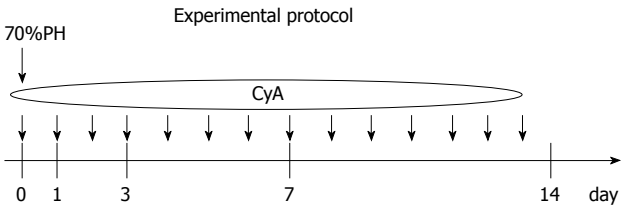

Figure 1 Administration schedule of CyA. Rats underwent a 70% hepatectomy immediately followed by the daily administration of CyA for up to 14 d per os. Blood samples were collected on post operative day 1, 3, 7 and 14.

Statistical analysis

The data are expressed as the mean ± SD (n = 5). Statistical analyses were performed by unpaired, two tailed Student's t-test. A P value less than 0.05 was considered to be significant.

RESULTS

Changes of serum concentration of CyA during liver regeneration

Figure 2 shows that the concentration of CyA reached a maximum during 3 to 7 d, and gradually declined thereafter. The levels of CyA in the PH group were significantly higher than that in control group.

The effect of CyA on liver regeneration ratio

As shown in Figure 3, the lower concentration of CyA (5 mg) did not affect the liver regeneration potential during the observation period; however, the rate of liver regeneration was significantly higher than that in the low CyA group on postoperative day 7.

Changes of hepatocyte specific gene expression during liver regeneration

Alb mRNA expression remained constant during liver regeneration, while hepatocyte specific p450 activity-CYP3A2 was significantly reduced on postoperative day 14 (Figure 4).

The effect of CyA on liver function

Rats were anesthetized and blood samples were collected through the tail vein at the indicated time points. ALT and T-Bil levels were measured as indicators of liver function. On day 1, plasma ALT concentrations increased during the first 24 h after the hepatectomy and then decreased gradually returning to the preoperative values at 72 h. There was no significant difference between the groups (Figure 5).

As shown in Figure 5, the ALT level in control animals were slightly increased, and thereafter gradually reduced. There was no statistically significant difference in any of the groups.

Table 1 Primers used in the present study. The hepatocyte specific gene expression levels were determined by using RT-PCR method. As hepatocyte specific parameters, albumin and CYP3A2 were selected. As an internal control, GAPDH gene expression was also examined. The primer sequences and optimal PCR conditions were summarized

Gene	Sequence (5'-3' sense/antisense)	Reaction condition			Product size	Cycles
		Denaturation	Annealing	Elongation		
GAPDH	TTCAACGGCACAGTCAAG CACACCCATCACAAACAT	95℃, 1 min	60℃, 1 min	72℃, 2 min	240 bp	26
CYP3A2	TACTACAAGGGCTTAGGGAG CTTGCCTGTCTCCGCCTCTT	94℃, 1 min	60℃, 1 min	72℃, 2 min	348 bp	27
ALB	ATACACCCAGAAAGCACCTC CAGAGTGGAAGGTGAAGGTC	94℃, 1 min	60℃, 1 min	72℃, 2 min	305 bp	27

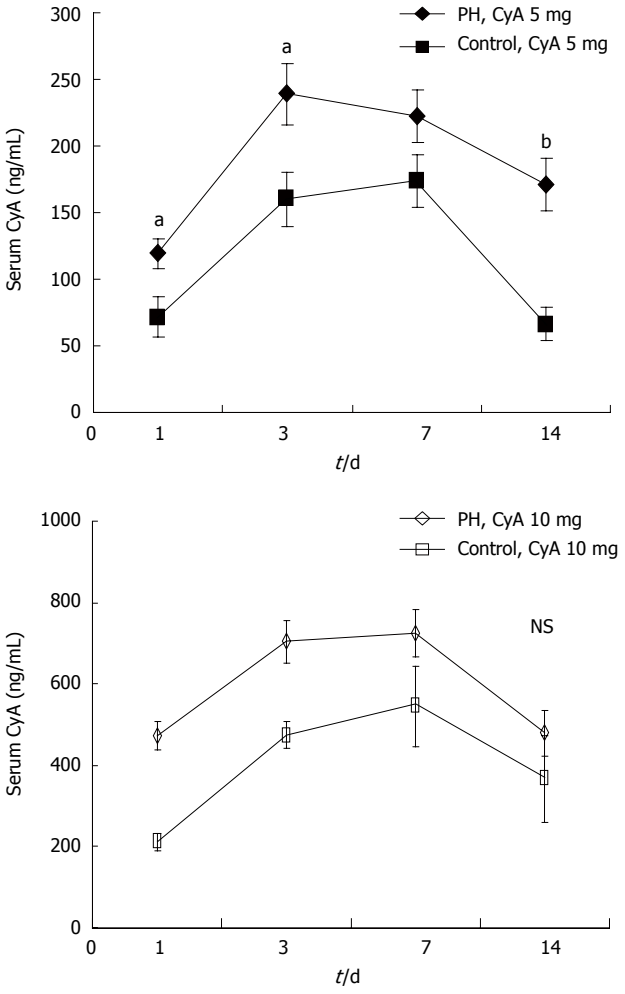

Figure 2 Changes in the serum concentration of CyA during liver regeneration. The values are expressed as the mean ± SD of 5 samples in each group. The concentration of CyA reached a maximum during 3 to 7 d, and gradually declined thereafter. The level of CyA in the PH group was significantly higher than that in control group. $^{a}P < 0.05$, $^{b}P < 0.01$.

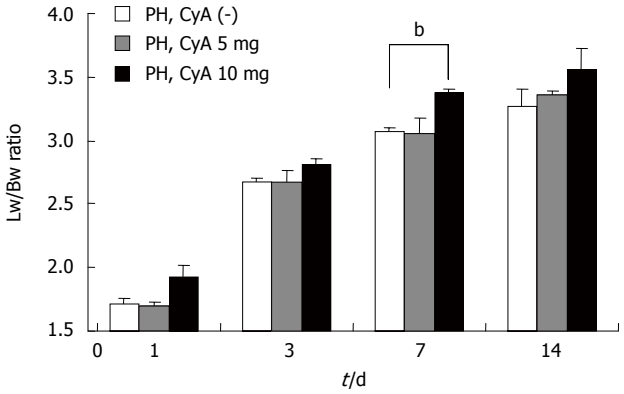

Figure 3 The effect of CyA on the liver regeneration ratio. The values are expressed as the mean ± SE of 5 samples in each group. The low concentration of CyA (5 mg) did not affect the liver regeneration potential during the observation period; however, the rate of liver regeneration was significantly higher than that in the-low CyA group on postoperative day 7. $^{b}P < 0.01$.

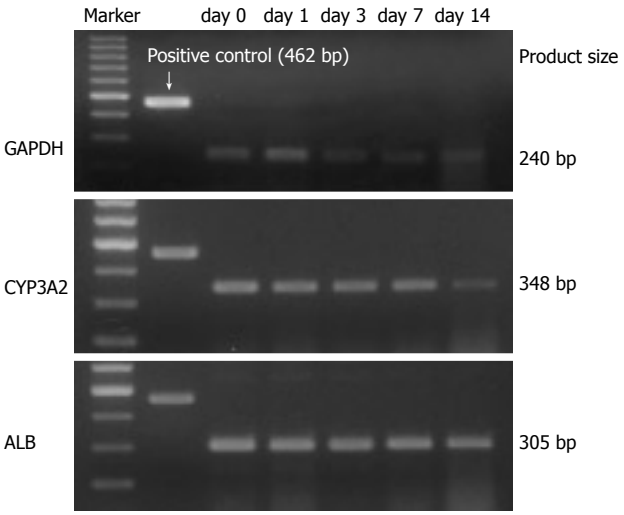

Figure 4 Changes of hepatocyte specific gene expression during liver regeneration. Alb mRNA expression remained constant during liver regeneration, while the hepatocyte specific p450 activity-CYP3A2 significantly decreased on postoperative day 14.

DISCUSSION

The present study, investigated the pharmacokinetics of the CyA in a rat two thirds hepatectomy model, for the first time. The results yielded important information concerning the interrelationship between the CyA and regenerating liver. (1) The metabolism is retarded in a regenerating liver, which is actually seen in clinical partial liver transplantation. (2) CyA has possible hepatotrophic effect on the regenerating liver in a CyA-dose dependent manner. (3) The p450 activity of the regenerating liver was down-regulated after CyA administration.

As expected, the serum concentrations of CyA after

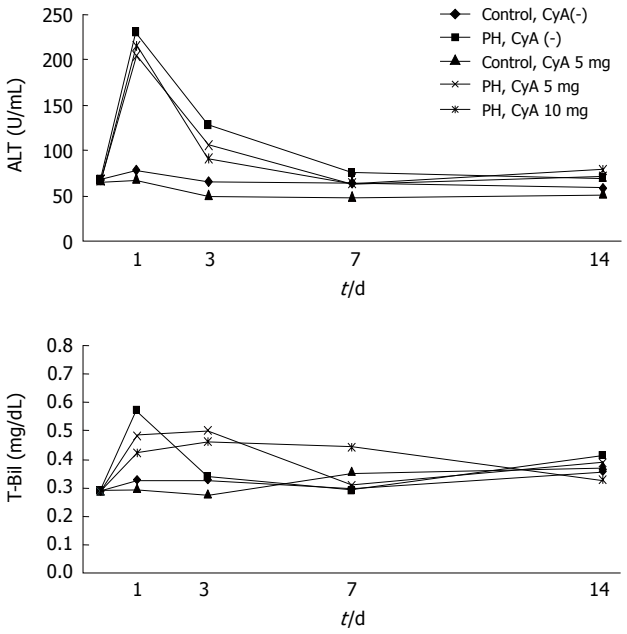

Figure 5 The effect of CyA on liver function. Rats were anesthetized and blood samples were collected through the tail vein at the indicated time points. ALT and T-Bil level were measured as indicators of liver function. On day 1, ALT level were significantly increased and thereafter gradually reduced. There was no statistically significant difference in each group ($P > 0.05$).

a hepatectomy were significantly higher than that seen in the sham operated group as previously reported in clinical settings. There are several possible explanations for this, including increased absorption, decreased volume of distribution, or decreased clearance.

However, an increased absorption is not likely. The CyA used in this study was the microemulsified type, and the absorption is bile independent. Therefore, absorption of the CyA was table in both groups[9]. The volume of distribution should be smaller in the partially hepatectomized rats. In other words, a smaller volume of distribution could increase the relative blood level of an immunosuppressant for a given dose.

Another possibility for the higher levels of CyA after the partial hepatectomy is reduced hepatic immunosuppressive clearance, which may be explained by two possible mechanisms. One is simply because of the reduced hepatic mass available to metabolize the drugs. Another possibility is that immediately after hepatectomy, the hepatic mass is reduced because of the surgical excision of hepatic tissue. As a result, the ability to clear substances through the liver is reduced. For instance, the indocyanine green half-life is increased four-fold after a 60% hepatectomy and by 33% after a 40% hepatectomy[10]. In the rats after a two thirds hepatectomy, the whole-organ reduced form of-cytochrome c reductase and cytochrome p-450 activity are reduced by half. After a 90% hepatectomy, galactose clearance in rats was reduced by 90% within 24 h after surgery. The genetic data regarding the cytochrome p-450 gene suggested that the metabolic activity of specific enzymes responsible for drug metabolism is reduced in the remaining hepatic tissue. Marie *et al* reported that the cytochrome p-450 activity decreased soon after a two-thirds hepatectomy in rats, returning to 90% of the initial activity 2 wk postoperatively. In the present study, the expression analysis of cytochrome p450 activity was performed using RT-PCR method. The results showed the cytochrome p-450 activity remained at the initial stage of liver regeneration, finally declined in the late stages. Although, no other liver specific enzymes were examined, a previous study demonstrated that the levels of mRNA for enzymes responsible for gluconeogenesis and the acute phase proteins are increased up to four fold after a hepatectomy[11]. Therefore, there are adaptive changes in the hepatic tissue after a partial hepatectomy. Collectively, the activity of enzymes that support hepatic regeneration is increased, whereas the activity of the enzymes responsible for drug metabolism is reduced.

The present data also suggest that cyclosporine enhances the hepatic regenerative response without affecting the individual hepatocellular function. Among immunosuppressive drugs currently in clinical use, azathioprine and steroids have been reported to exert an antiproliferative action on the regenerating liver. Azathioprine inhibits the DNA or RNA synthesis of hepatocytes, acting as an antimetabolite, whereas the action of steroids is more complex. Several investigators have suggested a functional linkage between lymphoid tissues and hepatocytes. Craddock *et al* reported that a partial hepatectomy induced proliferation of hepatocytes as well as lymphoid tissues. Another study recently suggested a very close and positive interrelationship between hepatocyte replication and lymphocyte activities[12]. The new potent immunosuppressor, cyclosporin A has been extensively compared with azathioprine and steroids. It primarily inhibits T-lymphocyte responses, and has no functional effects on other hematopoietic cells or phagocytic cells[13-16]. The present study on hepatectomized rats confirmed the antimitotic action of these immunosuppressants on hepatocytes, although the degree of suppression was less than that seen in previous reports.

Notably, there seems to be some discrepancy in these data showing that the statistical difference of the serum concentration of CyA between 5 mg treated and control animals was not observed in the groups treated with 10 mg of CyA treated as demonstrated in Figure 2. However, this is probably a reflection of-the fact that the liver regenerative effect of CyA at a higher dose may improve the impaired metabolic potential for CyA itself.

The limitation of this study is that this model potentially does not require immunosuppresion; therefore, further research will be needed to elucidate the underlining mechanism for these findings in a partial liver transplant model.

In conclusion, these results indicate that CyA levels in hepatectomized rats were significantly higher in control rats without a hepatectomy, probably because of the decreased volume of distribution, and/or decreased clearance by reduced metabolic activity. The possible hepatotrophic effect of CyA on the regenerating liver has also been confirmed.

REFERENCES

1 **Chen CL**, Fan ST, Lee SG, Makuuchi M, Tanaka K. Living-donor liver transplantation: 12 years of experience in Asia. *Transplantation* 2003; **75**: S6-S11

2 **Burton JR Jr**, Rosen HR. Diagnosis and management of allograft failure. *Clin Liver Dis* 2006; **10**: 407-435, x

3 **Said A**, Einstein M, Lucey MR. Liver transplantation: an update 2007. *Curr Opin Gastroenterol* 2007; **23**: 292-298

4 **Pichlmayr R**, Neuhaus P, Ringe B, Wonigeit K, Burdelski M, Verner L, Lauchart W, Schmidt FW. Developments in liver transplantation. *Jpn J Surg* 1985; **15**: 409-419

5 **Higgins G**, Anderson R. Experimental pathology of the liver. *Arch Pathol* 1931; **12**: 186-202

6 **Morii Y**, Kawano K, Kim YI, Aramaki M, Yoshida T, Kitano S. Augmentative effect of cyclosporin A on rat liver regeneration: influence on hepatocyte growth factor and transforming growth factor-beta(1). *Eur Surg Res* 1999; **31**: 399-405

7 **Sabate I**, Liron FJ, Gonzalez Alba JM, Ginard M, Virgili J, Baro S, Figueras J, Gonzalez Segura C, Jaurrieta E. Comparison of cyclosporine immunoassays (AxSYM and RIA) for assessing pharmacokinetic parameters in liver transplant patients. *Transplant Proc* 1999; **31**: 2421-2422

8 **Konno Y**, Sekimoto M, Nemoto K, Degawa M. Sex difference in induction of hepatic CYP2B and CYP3A subfamily enzymes by nicardipine and nifedipine in rats. *Toxicol Appl Pharmacol* 2004; **196**: 20-28

9 **Tredger JM**. Using cyclosporine Neoral immediately after liver transplantation. United Kingdom Neoral Pilot Study Group. *Ther Drug Monit* 1995; **17**: 638-641

10 **Prasse KW**, Bjorling DE, Holmes RA, Cornelius LM. Indocyanine green clearance and ammonia tolerance in partially hepatectomized and hepatic devascularized, anesthetized dogs. *Am J Vet Res* 1983; **44**: 2320-2323

11 **Tygstrup N**, Jensen SA, Krog B, Pietrangelo A, Shafritz DA. Expression of messenger RNA for liver functions following 70% and 90% hepatectomy. *J Hepatol* 1996; **25**: 72-78

12 **Sakai A**, Pfeffermann R, Kountz SL. Liver regeneration and lymphocyte activation. *Surg Gynecol Obstet* 1976; **143**: 914-918

13 **White DJ**, Calne RY, Plumb A. Mode of action of cyclosporin A: a new immunosuppressive agent. *Transplant Proc* 1979; **11**: 855-859

14 **Hellman A**, Goldman JM. Effects of cyclosporin A on human granulopoiesis in vitro. *Transplantation* 1980; **30**: 386-387

15 **White DJ**, Plumb AM, Pawelec G, Brons G. Cyclosporin A: an immunosuppressive agent preferentially active against proliferating T cells. *Transplantation* 1979; **27**: 55-58

16 **Larsson EL**. Cyclosporin A and dexamethasone suppress T cell responses by selectively acting at distinct sites of the triggering process. *J Immunol* 1980; **124**: 2828-2833

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RAPID COMMUNICATION

Protective effects of anti-ricin A-chain RNA aptamer against ricin toxicity

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Abstract

AIM: To investigate the therapeutic potential of an RNA ligand (aptamer) specific for the catalytic ricin A-chain (RTA), the protective effects of a 31-nucleotide RNA aptamer (31RA), which formed a high affinity complex with RTA, against ricin-induced toxicity in cell-based luciferase translation and cell cytotoxicity assays were evaluated.

METHODS: To test the therapeutic potential of anti-RTA aptamers in Chinese hamster ovary (CHO) AA8 cells stably transfected with a tetracycline regulatable promoter, ricin ribotoxicity was measured using luciferase and ricin-induced cytotoxicity was ascertained by MTS cell proliferation assay with tetrazolium compound [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium].

RESULTS: Inhibition of protein synthesis by ricin in CHO AA8 cells resulted in diminished luciferase activity and treatment with polyclonal antibody against deglycosylated RTA (dgA) neutralized the inhibitory effects of ricin on luciferase activity and protected against ricin-induced cytotoxicity as measured by MTS assay. The 31RA anti-RTA aptamer inhibited the translation of luciferase mRNA in cell-free reticulocyte translation assay. 31RA aptamer also partially neutralized the inhibitory effects of ricin on luciferase activity and partially protected against ricin-induced cytotoxicity in CHO AA8 cells.

CONCLUSION: We have shown that anti-RTA RNA aptamer can protect against ricin ribotoxicity in cell-based luciferase and cell cytotoxicity assays. Hence, RNA aptamer that inhibits RTA enzymatic activity represents a novel class of nucleic acid inhibitor that has the potential to be developed as a therapeutic agent for the treatment of ricin intoxication.

Key words: Ricin inhibitor; RNA aptamer; Luciferase assay

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INTRODUCTION

Ricin, a lectin from the castor bean plant *R. communis* is considered one of the most potent plant toxins. Ricin poisoning can cause severe tissue damage and inflammation and can result in death. More than 750 cases of accidental or deliberate ricin poisoning have been described in humans. Most accidental exposures occur by ingestion of the seeds of castor beans whereby the toxin is released after the seed coat is damaged. The ingested toxin causes severe gastrointestinal damage with symptoms including nausea, vomiting, diarrhea, and abdominal pain and may progress to hypotension, liver failure, renal dysfunction, and death due to multiorgan failure or cardiovascular collapse[1]. Ricin administered

intragastrically to mice induced villus atrophy and epithelial damage in the proximal small intestine[2]. In experimental exposed animals, intravenous injection of ricin to mice induced severe inflammatory responses and clinical symptoms resembling hemolytic uremic syndrome (HUS), including thrombotic microangiopathy, hemolytic anemia, thrombocytopenia, and acute renal failure[3].

Ricin belongs to a group of toxins referred to as ribosome-inactivating proteins (RIPs)[4,5] and is composed of two glycoproteins, the A-chain (RTA) and the B-chain (RTB), linked by a disulfide bond[6]. RTB binds to galactose residues on cell surface receptors and cell binding is followed by endocytic uptake. A proportion of the ricin moves from early endosomes to the trans-Golgi network and to the endoplasmic reticulum (ER) lumen. In the ER lumen, RTA and RTB dissociate and RTA is retrograde transported across the ER membrane into the cytosol[7]. RTA is a ribotoxin that possesses RNA N-glycosidase activity that disables protein translation by depurinating a single adenine in the 28 S eukaryotic ribosomal RNA[8] which prevents the binding of elongation factor 2, thereby terminating protein synthesis[9,10]. The irreversible poisoning of the ribosome and inhibition of protein synthesis may lead to eventual cell death.

Since currently there is no antidote or specific therapy available for ricin poisoning, the discovery of antitoxins is a high priority. Ricin ribotoxicity can be counteracted by several different types of antitoxins including neutralizing anti-ricin antibodies, small molecule RTA inhibitors, polynucleotide active site inhibitors and polynucleotide substrate analogues[11,12]. *In vitro* selection had been used to generate RNA ligands (aptamers) specific for the catalytic ricin A-chain[13]. An initial 80-nucleotide RNA ligand was minimized to a 31-nucleotide RNA aptamer (31RA) that contained all sequences and structures necessary for forming high affinity complexes with RTA and blocking enzymatic activity of RTA *in vitro*. A transient cell-based luciferase assay had been utilized for quantifying protein synthesis inhibition by bacterial toxins[14]. In this report, we utilized a stable cell-based luciferase assay and showed that 31RA aptamer also neutralized the inhibitory effects of ricin on translation inhibition in cell-free and cell-based luciferase assays and ricin-induced cytotoxicity assay. The use of a stably transfected cell-based luciferase assay will facilitate the development of high throughput screening for inhibitors of ricin as potential antidotes for the treatment of ricin intoxication.

MATERIALS AND METHODS

Cell-free luciferase translation assay

Ricin (*Ricinus communis* agglutinin Ⅱ) and ricin A-chain (RTA) were purchased from Vector Laboratories, Inc. (Burlington, CA). Anti-deglycosylated ricin A-chain (anti-dgA) antibody was IgG purified by protein-A sepharose from pooled polyclonal antisera obtained from mice hyperimmunized with dgA (USAMRIID, Fort Detrick, MD). The anti-RTA RNA aptamer (31RA) (GGCGAAUUCAGGGGACGUAGCAAUGACUGCC)[13] was synthesized by Sigma-Genosys. Rabbit reticulocyte lysate (nuclease treated), amino acid (complete) mixture, luciferase control RNA, RNasin inhibitor, nuclease-free water, and luciferin substrate (CFT luciferase reporter buffer) were purchased from Promega (Madison, WI). 31RA aptamer was diluted and heated for 3 min at 65℃, cooled to 25℃ and incubated at 25℃ for 10 min. After incubation, aptamer and toxin were mixed together and incubated at 25℃ for an additional 10 min. As a standard control, ricin (1.6 to 200 ng/mL) and RTA (0.4 to 50 ng/mL) diluted in PBS buffer were added to a V-shaped 96-well microtiter plate. Rabbit reticulocyte lysate, RNAsin, amino acid complete mixture, nuclease-free deionized water, and luciferase mRNA were mixed together and kept on ice. Five μL of each standard and treatment group were added to microtiter plate, and then 25 μL of the lysate mixture was added to each well. The plate was wrapped in a damp paper towel, placed in a plastic bag and incubated for 90 min at 37℃. After incubation, 5 μL of reaction mixture was transferred to a black microtiter plate and 45 μL of the luciferin reaction buffer was added to each well. Luminescence was measured as counts per second (CPS) using a SpectraMax Luminometer (Molecular Devices). Data were presented as the % of control (PBS only or no treatment) [CPS experimental/CPS PBS control × 100] as previously described[15]. Statistical analyses were calculated using Microsoft Excel 7.0 and SigmaPlot™ V3.01. Three separate assays were performed for each experimental group.

Cell-based luciferase and cytotoxicity assays

Chinese Hamster Ovary AA8 (CHO AA8) cells offered a stably transfected luciferase reporter cell system, whereby expression of the luciferase gene was under the transcriptional control of a tetracycline-repressible promoter system (Tet-Off™ Expression System from Clontech/BD Biosciences). CHO AA8 cells were cultured in Dulbecco's modified Eagle's Medium (DMEM) supplemented with 10% FBS, penicillin and streptomycin and incubated at 37℃ in a humidified 5% CO_2 incubator. Isotype control mouse IgG, anisomycin and doxycycline were obtained from Sigma-Aldrich.

To suppress luciferase expression, CHO AA8 cells were cultured in DMEM containing 10% FBS and doxycycline (Dox) (1 μg/mL). For the kinetics of induction of luciferase activity, cells were trypsinized and seeded at 2×10^5 cells/well in 6-well plate without Dox and 3 h after cell plating, residual and cell-attached Dox was removed by several washes with PBS. Cells were subjected to the indicated treatment and incubated for different lengths of time before cell lysis. As control, cells were treated with anisomycin (10 μg/mL). Equal amounts of protein (20 μg) were assayed for luciferase activity using the Bright-Glo™ Luciferase Assay System (Promega) and luminescence was measured with EG&G Berthold microplate luminometer (MicroLumat Plus LB96V).

To evaluate the protective effects of anti-dgA Ab

and 31RA aptamer, 5000 CHO AA8 cells were plated in 96-well plates in the absence of Dox overnight. Ricin was co-incubated with anti-dgA IgG or control IgG at a ratio of 1 μg ricin to 10 μg antibody for 30 min at 37℃ in 5% CO_2 as previously described[16]. The ricin-antibody mixture was added to CHO AA8 cells in triplicate wells. Prior to use, the 31RA aptamer was heated at 65℃ for 3 min and cool at 25℃ for 10 min. Various dilutions of the 31RA aptamer were added to ricin and incubated at 37℃ for 30-40 min before incubation with cells. Cells were harvested 24 h after treatment for luciferase assay.

For ricin-induced cytotoxicity assay, CHO AA8 cells were seeded in 96-well flat-bottom plates at 5000 cells/well in 100 μL DMEM plus 10% FBS and incubated at 37℃ overnight. Ricin was pre-incubated with anti-ricin Ab or aptamer as described above and cell viability was assayed at 48 h post-treatment. Cytotoxicity of CHO AA8 cells induced by ricin was quantitated in triplicate wells using the CellTiter 96® Aqueous Non-Radioactive Cell Proliferation (MTS) Assay (Promega) and the plate was read using a 492 nm absorbance filter in a Perkin Elmer HTS700 BioAssay plate reader.

RESULTS

Hale ML[15] developed a cell-free *in vitro* translation assay for measuring the ribotoxicity of ribosome-inactivating toxins based on inhibition of protein translation of the luciferase mRNA in a rabbit reticulocyte assay. As shown in Figure 1 (upper panels), the amount of luciferase translated, as measured by luminescence, was inversely proportional to the concentration of RTA and ricin holoenzyme. The protective effects of 31RA aptamer were first evaluated using the cell-free translation assay. Preincubation of RTA (5 ng/mL) with 31RA aptamer at both 1 and 5 ng/mL protected equally against RTA-induced translation inhibition while dose-dependent protection was observed with a higher dose of RTA (10 ng/mL) (Figure 1A, lower panel). When preincubated with the ricin holoenzyme, 31RA aptamer partially neutralized protein synthesis inhibition by ricin (Figure 1B, lower panel).

To evaluate the protective effects of 31RA aptamer against ricin ribotoxicity in cells, we utilized a cell-based luciferase assay to complement the cell-free luciferase translation assay. For the cell-based luciferase assay, CHO AA8 cells stably transfected with a luciferase reporter gene under a tetracycline-repressible promoter were used to measure the ribotoxicity of ricin. In the presence of ATP, the luciferase enzyme catalyzes the oxidation of D-luciferin to produce light and the light output corresponds to the concentration of the luciferase enzyme and activity. The light output (Relative light unit, RLU) was used to measure the dose-dependent inhibition of luciferase activity by ricin compared to anisomycin, a small molecule protein synthesis inhibitor known to target ribosomal RNA (rRNA) at an adjacent site distinct from that targeted by ricin[10]. First, the kinetics of induction of expression of the luciferase reporter gene after removal of doxycycline

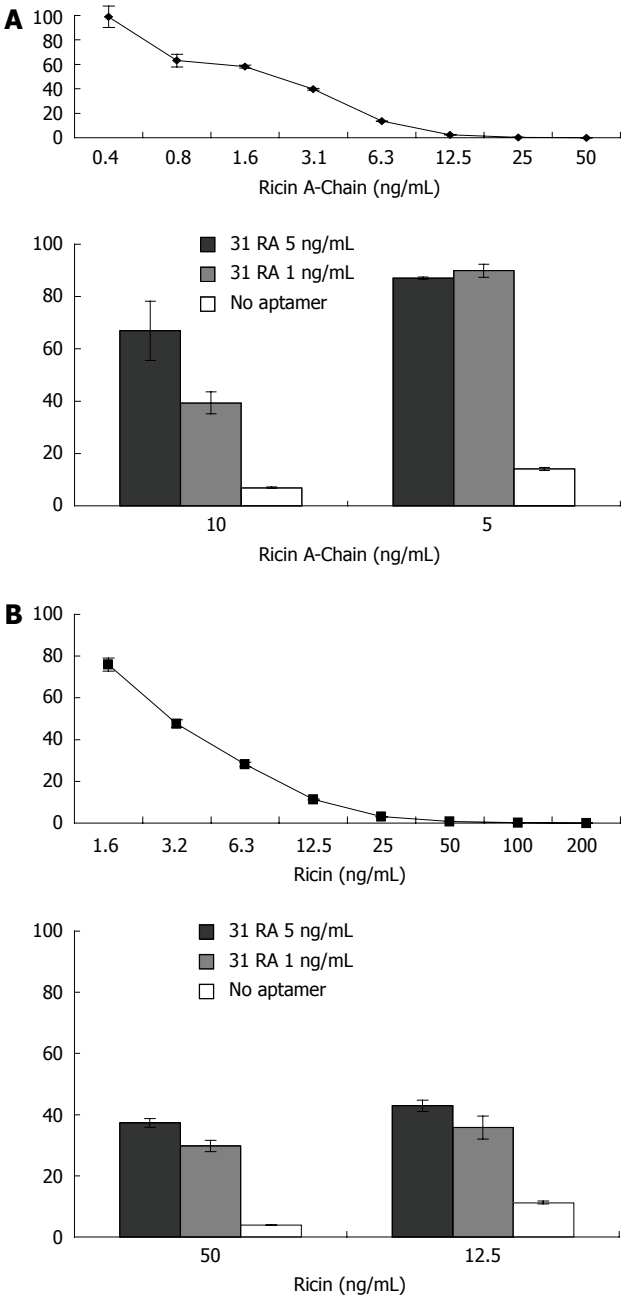

Figure 1 Cell-free luciferase translation assay for biological activity of ricin and protective effects of anti-RTA 31RA aptamer. A: Effects of increasing concentrations of RTA on luciferase activity in cell-free translation assay (upper panel) and neutralization by 31RA aptamer (lower panel); B: Effects of increasing concentrations of ricin holoenzyme on luciferase activity in cell-free translation assay (upper panel) and neutralization by 31RA aptamer (lower panel).

was determined by measuring luciferase activity over a 24-h period in the absence and presence of ricin or anisomycin (Figure 2A) The increase in luciferase activity was observed at 3 h after induction and ricin (1 μg/mL) and anisomycin (10 μg/mL) completely inhibited the increase in luciferase activity. Treatment of CHO AA8 cells with increasing concentrations of ricin resulted in increased inhibition of luciferase activity in a dose-dependent manner compared to untreated control and maximal inhibition was obtained with > 0.5 μg/mL ricin (Figure 2B).

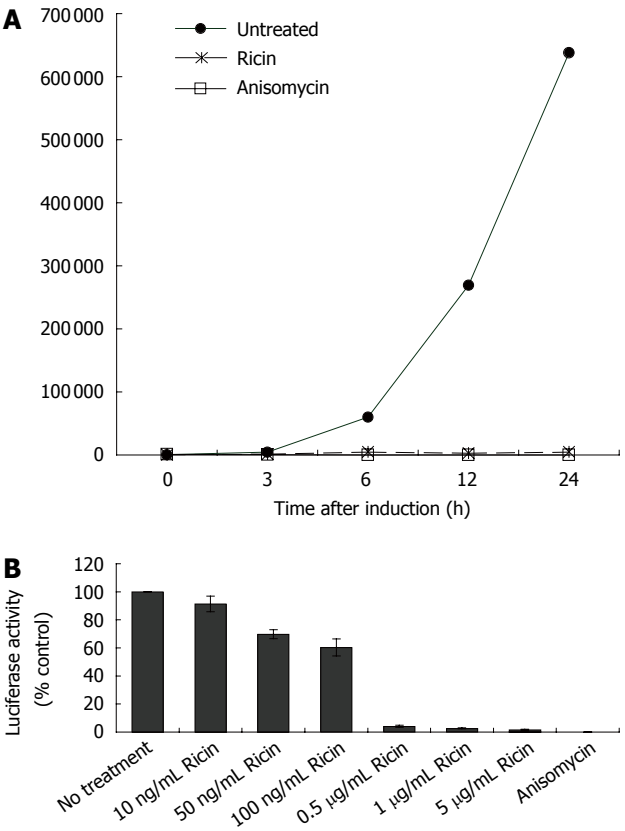

Figure 2 Cell-based luciferase assay for biological activity of ricin in CHO AA8 cells. A: Kinetics of induction of luciferase activity in CHO AA8 cells and inhibition by ricin and anisomycin. CHO AA8 cells were cultured in medium containing doxycycline (Dox) to suppress luciferase expression. For induction of luciferase expression, medium containing Dox was removed and replaced with medium (untreated) or medium containing ricin (1 μg/mL) or anisomycin (10 μg/mL). At various timepoints (0, 3, 6, 12, 24 h) after treatment, luciferase activity was measured. Light output is expressed as RLU; B: Dose-dependent inhibition of luciferase activity by increasing concentrations of ricin or anisomycin as control.

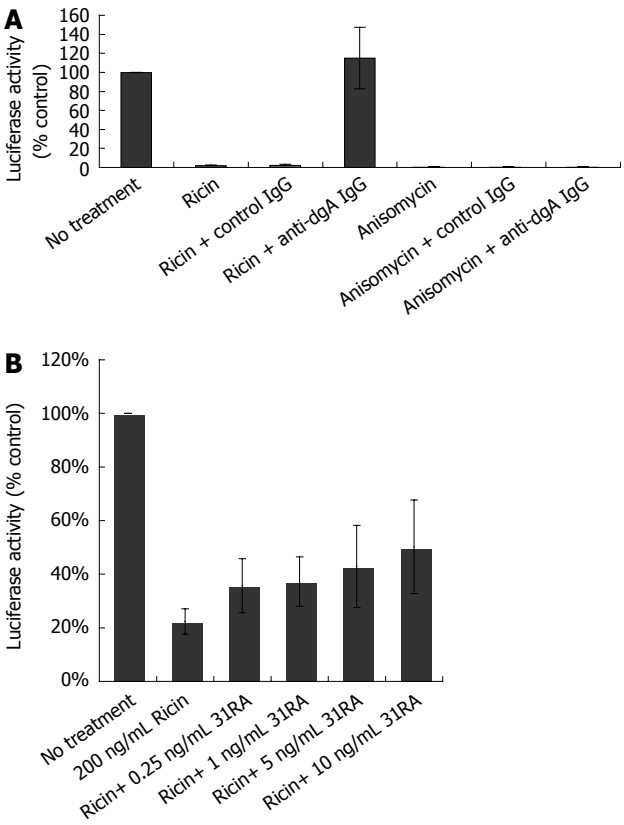

Figure 3 Protective effects of polyclonal anti-dgA IgG and anti-RTA 31RA aptamer on ricin-inhibited luciferase activity. A: Protective effects of anti-dgA IgG against ricin-inhibited luciferase activity, but not anisomycin-inhibited luciferase activity; B: Protective effects of various concentrations of 31RA aptamer against ricin-inhibited luciferase activity.

To determine the utility of this cell-based assay for testing specificity of antitoxins against ricin toxicity, the effects of polyclonal anti-deglycosylated ricin A chain (dgA) antibody in neutralizing the inhibitory effects of ricin on luciferase activity were determined. We have previously shown that anti-dgA Ab protected against ricin-induced cytotoxicity of RAW 264.7 mouse macrophage cells and ricin-induced lung injury and lethality[17]. CHO AA8 cells were treated with ricin or anisomycin in the presence of control IgG or IgG purified from the sera from dgA-immunized mice (anti-dgA IgG) and luciferase activity was measured 24 h later. As depicted in Figure 3A, anti-dgA IgG specifically neutralized the inhibitory effects of ricin on luciferase activity, but not that of anisomycin. Compared to anti-dgA IgG, 31RA aptamer partially protected against ricin-induced ribotoxicity as assessed by luciferase assay (Figure 3B).

To examine the effects of increasing doses of ricin on viability of CHO AA8 cells using the MTS assay. As shown in Figure 4A, treatment of CHO AA8 cells with increasing concentrations of ricin resulted in decreased cell survival. Interestingly, similar to the dose-dependent inhibition of luciferase activity (Figure 2B), ricin (100 ng/mL) induced ~50% cell death while > 0.5 μg/mL induced > 80% cell death (Figure 4A). Treatment with anti-dgA IgG, but not control IgG protected against cytotoxicity induced by ricin, and not anisomycin in CHO AA8 cells (Figure 4A). Pretreatment of ricin with 31RA aptamer also neutralized ricin-induced cytotoxicity in CHO AA8 cells (Figure 4B) and RAW264.7 mouse macrophage cells (data not shown).

DISCUSSION

In vitro selection is a powerful molecular tool for the generation of ligands for a wide variety of targets for therapeutic purposes. RNA aptamers that bind to human immunodeficiency virus type I Rev also inhibit viral replication[18]. Therefore, aptamers that recognize and inhibit ricin might be useful therapeutic agents. Interestingly, although the 31RA aptamer specific for the catalytic RTA bore no resemblance to the normal RTA substrate, i.e. the sarcin-ricin loop (SRL) and was not depurinated by RTA[13], it contained all sequences and structures necessary for interacting with RTA. This minimal 31-nucleotide RNA formed high affinity complexes with RTA (K_d = 7.3 nmol/L) which could compete with SRL for binding to RTA and inhibited RTA depurination of the SRL and could partially protect protein translation from RTA inhibition in *in vitro* translation assay. The IC_{50} of the aptamer for

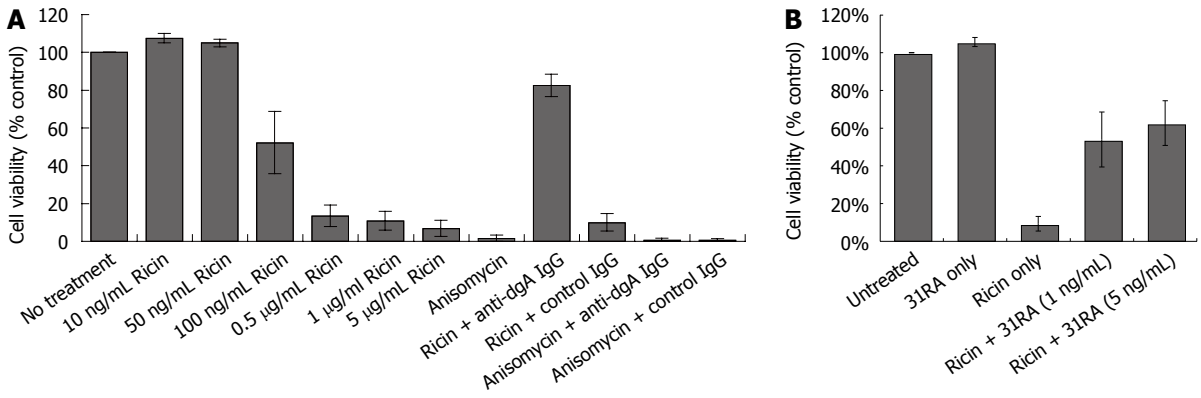

Figure 4 Protective effects of polyclonal anti-dgA IgG and anti-RTA 31RA aptamer on ricin-induced cell cytotoxicity as measured by MTS assay. A: Protective effects of anti-dgA IgG against ricin-induced cytotoxicity, but not anisomycin-induced cytotoxicity; B: Protective effects of 31RA aptamer against ricin-induced cytotoxicity.

RTA in the latter assay was 100 nmol/L, roughly 3 orders of magnitude lower than a small molecule inhibitor of ricin, pteroic acid[19]. Here we showed that 31RA aptamer can inhibit ricin ribotoxicity in cell-free and cell-based luciferase translation assays and cell cytotoxicity assay. It will also be interesting to determine if the 31RA aptamer will be effective against ricin after cell internalization which will be relevant for post-exposure treatment. We are currently testing various methods for intracellular delivery of the 31RA aptamer to determine if internalized aptamer can block toxicity of intracellular ricin A-chain. Hence, anti-RTA aptamers have the potential to be developed as ricin inhibitors and the therapeutic effects of aptamers *in vivo* in animal models of ricin intoxication remain to be determined.

Luciferase and luminescence assays, commonly used readouts for small molecule screens because of high sensitivity and linear signal response, can readily be adapted for high throughput screening of large libraries of compounds[20]. We have utilized a CHO Tet-Off luciferase system for measuring the biological activity of ricin based on protein synthesis inhibition and have shown that this assay can be used for measuring the protective effects of ricin inhibitors including anti-RTA neutralizing antibodies and an anti-RTA RNA aptamer. Compared to the transient luciferase-based assay described by Zhao *et al*[14] which utilized adenoviral transduction to deliver the luciferase gene, our cell-based assay is a stable cell system whereby the luciferase gene is stably integrated and its expression can be regulated by removal of tetracycline or doxycycline. Another advantage of using a stable cell system compared to adenoviral transduction is that the variability due to batch-to-batch variations of viral titers and infection efficiency are avoided. The conventional assays for protein synthesis utilize radioactive amino acids, but these assays suffer from significant sample-to-sample variability and insufficient sensitivity for high throughput assays. Hence, the adaptation of the stable luciferase-based assay in combination with cell cytotoxicity assay will be useful for high throughput screening of compounds for inhibitors of ricin and other related ribotoxins.

COMMENTS

Background

Ricin, a lectin from the castor bean plant *Ricinus communis* is considered one of the most potent plant toxins. Ricin poisoning can cause severe tissue damage and inflammation and can result in death. More than 750 cases of accidental or deliberate ricin poisoning have been described in humans. Most accidental exposures occur by ingestion of the seeds of castor beans whereby the toxin is released after the seed coat is damaged. The ingested toxin causes severe gastrointestinal damage with symptoms including nausea, vomiting, diarrhea, and abdominal pain and may progress to hypotension, liver failure, renal dysfunction, and death due to multiorgan failure or cardiovascular collapse.

Research frontiers

Since currently there is no antidote or specific therapy available for ricin poisoning, the discovery of antitoxins is a high priority. Ricin ribotoxicity can be counteracted by several different types of antitoxins including neutralizing anti-ricin antibodies, small molecule RTA inhibitors, polynucleotide active site inhibitors and polynucleotide substrate analogues. The development of specific inhibitors of RTA will offer novel insights into the development of effective therapeutics against ricin poisoning.

Innovations and breakthroughs

In vitro selection had been used to generate RNA ligands (aptamers) specific for the catalytic ricin A-chain. An initial 80-nucleotide RNA ligand was minimized to a 31-nucleotide RNA aptamer (31RA) that contained all sequences and structures necessary for forming high affinity complexes with RTA and blocking enzymatic activity of RTA *in vitro*. In this report, authors utilized a stable cell-based luciferase assay and showed that 31RA aptamer also neutralized the inhibitory effects of ricin on translation inhibition in cell-free and cell-based luciferase assays and ricin-induced cytotoxicity assay.

Applications

The use of a stably transfected cell-based luciferase assay will facilitate the development of high throughput screening for inhibitors of ricin as potential antidotes for the treatment of ricin intoxication.

Peer review

The manuscript deals with the inhibitory effects of a previously described aptamer against ricin toxicity. The authors employ an *in vitro* translation and a cell-based luciferase assay. This is an interesting paper.

REFERENCES

1 **Audi J**, Belson M, Patel M, Schier J, Osterloh J. Ricin poisoning: a comprehensive review. *JAMA* 2005; **294**: 2342-2351

2 **Yoder JM**, Aslam RU, Mantis NJ. Evidence for widespread epithelial damage and coincident production of monocyte chemotactic protein 1 in a murine model of intestinal ricin intoxication. *Infect Immun* 2007; **75**: 1745-1750

3 **Korcheva V**, Wong J, Corless C, Iordanov M, Magun B. Administration of ricin induces a severe inflammatory

response via nonredundant stimulation of ERK, JNK, and P38 MAPK and provides a mouse model of hemolytic uremic syndrome. *Am J Pathol* 2005; **166**: 323-339
4 **Olsnes S**, Refsnes K, Pihl A. Mechanism of action of the toxic lectins abrin and ricin. *Nature* 1974; **249**: 627-631
5 **Stirpe F**, Barbieri L. Ribosome-inactivating proteins up to date. *FEBS Lett* 1986; **195**: 1-8
6 **Lord JM**, Roberts LM, Robertus JD. Ricin: structure, mode of action, and some current applications. *FASEB J* 1994; **8**: 201-208
7 **Lord JM**, Deeks E, Marsden CJ, Moore K, Pateman C, Smith DC, Spooner RA, Watson P, Roberts LM. Retrograde transport of toxins across the endoplasmic reticulum membrane. *Biochem Soc Trans* 2003; **31**: 1260-1262
8 **Endo Y**, Tsurugi K. The RNA N-glycosidase activity of ricin A-chain. The characteristics of the enzymatic activity of ricin A-chain with ribosomes and with rRNA. *J Biol Chem* 1988; **263**: 8735-8739
9 **Olsnes S**, Pihl A. Different biological properties of the two constituent peptide chains of ricin, a toxic protein inhibiting protein synthesis. *Biochemistry* 1973; **12**: 3121-3126
10 **Wool IG**, Gluck A, Endo Y. Ribotoxin recognition of ribosomal RNA and a proposal for the mechanism of translocation. *Trends Biochem Sci* 1992; **17**: 266-269
11 **Rainey GJ**, Young JA. Antitoxins: novel strategies to target agents of bioterrorism. *Nat Rev Microbiol* 2004; **2**: 721-726
12 **Mantis NJ**. Vaccines against the category B toxins: Staphylococcal enterotoxin B, epsilon toxin and ricin. *Adv Drug Deliv Rev* 2005; **57**: 1424-1439
13 **Hesselberth JR**, Miller D, Robertus J, Ellington AD. In vitro selection of RNA molecules that inhibit the activity of ricin A-chain. *J Biol Chem* 2000; **275**: 4937-4942
14 **Zhao L**, Haslam DB. A quantitative and highly sensitive luciferase-based assay for bacterial toxins that inhibit protein synthesis. *J Med Microbiol* 2005; **54**: 1023-1030
15 **Hale ML**. Microtiter-based assay for evaluating the biological activity of ribosome-inactivating proteins. *Pharmacol Toxicol* 2001; **88**: 255-260
16 **Dertzbaugh MT**, Rossi CA, Paddle BM, Hale M, Poretski M, Alderton MR. Monoclonal antibodies to ricin: in vitro inhibition of toxicity and utility as diagnostic reagents. *Hybridoma* (Larchmt) 2005; **24**: 236-243
17 **Pratt TS**, Pincus SH, Hale ML, Moreira AL, Roy CJ, Tchou-Wong KM. Oropharyngeal aspiration of ricin as a lung challenge model for evaluation of the therapeutic index of antibodies against ricin A-chain for post-exposure treatment. *Exp Lung Res* 2007; **33**: 459-481
18 **Symensma TL**, Giver L, Zapp M, Takle GB, Ellington AD. RNA aptamers selected to bind human immunodeficiency virus type 1 Rev in vitro are Rev responsive in vivo. *J Virol* 1996; **70**: 179-187
19 **Miller DJ**, Ravikumar K, Shen H, Suh JK, Kerwin SM, Robertus JD. Structure-based design and characterization of novel platforms for ricin and shiga toxin inhibition. *J Med Chem* 2002; **45**: 90-98
20 **de Wet JR**, Wood KV, DeLuca M, Helinski DR, Subramani S. Firefly luciferase gene: structure and expression in mammalian cells. *Mol Cell Biol* 1987; **7**: 725-737

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RAPID COMMUNICATION

Chronic hepatitis C is a common associated with hepatic granulomas

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Abstract

AIM: To determine the most frequent etiologies of hepatic epithelioid granulomas, and whether there was an association with chronic hepatitis C virus (HCV).

METHODS: Both a retrospective review of the pathology database of liver biopsies at our institution from 1996 through 2006 as well as data from a prospective study of hepatic fibrosis markers and liver biopsies from 2003 to 2006 were reviewed to identify cases of hepatic epithelioid granulomas. Appropriate charts, liver biopsy slides, and laboratory data were reviewed to determine all possible associations. The diagnosis of HCV was based on a positive HCV RNA.

RESULTS: There were 4578 liver biopsies and 36 (0.79%) had at least one epithelioid granuloma. HCV was the most common association. Fourteen patients had HCV, and in nine, there were no concurrent conditions known to be associated with hepatic granulomas. Prior interferon therapy and crystalloid substances from illicit intravenous injections did not account for the finding. There were hepatic epithelioid granulomas in 3 of 241 patients (1.24%) with known chronic HCV enrolled in the prospective study of hepatic fibrosis markers.

CONCLUSION: Although uncommon, hepatic granulomas may be part of the histological spectrum of chronic HCV. When epithelioid granulomas are found on the liver biopsy of someone with HCV, other clinically appropriate studies should be done, but if nothing else is found, the clinician can be comfortable with an HCV association.

Key words: Hepatitis C; Liver; Granulomas; Liver biopsy

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INTRODUCTION

Epithelioid granulomas are found in 1%-10% of liver biopsy specimens[1-3]. They tend to fall into three broad categories which are systemic granulomatous disease, primary liver disease, or miscellaneous conditions that fit neither category. Hepatic granulomas are sometimes a surprise finding that may trigger an extensive search for an etiology. In some cases the granulomas are associated with a clinical picture which includes a high alkaline phosphatase and hepatomegaly; but these features are frequently absent. A significant percentage of liver biopsies performed today are for the staging of chronic hepatitis C. Granulomas have been reported with possible increased frequency in patients with hepatitis C with both mild disease as well as in explants[4-8]. It has been postulated that hepatitis C virus (HCV) may have a role in granuloma formation[9]. We decided to undertake a retrospective review of cases of hepatic granulomas at our institution during the last decade in order to determine the most frequent etiologies, and also to determine if there was an association with HCV.

MATERIALS AND METHODS

Patients studied

Following approval by our institutional review board, we used the data information system in our surgical pathology information system to identify all patients

Table 1 Patients with hepatic granuloma by diagnosis

Diagnosis	Number of patients
HCV	9
Histoplasmosis	4
Sarcoidosis	3
HIV alone	2
HCV/HBV	2
HCV/HIV	2
Mycobacterium avium	2
Primary biliary cirrhosis	2
Unknown	2
Tuberculosis	1
Coccidiomycosis	1
Cryptococcus	1
HBV	1
Hodgkin's lymphoma	1
Q Fever	1
Mucormycosis	1
Drug induced	1

from 1995 through 2005 that had a percutaneous or surgical liver biopsy, and also had the word granuloma or granulomatous in the final diagnosis or in the pathologists description. This data base includes all liver biopsies that are performed at our institution. The University of Texas Medical Branch is a tertiary care institution that consists of 5 hospitals and an outpatient center in Galveston, Texas, USA. Each year it serves patients from most of the counties in Texas, although two thirds of the patients are residents of the Texas Upper Gulf of Mexico Coast. One of the hospitals provides out patient and hospital care to a majority of the inmates in the Texas prison system. No explanted livers were included. We also excluded cases where the biopsy was performed at another institution, but slides were reviewed for a second opinion, and we also excluded several cases where biopsy of ossified nodules at surgery revealed old burned out or inactive granulomas. Autopsies were also excluded. Patients that had only lipogranulomas or small, poorly organized granulomas on their original pathology report were excluded from the study.

Since March 2003, the hepatology service at our institution has been in the midst of a prospective study of hepatic fibrosis markers in patients undergoing pre-treatment liver biopsies in chronic HCV. Some of the results of this study have been published[10,11]. This data base was searched as well for patients with evidence of hepatic granulomas on liver biopsy.

Parameters assessed

When appropriate, charts, reports, and the liver biopsy slides were reviewed. We tabulated information on special stains and cultures that were obtained on the specimens. All patients with hepatic granulomas had their biopsies examined with polarized light for crystalloid particles[12]. Clinical data was assessed to determine HCV, hepatitis B virus (HBV), and human immunodeficiency virus (HIV) status. The diagnosis of HCV was based on the presence of serum HCV RNA[13]. Information was also recorded regarding the patient's HCV genotype and routine liver tests. The stage of fibrosis based on the Batts Ludwig (F0-F4) staging system[14] was also noted.

Table 2 Patients with HCV only

Age (yr)	Gender	Fibrosis stage	Granuloma location	Comments
46	Female	F3	Portal tract	Multiple
54	Male	F2	Lobule	Multiple
46	Male	F2	Portal tract	Single
52	Female	F1	Diffuse	↑ alkaline phos
51	Female	F1	Portal tract	Single
45	Male	F1	Lobule	Multiple
67	Male	F1	Portal tract	Multiple, refractile Crystals
47	Male	F1	Portal tract	Multiple
37	Female	F2	Portal tract	Single

RESULTS

There were a total of 4578 liver biopsies performed at our institution during this time. There were 36 (0.79%) patients identified from the surgical pathology review that had at least one hepatic epithelioid granuloma (Table 1). Special stains were performed in 26 f and tissue cultures in five. The associated diagnoses are listed in Table 1. The most common association was HCV. Fourteen (36.1%) had HCV, and nine of these had no other clinical associations to explain the granulomas. Five of the HCV patients had other confounding associations including chronic hepatitis B, sarcoidosis, histoplasmosis, and HIV (2 patients). The etiologies with more than one case in the non HCV patients were sarcoidosis (3), histoplasmosis (4), primary biliary cirrhosis (3), HIV only (2), mycobacterium avis complex (2), and primary biliary cirrhosis (2), and unidentifiable (2).

There were 241 patients with chronic HCV that had liver biopsies while enrolled in the prospective hepatic fibrosis study during 2003-2006. Three (1.24%) had granulomas, and all of these had been identified in the above database search.

Patients with HCV and granulomas

Table 2 summarizes the results of the 9 patients with HCV, hepatic granulomas, and no other identifiable associations. All of the patients had special stains performed on their liver biopsies, and only one had a crystalloid substance noted on polarizing light. Eight of the patients had genotype 1, and the fibrosis stages F1, F2, and F3 were all present in at least one patient. None of the patients had cirrhosis. One patient with diffuse granulomas had an alkaline phosphatase consistently twice normal. Otherwise the liver function tests were typical of chronic HCV.

Characteristics of granulomas in HCV

The histologic findings in the patients with HCV only were variable. Figure 1 shows a large granuloma in an asymptomatic patient with mild elevation of ALT that had a staging liver biopsy. The granulomas were present in the

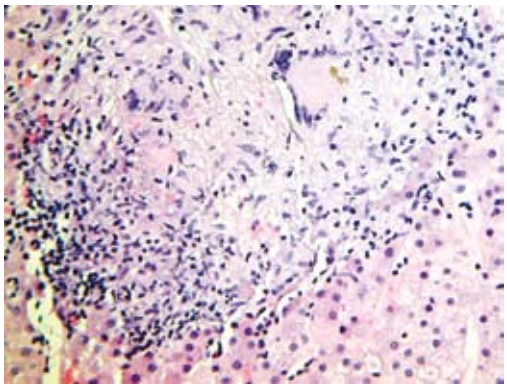

Figure 1 Large granuloma in the hepatic lobule noted in an asymptomatic patient with chronic HCV and mildly elevated ALT (HE, × 40).

portal area in 6 patients, the hepatic lobule in 2 patients, and diffusely in both the portal tract and lobule in one patient. In three patients, there was only a solitary granuloma in the portal tract, while in the others there were multiple granulomas. The case that had crystalloid substance on polarized light had multiple portal granulomas.

DISCUSSION

We found granulomas in less than 1% of our liver biopsies. Other reports have found granulomas in up to 10% of liver biopsies[1-3]. The frequency of detection as well as the frequency of various diagnoses can depend upon the geographic location of the center and the diseases endemic in that area as well as the diligence of the histopathology laboratory and the pathologists that prepare and examine the slides. Previous studies of hepatic granulomas have primarily been from eras before HIV and widespread organ transplantation, and before HCV became prominent. Although the percentages varied, sarcoidosis and tuberculosis tended to be most common associated diseases reported. A study from our institution from another era found that tuberculosis accounted for 53% and sarcoidosis 12% of the cases of hepatic epithelioid granulomas while in 20% no etiology could be determined[1]. In a large series of 565 cases of epithelioid hepatic granulomas in over 6000 biopsies accumulated over 4 decades, Klatskin[2] found that 36% had sarcoidosis, 12% had tuberculosis and 7% were undiagnosed. While our study indeed found 3 patients with sarcoidosis and another with *M. tuberculosis*, most cases were associated with HCV or HIV with associated infections.

There have been several reports of granulomas in the liver biopsies of patients with HCV and no other known systemic or hepatic diseases. This may not be unexpected since the most common reason for liver biopsies at most institutions today is staging of chronic HCV, and some unexplained granulomas have long been found on liver biopsies. On the other hand, the concurrence of HCV and granulomas may be frequent enough to not be simply explained by chance. A large recent retrospective review found epithelioid granulomas in 63 of 1662 liver biopsies[5]. While primary biliary cirrhosis (23.8%), sarcoidosis (11.1%), and unknown (11.1%) were the most common associations, HCV was associated with 9.5% of the cases. Emile *et al*[9] found that the explants of 5 of 52 patients undergoing liver transplantation for cirrhosis from HCV had epithelioid granulomas, and none of these patients had evidence of any other diseases such as tuberculosis or sarcoidosis. Goldin *et al*[4] found in a retrospective study that there were granulomas in the liver biopsy specimens of 14/155 patients with HCV compared with only 3/151 with HBV and 3/129 with alcoholic liver disease. Yamamoto *et al*[6] found unexplained granulomas in 2/273 (0.73%) liver biopsies of patients with chronic HCV. In a more recent study from Turkey, 8/605 (1.3%) of patients with chronic hepatitis C had unexplained granulomas on liver biopsy[7]. This is the same percentage that we found among chronic HCV patients in our prospective study of hepatic fibrosis markers that underwent liver biopsies over a three year period. Consequently, it has been postulated that hepatic granulomas may themselves be part of the histologic picture of chronic HCV, albeit uncommonly. Our finding that 9 of 36 patients with hepatic epithelioid granulomas had HCV as their only disease association would support this. We cannot rule out that some of these patients could have had other undiagnosed disorders such as sarcoidosis; but in follow up, none of them have manifested pulmonary or systemic findings to support another diagnosis.

Alpha interferon in regular or pegylated form has been used in the therapy of chronic HCV since the discovery of the virus[13]. It has been speculated that interferon itself can stimulate granuloma production, and there are several reported cases of the development of sarcoidosis in patients receiving interferon therapy[15-19]. This is thought to be related to the ability of interferon to stimulate a Th 1 immune response which is felt to be responsible for the granulomas in sarcoidosis[20]. Both the sarcoidosis and granulomas have usually regressed when interferon was stopped. It is unlikely that interferon usage played a major factor in our patients since only one subject with chronic HCV and granulomas had received interferon prior to liver biopsy.

Intravenous drug use is the most common route of transmission of chronic hepatitis C[21]. Since granulomas can be induced by foreign substances that might be used in a "street prepared" illicit drug mixture, we examined all the slides of patients with HCV and granulomas with polarized light, and a crystalloid substance was found in only one patient. Therefore, foreign body induced granulomas do not appear to be an explanation for most of the granulomas found in our patients with HCV.

We realize that there are problems interpreting the results of a retrospective study such as ours. The majority of the liver biopsies that we perform are on patients that are being staged prior to proposed treatment for chronic HCV. Therefore, the finding of otherwise unexplained granulomas in multiple patients with chronic HCV may not be that unusual or surprising. Nevertheless, in this study the most common association of hepatic granulomas was chronic HCV.

We would recommend if a patient with chronic HCV should have granulomas on liver biopsy that a search

for another disease should be made by utilizing special stains and other clinically appropriate tests such as a chest X-ray. However, if no other abnormality is found, the clinician should be comfortable associating the granuloma(s) with the chronic HCV.

COMMENTS

Background

Granulomas have long been curious findings on liver biopsies, and sometimes can trigger exhaustive searches for the etiology. Although there are many causes, sarcoidosis and tuberculosis have been the most frequent associations in previous series.

Research frontiers

Recent papers have reported finding granulomas in chronic hepatitis C virus (HCV) patients, and it has been speculated that granulomas may be an uncommon part of the immune response in chronic HCV. This study looked retrospectively at a decade of liver biopsies at the large institution to see what the most common disease associations with hepatic granulomas were. Authors also looked prospectively at the prevalence of granulomas in a series of chronic HCV patients undergoing staging liver biopsies.

Innovations and breakthroughs

The most common association of hepatic granulomas at the institution is chronic hepatitis C. The granulomas were both in the portal area and the lobule, and they were both single and multiple. Although present in only about 1% of liver biopsies of patients with hepatitis C, they should be considered as part of the histologic spectrum of the disease.

Applications

The finding of hepatic granulomas on the liver biopsy of someone with chronic HCV does not necessitate an extensive workup. Special stains and pertinent tests such as a chest x-ray should be done, but the clinician should be comfortable with the association if nothing is found.

Terminology

An epithelioid granuloma is a complex of transformed macrophages together with inflammatory cells and often multinucleated giant cells. It is a manifestation of delayed hypersensitivity.

Peer review

This is a very good retrospective examination of characteristics associated with hepatogranulomas, with the added strength of the prospective surveillance.

REFERENCES

1 **Guckian JC**, Perry JE. Granulomatous hepatitis. An analysis of 63 cases and review of the literature. *Ann Intern Med* 1966; **65**: 1081-1100

2 **Klatskin G**. Hepatic granulomata: problems in interpretation. *Mt Sinai J Med* 1977; **44**: 798-812

3 **Cunnigham D**, Mills PR, Quigley EM, Patrick RS, Watkinson G, MacKenzie JF, Russell RI. Hepatic granulomas: experience over a 10-year period in the West of Scotland. *Q J Med* 1982; **51**: 162-170

4 **Goldin RD**, Levine TS, Foster GR, Thomas HC. Granulomas and hepatitis C. *Histopathology* 1996; **28**: 265-267

5 **Gaya DR**, Thorburn D, Oien KA, Morris AJ, Stanley AJ. Hepatic granulomas: a 10 year single centre experience. *J Clin Pathol* 2003; **56**: 850-853

6 **Yamamoto S**, Iguchi Y, Ohomoto K, Mitsui Y, Shimabara M, Mikami Y. Epitheloid granuloma formation in type C chronic hepatitis: report of two cases. *Hepatogastroenterology* 1995; **42**: 291-293

7 **Ozaras R**, Tahan V, Mert A, Uraz S, Kanat M, Tabak F, Avsar E, Ozbay G, Celikel CA, Tozun N, Senturk H. The prevalence of hepatic granulomas in chronic hepatitis C. *J Clin Gastroenterol* 2004; **38**: 449-452

8 **Mert A**, Tabak F, Ozaras R, Tahan V, Senturk H, Ozbay G. Hepatic granulomas in chronic hepatitis C. *J Clin Gastroenterol* 2001; **33**: 342-343

9 **Emile JF**, Sebagh M, Feray C, David F, Reynes M. The presence of epithelioid granulomas in hepatitis C virus-related cirrhosis. *Hum Pathol* 1993; **24**: 1095-1097

10 **Snyder N**, Gajula L, Xiao SY, Grady J, Luxon B, Lau DT, Soloway R, Petersen J. APRI: an easy and validated predictor of hepatic fibrosis in chronic hepatitis C. *J Clin Gastroenterol* 2006; **40**: 535-542

11 **Snyder N**, Nguyen A, Gajula L, Soloway R, Xiao SY, Lau DT, Petersen J. The APRI may be enhanced by the use of the FIBROSpect II in the estimation of fibrosis in chronic hepatitis C. *Clin Chim Acta* 2007; **381**: 119-123

12 **Ishak KG**. Light microscopic morphology of viral hepatitis. *Am J Clin Pathol* 1976; **65**: 787-827

13 **National Institutes of Health Consensus Development Conference Statement: Management of hepatitis C: 2002--June 10-12, 2002**. *Hepatology* 2002; **36**: S3-S20

14 **Batts KP**, Ludwig J. Chronic hepatitis. An update on terminology and reporting. *Am J Surg Pathol* 1995; **19**: 1409-1417

15 **Ryan BM**, McDonald GS, Pilkington R, Kelleher D. The development of hepatic granulomas following interferon-alpha2b therapy for chronic hepatitis C infection. *Eur J Gastroenterol Hepatol* 1998; **10**: 349-351

16 **Gitlin N**. Manifestation of sarcoidosis during interferon and ribavirin therapy for chronic hepatitis C: a report of two cases. *Eur J Gastroenterol Hepatol* 2002; **14**: 883-885

17 **Butnor KJ**. Pulmonary sarcoidosis induced by interferon-alpha therapy. *Am J Surg Pathol* 2005; **29**: 976-979

18 **Ubina-Aznar E**, Fernandez-Moreno N, Rivera-Irigoin R, Navarro-Jarabo JM, Garcia-Fernandez G, Perez-Aisa A, Vera-Rivero F, Fernandez-Perez F, Moreno-Mejias P, Mendez-Sanchez I, de Sola-Earle C, Sanchez-Cantos A. [Pulmonary sarcoidosis associated with pegylated interferon in the treatment of chronic hepatitis C] *Gastroenterol Hepatol* 2005; **28**: 450-452

19 **Menon Y**, Cucurull E, Reisin E, Espinoza LR. Interferon-alpha-associated sarcoidosis responsive to infliximab therapy. *Am J Med Sci* 2004; **328**: 173-175

20 **Alfageme Michavila I**, Merino Sanchez M, Perez Ronchel J, Lara Lara I, Suarez Garcia E, Lopez Garrido J. [Sarcoidosis following combined ribavirin and interferon therapy: a case report and review of the literature] *Arch Bronconeumol* 2004; **40**: 45-49

21 **Armstrong GL**, Wasley A, Simard EP, McQuillan GM, Kuhnert WL, Alter MJ. The prevalence of hepatitis C virus infection in the United States, 1999 through 2002. *Ann Intern Med* 2006; **144**: 705-714

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RAPID COMMUNICATION

Histological abnormalities of the small bowel mucosa in cirrhosis and portal hypertension

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Abstract

AIM: To study the small bowel (SB) mucosa on biopsy in cirrhotic patients with portal hypertension and in non-cirrhotic controls and grade findings according to the Marsh criteria.

METHODS: We prospectively enrolled 51 consecutive patients undergoing an upper endoscopy for their routine medical care. Twenty five patients with cirrhosis and portal hypertension were compared to 26 controls. We obtained coeliac serology and multiple upper small bowel biopsies on all 51 patients. A GI pathologist interpreted biopsies and graded findings according to the Marsh criteria. We assessed equivalence in Marsh grade between cirrhotic and non-cirrhotic controls using the Mann-Whitney test for equivalence.

RESULTS: Gender, ethnicity and age were similar between both groups. Marsh grades were equivalent between the groups. Grade of 0 was present in 96% and grade of 1 was present in 4% of both groups and there was no villus atrophy or decrease in villus/crypt ratio in patients with portal hypertension.

CONCLUSION: This study provides evidence for the lack of villus atrophy in patients with cirrhosis and portal hypertension, and supports the continuous reliance on the Marsh criteria when the diagnosis of coeliac disease is to be made in the presence of cirrhosis.

Key words: Cirrhosis; Portal hypertension; Coeliac disease; Marsh criteria; Small bowel mucosa

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INTRODUCTION

Studies of small bowel (SB) mucosa in cirrhosis and portal hypertension report a diverse spectrum of histological abnormalities[1-5]. These include increased capillary angiogenesis, mucosal edema, decreased villus to crypt ratio, villus atrophy and decreased total absorptive surface. Villus abnormalities resemble the abnormalities of coeliac disease (CD) and this may affect the interpretation of small bowel biopsy, and lead to confusion when CD is to be excluded in patients with cirrhosis and portal hypertension.

The diagnosis of CD is increasingly considered and work up recommended during the evaluation of abnormal liver enzymes[6-7]. This is in part due to the heightened awareness of the association of CD with a variety of liver disorders. Most commonly described associated liver disorders include autoimmune hepatitis, primary biliary cirrhosis, non-alcoholic fatty liver disease, unexplained abnormal liver tests and cirrhosis[8-15]. The mechanisms of this association are not clear and the prevalence of CD in patients with liver disease is variable depending on the associated liver disease. For example, while CD affects 1% of the general population, one study reports that about 4% of 185 cirrhotic patients who had undergone liver transplantation were found to have CD, and 3 of 4 pa-

tients presenting with severe liver disease were diagnosed with CD and were remitted as possible candidates for liver transplantation when placed on a gluten free diet[16]. Approximately 40% of patients with CD have abnormal liver enzymes, and these return to normal in 75% to 95% when a gluten free diet is instituted[16,17]. Based on these and other studies, it is recommended that clinicians should have a low threshold for testing for CD in patients with abnormal liver blood tests[6].

The diagnosis of CD is based on initial screening with coeliac serological tests (endomysial EMA and human tissue transglutaminase hTTG antibodies); but histological examination of SB biopsy is required for establishing a definite diagnosis[6,10]. However, in patients with cirrhosis, the diagnosis of coexisting CD is a challenge because of the reported similar changes on SB mucosa in both cirrhosis and CD. CD may, therefore, be underdiagnosed in cirrhosis. Studies of SB biopsy in cirrhosis[1-5], have thus shed doubt on the validity of biopsy in the diagnosis of CD in cirrhotic patients; but reported findings are poorly characterized, lack standardized grading, and of unclear significance. The current study was undertaken to determine if SB biopsies in cirrhosis show features that might mimic CD. Findings would determine if SB biopsy should be used in the diagnosis of CD in patients with cirrhosis and portal hypertension.

The aim of the study was to prospectively assess the histological abnormalities of the SB mucosa in patients with and without cirrhosis and portal hypertension by grading findings according to the grading system defined by Marsh[6].

MATERIALS AND METHODS

Selection of patients and data collection

This is a prospective case control study approved by the Institutional Board Review at the Cleveland Clinic. Eighty consecutive patients scheduled for an upper endoscopy EGD at the Cleveland Clinic between 9/1/2005 to 11/30/2005 were identified. Medical records were reviewed. Of 80 patients, 25 with cirrhosis and portal hypertension and 26 without cirrhosis, portal hypertension or liver disease fulfilled inclusion and exclusion criteria and were enrolled in the study.

Records reviewed included age, ethnicity, gender, indications for upper endoscopy EGD, imaging studies and laboratory tests. Laboratory tests obtained within the preceding 6 mo of the date of enrollment were reviewed for liver transaminases, alkaline phosphatase, protime/INR, celiac serology panel, complete blood count and differential, iron saturation and ferritin, and viral hepatitis panel.

Patients were included if they were older than 18 years old and able to give informed consent. Patients with cirrhosis and undergoing EGD were included regardless of the etiology of cirrhosis. Patients without cirrhosis and undergoing EGD for acid reflux, abdominal pain, dysphagia or vomiting were included.

Cirrhosis was defined histologically according to Batts and Ludwig staging system[18]. In patients without a liver biopsy, diagnosis of cirrhosis and portal hypertension was based on a combination of clinical data (jaundice, cutaneous spider angiomas, muscle wasting, ascites, and palmar erythema), biochemical data (decreased serum albumin and prolonged protime), imaging study (nodular surface on ultrasound or CT scan), and manifestations of portal hypertension (low platelets, splenomegaly, esophageal varices, hepatic encephalopathy or ascites) in the setting of chronic liver disease.

Patients were excluded if they were pregnant, on dialysis, had a bleeding disorder or were actively bleeding at the time of endoscopy, taking anticoagulants or had INR greater than > 1.5 or platelet count less than $30 \times 10^3/mm^3$. Patients with a diagnosis of malabsorption, coeliac disease, patients taking corticosteroids or immunosuppressant drugs, patients with a history of Crohn's disease, organ transplant, graft versus host disease, food allergies, iron deficiency anemia, osteoporosis, ataxia, and autoimmune disorders that could potentially be associated with CD such as thyroid disorders, dermatitis herpetiformis and type 1diabetes were excluded. In the control group, additional exclusions encompass individuals with a history of chronic liver disease or a history of abnormal liver tests.

Informed consent for SB biopsy and for blood draw for coeliac serology panel was obtained on all 51 patients. Severity of cirrhosis was assessed by calculating Child Turcotte Pugh (CTP) and Model for End-Stage Liver Disease (MELD) scores for all cirrhotic patients.

Laboratory assessment

Coeliac serology panel included antibodies to human tissue transglutaminase (IgG and IgA for hTTG, QUANTA lite™ ELISA, Inova diagnostics, San Diego CA), endomysial antibodies (IgA EMA, Immunofluorescence Inova diagnostics San Diego CA), and total IgA levels by nephelometry (Beckman Coulter Immage/image 800 Immunochemistry system and Calibrator 1, Fullerton CA). Tests were consecutively analyzed in the immunology laboratory at the Cleveland Clinic. These tests are reported to be highly sensitive and specific in the diagnosis of CD in the general population with sensitivities and specificities above 85%, and they supplant the use of gliadin antibody testing as the preferred mean of serological detection[6]. Abnormal serology panel is any value for IgA EMA above 1:10 dilution, or any value for either IgG hTTG or IgA hTTG above 20 U.

Histological assessment

Upper endoscopies were performed by gastroenterologists at the Cleveland Clinic. The gastroenterologists were not blinded to the study, and they were asked to obtain at least 3 biopsies from the second part of the duodenum or beyond on all subjects. Biopsy specimens were placed in vials containing 10% of buffered formalin solution for fixation. Paraffin sections were prepared and stained by hematoxylin and eosin (HE) stain. Pathology slides were interpreted by a Cleveland Clinic pathologist (A.B.) experienced with the spectrum of mucosal changes in CD. The pathologist was blinded to names

and diagnosis. All slides were batched and read after samples from all 51 subjects collected and processed. The pathologist graded findings according to the Marsh grading system[6]. Marsh 0 is defined by normal mucosal and villus architecture, Marsh I is defined by normal villus architecture, but increased numbers of intraepithelial lymphocytes, Marsh Ⅱ shows increased intraepithelial lymphocytes, enlarged crypts and increased crypt cell division, Marsh Ⅲ is defined by villus atrophy, shortened blunt villi and enlarged hyperplastic crypts. Marsh Ⅳ demonstrates hypoplastic mucosa.

Statistical analysis

The sample size estimation was based on the inference that the standard deviation of the Marsh grade would be equal to 1 for both groups. The study was designed to establish equivalence in small bowel mucosa (defined as a difference in mean Marsh grades no greater than1) between cirrhotics and non-cirrhotics with a significance level of 0.05 and a power of at least 90%. Therefore, it was estimated that a total of 25 subjects would be required in each group.

Descriptive statistics, such as frequencies for categorical factors and mean (SD) for continuous factors, were computed for all variables. A Student's *t*-test was used to assess differences in age between cirrhotics with portal hypertension and non-cirrhotic controls. In addition, Pearson's χ^2 and Fisher's exact test were used to compare gender and race between the groups.

In order to assess whether the SB mucosal architecture in cirrhotic patients with portal hypertension was indistinguishable from the mucosal architecture in non-cirrhotic controls, the Mann-Whitney test for equivalence was used[19]. It tests the null hypothesis (H_0) of difference in SB mucosal architecture between groups versus the research hypothesis (H_a) of equivalent SB mucosal architecture between groups. A *P*-value < 0.05 was considered statistically significant. SAS version 9.1 software (SAS institute, Carey, NC) was used to carry out all analyses.

RESULTS

Study populations

A total of 51 patients were enrolled. Twenty five had cirrhosis and portal hypertension and 26 controls had no evidence of liver disease or portal hypertension. Fifty patients had normal coeliac serology and one patient in each group had abnormal biopsy.

Baseline demographic characteristics of patients who fulfilled inclusion criteria are shown in Table 1. The mean age was 57 (± 10) years in the portal hypertension with cirrhosis group and 52 (± 15) years in the control group. Fifty two percent were males and 92% were Caucasians in the former group *versus* 42% and 84% respectively in the control group. There was no evidence to suggest statistically significant differences in the demographic characteristics between the groups ($P > 0.05$).

The most common indications for upper endoscopy in cirrhotic patients with portal hypertension were screening for or banding of esophageal varices (100%). In the control group, the most common indications were acid reflux (30.8%), dysphagia (23.1%), epigastric pain (23.1%), and nausea and vomiting in (15.4%).

Table 1 Demographic characteristics of study subjects (mean ± SD) *n* (%)

	Cirrhotic	Non-cirrhotic	*P* value
n	25	26	-
Age	57.5 ± 9.7	51.9 ± 15.3	0.12
Gender			0.49
Male	13 (52)	11 (42.3)	
Female	12 (48)	15 (57.7)	
Ethnicity			0.67
Caucasian	23 (92)	22 (84.6)	
Other	2 (8)	4 (15.4)	

All 25 patients with cirrhosis had evidence of portal hypertension, esophageal varices, thrombocytopenia and an imaging study showing cirrhotic liver. Fifteen patients had a liver biopsy. Twelve patients had CTP score A, 11 had CTP score B and 2 had CTP score C. The mean platelet count was $89.76 \times 10^3/mm^3$, with a range between 45 and $148 \times 10^3/mm^3$. The mean MELD score was 10 with a range between 5 and 17. The most common etiologies for cirrhosis were nonalcoholic steatohepatitis (24%), hepatitis C (24%) and cryptogenic cirrhosis (24%).

Histological findings in patients with and without cirrhosis

There was strong evidence to suggest that based on Marsh grade, cirrhotics and non-cirrhotics have indistinguishable SB mucosa (Mann-Whitney test of equivalence: $P < 0.01$). One patient in each group (4%) had an abnormal small bowel biopsy and 96% of patients from each group had a normal small bowel biopsy (Figures 1 and 2). Both patients with abnormal biopsy were females with normal coeliac serology. The two women had Marsh grade I based on 3 SB biopsies obtained for each. One of these women was 57 years old, and had a liver biopsy that was consistent with nonalcoholic steatohepatitis and cirrhosis. Her platelet level was $130 \times 10^3/mm^3$, her MELD score was 10 and her CTP score was B. The other woman with Marsh grade I on SB biopsy did not have cirrhosis. She was 51 years old and was scheduled for EGD for epigastric pain and bloating.

DISCUSSION

Portal hypertension and cirrhosis due to a variety of parenchymal liver diseases are associated with malnutrition[20,21]. The pathophysiologic mechanisms are not totally understood and several factors may be involved. Malabsorption has been implicated and this was presumed to be related to changes in the SB villi[2,4,5]. For example, Such *et al*[4] investigated 6 patients with cirrhosis using jejunal biopsies, and studied the mucosa under electron microscopy. The authors observed that "the microvilli were reduced in number and appeared shorter

Figure 1 Percentage of patients with Marsh grade 0 and Marsh grade 1 in cirrhosis and no cirrhosis.

and thicker when compared to controls", and their conclusion was that "the total absorptive surface may be reduced in cirrhotic patients". Misra[2] found a significant decrease in villus/crypt ratio in cirrhotic patients when compared to healthy volunteers. On the other hand, Nagral[22] reports a significant number of patients with large vessels in duodenal mucosa of patients with portal hypertension in comparison with controls, but did not find a statistical difference in severity and type of infiltrate, edema of lamina propria or villus/crypt ratio between the groups. In the study of Barakat[5], abnormal villus changes were present in 11.4% of portal hypertensive patients. These were described as "shortened villi, decreased or even reversed villus to crypt ratio down to total villus atrophy". The authors implied that these changes might have an effect on the intestinal absorptive functions, and in turn, might have a share in the pathogenesis of nutritional derangements in portal hypertensive patients.

Results of these studies implicate SB villus shortening and atrophy as underlying factors in the malabsorption and malnutrition observed in cirrhosis. Such conclusions need further validation and other diseases that may affect the SB mucosa should be considered in the differential diagnosis. Furthermore, histological abnormalities were reported descriptively and without systematic classification. Lack of a proper classification of abnormalities was once described by Marsh[23] as follows: "The system of qualitative terminology is not only inappropriate but also seems to have paralyzed any new intellectual activity that might elucidate afresh the immunopathogenic basis of mucosal response". And in 1992, Marsh described the mucosal abnormalities of the SB by establishing a classification system that utilized inflammatory and atrophic grades. This grading system has since been adopted by clinicians and pathology researchers in the diagnosis and study of CD, and is the only available system to systematically grade mucosal abnormalities of the SB. In this unprecedented study, we prospectively analyzed and graded changes of SB mucosa on biopsy according to the unified and accepted grading system characterized by Marsh[6,10].

We found no difference in Marsh grade between the study groups. Our patients had either a grade of 0 or a grade of I . One patient in each group had a Marsh grade of I , but neither had abnormal coeliac serology. The presence of Marsh grade I or intraepithelial lymphocytes is a non-specific finding especially in the absence of coexistent abnormal serology. We did not find

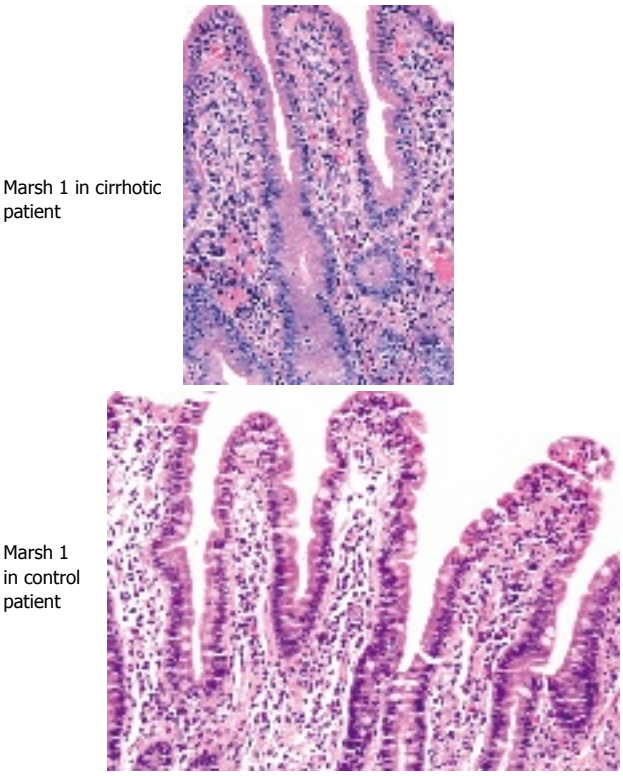

Figure 2 Small bowel mucosa in the cirrhotic and in the control patient, respectively.

any Marsh grades Ⅱ, Ⅲ or villus/crypt changes of CD among patients in either group. This provides evidence that histological abnormalities of the SB mucosa are equivalent between cirrhotic patients with portal hypertension and non-cirrhotic controls.

Since the etiology of cirrhosis has not been shown to influence the changes in mucosal architecture of the SB, we included all eligible cirrhotic patients regardless of the etiology of their cirrhosis. We excluded patients with clinical and laboratory evidence of malabsorption, patients with known SB mucosal disease and/or on therapy that included corticosteroids and immunosuppressants, and patients with autoimmune diseases that have the potential to be associated with CD or induce changes in the SB mucosa, because such cases may not necessarily yield the direct effects of cirrhosis on the SB mucosa.

We calculated the MELD and CTP scores on all cirrhotic patients. Although our study did not aim to examine whether the severity of liver disease would further exacerbate villus damage, we did not observe any correlation between Marsh grade and the degree of MELD and CTP scores. This has been reported previously[22].

An exact quantitative count for the intraepithelial lymphocytes IEL and immunohistochemical typing were not obtained because such stains are not utilized in routine clinical practice, and because one observer (pathologist A.B.) interpreted and analyzed all biopsy specimens.

Portal hypertensive enteropathy is receiving increased recognition in research studies of recent years. Studies have aimed at characterizing abnormalities on endoscopy, wireless capsule imaging, and SB biopsy. Small

bowel biopsy findings describe villus changes and villus atrophy. Contrary to previously reported studies, our study did not show shortened villi, reversal of villus to crypt ratio, villus atrophy, crypt hyperplasia or changes suggestive of CD in patients with portal hypertension. This concurs with the study of Nagral[22].

Our study is unique and different from other studies of the effects of cirrhosis and portal hypertension on the SB mucosa because we used the validated Marsh grading system to grade for abnormalities and we excluded subjects with CD and other diseases that would potentially affect the SB mucosa such as Crohn's and lymphoma *etc*, because we intended to study the sole effect of cirrhosis on the SB. SB villus changes and atrophy are characteristic, but are not specific for coeliac disease[24,25].

Coeliac serological tests have low sensitivities and specificities in chronic liver disease and cirrhosis[15,26-29] which emphasizes the importance of validating SB biopsy as the most important tool for the diagnosis of CD in this group of patients. Results of this study support the use of SB biopsy in patients with cirrhosis and portal hypertension when the diagnosis of CD is suspected. Furthermore, and since cirrhosis is not associated with significant inflammatory or atrophic changes of SB villi, we can extrapolate that there is a low probability that different results would be seen in the earlier stages of liver disease when cirrhosis has not yet developed.

Due to the estimated high prevalence of CD in patients with cirrhosis that is higher than in the general population[8,16,29,30], and the potential reversibility of liver dysfunction on a gluten free diet, we recommend prompt consideration and exclusion of SB mucosal diseases and CD (by using a combination of laboratory, clinical, pathologic and genetic examinations, and response to a gluten free diet), when shortened villi, reversal of villus to crypt ratio or villus atrophy are seen in patients with cirrhosis.

As to the mechanisms of malabsorption reported in patients with cirrhosis and portal hypertension, factors other than cirrhosis- related- villus changes should be considered. These may include bile salt deficiencies, motility disorders, protein losing enteropathy and other concomitant diseases of the SB. Further studies are needed.

In conclusion, our study provides evidence for the lack of villus atrophy in patients with cirrhosis and portal hypertension. Hence, small bowel biopsies can be interpreted reliably to exclude coeliac disease in these patients. Furthermore, findings of shortened villi and villus atrophy should trigger the exclusion of coeliac disease and other diseases of the small bowel in this group of patients.

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COMMENTS

Background

Cirrhosis affects the small bowel mucosa in ways not totally elucidated. Villus shortening and atrophy are described. But these findings are reported descriptively and without proper classification, which may affect the interpretation of small bowel biopsy when the diagnosis of coeliac disease is to be excluded in patients with cirrhosis. We aimed to study the small bowel mucosa on biopsy in cirrhotic patients with portal hypertension and in non-cirrhotic controls and grade findings according to the standardized grading system described by Marsh.

Research frontiers

Studies of recent years have shown that coeliac disease is increasingly recognized in association with chronic liver disease and cirrhosis, and coeliac disease is more common than previously thought but is under diagnosed. It is, therefore, recommended to exclude coeliac disease in patients who have cirrhosis and cryptogenic abnormality of liver tests, and the diagnostic tool of choice is a small bowel biopsy.

Innovations and breakthroughs

This study is different from other studies of the small bowel mucosa in cirrhosis and portal hypertension because we graded findings according to the standardized Marsh grading system and excluded subjects with diseases that could potentially affect the small bowel in order to assess the sole effect of portal hypertension on the small bowel mucosa. Additionally, we used the Mann-Whitney test of equivalence to support the equality of mucosal abnormalities in both study groups, and we estimated a sample size of 25 individuals in each group in order to confer a statistical power of at least 90%.

Applications

Today's medical practice emphasizes evidence based medicine. Our study provides evidence for the lack of villus atrophy in cirrhosis and portal hypertension; hence SB biopsies can be interpreted reliably to exclude coeliac disease in these patients and in future studies of the mechanisms of liver disease in patients with coeliac disease. Additionally, small bowel mucosal diseases should be excluded when villus shortening and atrophy are seen in patients with cirrhosis and portal hypertension

Peer review

This is a well designed study, and the data looks sound. Patients with liver disease of unknown etiology can have small bowel biopsies that reliably exclude coeliac disease as an association or cause of their hepatic disease.

REFERENCES

1 **Baraona E**, Orrego H, Fernandez O, Amenabar E, Maldonado E, Tag F, Salinas A. Absorptive function of the small intestine in liver cirrhosis. *Am J Dig Dis* 1962; **7**: 318-330

2 **Misra V**, Misra SP, Dwivedi M, Gupta SC. Histomorphometric study of portal hypertensive enteropathy. *Am J Clin Pathol* 1997; **108**: 652-657

3 **Astaldi G**, Strosselli E. Peroral biopsy of the intestinal mucosa in hepatic cirrhosis. *Am J Dig Dis* 1960; **5**: 603-612

4 **Such J**, Guardiola JV, de Juan J, Casellas JA, Pascual S, Aparicio JR, Sola-Vera J, Perez-Mateo M. Ultrastructural characteristics of distal duodenum mucosa in patients with cirrhosis. *Eur J Gastroenterol Hepatol* 2002; **14**: 371-376

5 **Barakat M**, Mostafa M, Mahran Z, Soliman AG. Portal hypertensive duodenopathy: clinical, endoscopic, and histopathologic profiles. *Am J Gastroenterol* 2007; **102**: 2793-2802

6 **AGA Institute Medical Position Statement on the Diagnosis and Management of Celiac Disease**. *Gastroenterology* 2006; **131**: 1977-1980

7 **Morisco F**, Pagliaro L, Caporaso N, Bianco E, Sagliocca L, Fargion S, Smedile A, Salvagnini M, Mele A. Consensus recommendations for managing asymptomatic persistent non-virus non-alcohol related elevation of aminotransferase levels: suggestions for diagnostic procedures and monitoring. *Dig Liver Dis* 2008; **40**: 585-598

8 **Bardella MT**, Valenti L, Pagliari C, Peracchi M, Fare M, Fracanzani AL, Fargion S. Searching for coeliac disease in

patients with non-alcoholic fatty liver disease. *Dig Liver Dis* 2004; **36**: 333-336
9 **Rubio-Tapia A**, Murray JA. The liver in celiac disease. *Hepatology* 2007; **46**: 1650-1658
10 **National Institutes of Health Consensus Development Conference Statement on Celiac Disease, June 28-30, 2004**. *Gastroenterology* 2005; **128**: S1-S9
11 **Kingham JG**, Parker DR. The association between primary biliary cirrhosis and coeliac disease: a study of relative prevalences. *Gut* 1998; **42**: 120-122
12 **Ludvigsson JF**, Elfstrom P, Broome U, Ekbom A, Montgomery SM. Celiac disease and risk of liver disease: a general population-based study. *Clin Gastroenterol Hepatol* 2007; **5**: 63-69.e1
13 **Volta U**, De Franceschi L, Lari F, Molinaro N, Zoli M, Bianchi FB. Coeliac disease hidden by cryptogenic hypertransaminasaemia. *Lancet* 1998; **352**: 26-29
14 **Hagander B**, Berg NO, Brandt L, Norden A, Sjolund K, Stenstam M. Hepatic injury in adult coeliac disease. *Lancet* 1977; **2**: 270-272
15 **Germenis AE**, Yiannaki EE, Zachou K, Roka V, Barbanis S, Liaskos C, Adam K, Kapsoritakis AN, Potamianos S, Dalekos GN. Prevalence and clinical significance of immunoglobulin A antibodies against tissue transglutaminase in patients with diverse chronic liver diseases. *Clin Diagn Lab Immunol* 2005; **12**: 941-948
16 **Kaukinen K**, Halme L, Collin P, Farkkila M, Maki M, Vehmanen P, Partanen J, Hockerstedt K. Celiac disease in patients with severe liver disease: gluten-free diet may reverse hepatic failure. *Gastroenterology* 2002; **122**: 881-888
17 **Bardella MT**, Fraquelli M, Quatrini M, Molteni N, Bianchi P, Conte D. Prevalence of hypertransaminasemia in adult celiac patients and effect of gluten-free diet. *Hepatology* 1995; **22**: 833-836
18 **Ludwig J**, Batts KP, Moyer TP, Poterucha JJ. Advances in liver biopsy diagnosis. *Mayo Clin Proc* 1994; **69**: 677-678
19 **Wellek S**. Testing Statistical Hypotheses of Equivalence. 1st ed. Boca Ration, London, New York, Washington, D.C.: Chapman & Hall/CRC Press, 2003: 106-111
20 **Henkel AS**, Buchman AL. Nutritional support in patients with chronic liver disease. *Nat Clin Pract Gastroenterol Hepatol* 2006; **3**: 202-209
21 **Hogenauer C**, Hammer HF. Maldigestion and Malabsorption. In: Feldman: Sleisenger & Fordtran's Gastrointestinal and Liver Disease. 8th ed. Saunders: An Imprint of Elsevier, 2006: 2200-2232
22 **Nagral AS**, Joshi AS, Bhatia SJ, Abraham P, Mistry FP, Vora IM. Congestive jejunopathy in portal hypertension. *Gut* 1993; **34**: 694-697
23 **Marsh MN**. Gluten, major histocompatibility complex, and the small intestine. A molecular and immunobiologic approach to the spectrum of gluten sensitivity ('celiac sprue'). *Gastroenterology* 1992; **102**: 330-354
24 **Green PH**, Cellier C. Celiac disease. *N Engl J Med* 2007; **357**: 1731-1743
25 **Farrell RJ**, Kelly CP. Celiac Sprue and Refractory Sprue. In: Feldman: Sleisenger & Fordtran's Gastrointestinal and Liver Disease. 8th ed. Saunders: An Imprint of Elsevier, 2006: 2277-2307
26 **Bizzaro N**, Tampoia M, Villalta D, Platzgummer S, Liguori M, Tozzoli R, Tonutti E. Low specificity of anti-tissue transglutaminase antibodies in patients with primary biliary cirrhosis. *J Clin Lab Anal* 2006; **20**: 184-189
27 **Vecchi M**, Folli C, Donato MF, Formenti S, Arosio E, de Franchis R. High rate of positive anti-tissue transglutaminase antibodies in chronic liver disease. Role of liver decompensation and of the antigen source. *Scand J Gastroenterol* 2003; **38**: 50-54
28 **Valera JM**, Hurtado C, Poniachik J, Abumohor P, Brahm J. [Study of celiac disease in patients with non-alcoholic fatty liver and autoimmune hepatic diseases] *Gastroenterol Hepatol* 2008; **31**: 8-11
29 **Lo Iacono O**, Petta S, Venezia G, Di Marco V, Tarantino G, Barbaria F, Mineo C, De Lisi S, Almasio PL, Craxi A. Anti-tissue transglutaminase antibodies in patients with abnormal liver tests: is it always coeliac disease? *Am J Gastroenterol* 2005; **100**: 2472-2477
30 **Volta U**. Pathogenesis and Clinical Significance of Liver Injury in Celiac Disease. *Clin Rev Allergy Immunol* 2009; **36:** 62-70

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RAPID COMMUNICATION

Expression of cell adhesion molecule CD44 in gastric adenocarcinoma and its prognostic importance

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Abstract

AIM: To evaluate the relation of cluster of differentiation 44 (CD44) expression with clinicopathological features of gastric adenocarcinoma, and also its effect on prognosis with an emphasis on the differences between intestinal and diffuse types.

METHODS: From 2000 to 2006, 100 patients with gastric adenocarcinoma, who had undergone total or subtotal gastrectomy without any prior treatment, were studied. Haematoxylin & eosin (HE) staining was used for histological evaluation, including the type (Lauren's classification) and grading of the tumor. The expression of CD44 in the gastric adenocarcinoma mucosa and the adjacent mucosa were determined by immunohistochemistry. The survival analysis was obtained using the Kaplan-Meier test.

RESULTS: Of 100 patients, 74 (74%) patients were male. The tumors were categorized as intestinal type (78%) or diffuse type (22%). Sixty-five percent of patients were CD44-positive. CD44 expression was not detected in normal gastric mucosa. Rather, CD44 was more commonly expressed in the intestinal subtype (P = 0.002). A significant relation was seen between the grade of tumor and the expression of CD44 (P = 0.014). The survival analysis showed a poor prognosis of patients with CD44-positive tumors (P = 0.008); and this was more prominent in the intestinal (P = 0.001) rather than diffuse type.

CONCLUSION: Cell adhesion molecule CD44 is highly expressed in gastric adenocarcinoma. CD44 expression is correlated with a poor prognosis in patients with the intestinal type of gastric adenocarcinoma. CD44 can, therefore, be utilized as a prognostic marker for this group of patients.

Key words: Gastric cancer; Immunohistochemistry; Cluster of differentiation 44; Cell adhesion molecules; Survival rate

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INTRODUCTION

Gastric malignancy is one of the leading types of cancer

and is the second most common cause of cancer-related death in the world[1,2]. According to reports from Iran's Ministry of Health, gastric cancer is the most common fatal gastrointestinal malignancy, while cancer is the third most common cause of mortality in this country[3,4]. Adenocarcinoma is the most prevalent type of gastric cancer. According to Lauren's histological classification, it is subdivided into diffuse and intestinal pathologic subtypes, each having different epidemiological and prognostic features[5,6]. Multiple genetic and epigenetic alterations in oncogenes, tumor-suppressor genes, cell-cycle regulators, cell adhesion molecules and DNA repair genes are implicated in the multistep process of human stomach carcinogenesis[6,7], and different genetic pathways have been proposed for these two subtypes of gastric carcinoma[2]. Depth of invasion and lymph node metastasis result from the polygene, and their protein expression in gastric carcinoma. The key step of the basic and clinical research of gastric carcinoma is to discover the related etiological biomarkers.

The loss of normal cellular adhesion is a significant event in human cancer development. Metastasis is characterized by a loss of adhesion that allows cancer cells to invade, and leave the site of origin, subsequently adhering to other sites, such as lymph nodes, liver, or peritoneum[8]. Cluster of differentiation 44 (CD44) is a transmembrane glycoprotein involved in cellular adhesion. This polymorphic integral membrane glycoprotein, which is expressed by many cell types, serves as the principal transmembrane hyaluronate receptor. It is considered a determinant of metastatic and invasive behavior in different malignancies, such as lung carcinoma, malignant melanoma, leukemia, breast cancer, as well as gastrointestinal carcinomas[2,9,10]. On the other hand, contradictory reports concerning the biological role of CD44 in tumorigenesis and its clinical value in prognosis have also been presented[9]. The expression of CD44 potentiates tumor cells to adhere to the extracellular matrix through ligands such as hyaluronan and facilitates the efficient formation of cell colonies[11,12].

The reported frequency of CD44 expression in human gastric carcinoma specimens varies widely from 31% to 72%, most likely reflecting the differences in the study population[13,14]. The intensity of CD44 expression has been reported to correlate with an increased depth of invasion[13], although different correlations have been reported in the two histological types of this cancer.

In this study, the expression of CD44 in patients with gastric carcinoma was measured by immunohistochemical method, with an aim to evaluate the relation of CD44 expression with clinicopathological features and also its effect on prognosis with an emphasis on the differences between intestinal and diffuse types.

MATERIALS AND METHODS

From 2000 through 2006, 100 gastric adenocarcinoma patients, who underwent total or sub-total gastrectomy without any prior treatment such as chemotherapy or radiation therapy at Omid Oncology Hospital (Mashhad, Iran), were enrolled in this study. All patients had been residing in the north-eastern provinces of Iran at the time of surgery. Demographic data of all patients were recorded and TNM staging was performed according to AJCC (American Joint Committee on Cancer) staging by supra- and infra-diaphragmatic imaging studies. Follow-up data were also gathered from patients, including local and distant recurrence and metastasis. The study protocol was approved by the Clinical Research Ethics Committee of the Mashhad University of Medical Sciences.

Formalin-fixed and paraffin wax-embedded gastric adenocarcinoma specimens from these patients were selected from the pathology archive. Specimen blocks were stained with HE and histological typing was determined according to the Lauren's classification and tumor grade (well-differentiated, moderately-differentiated and poorly-differentiated). The pathologist reviewed all the blocks and chose one block with more tumoral tissue and less necrotic tissue for immunostaining. Specimens were cut into 4-μm thick sections, and the sections were dewaxed and processed for immunohistochemical (IHC) staining. The sections were stained using the streptavidin-biotin-peroxidase complex method (Dako LSAB2 system, Denmark). Mouse anti-CD44 monoclonal antibody (1:50 dilution; clone DF1485, Dakocytomation, Denmark) which is able to detect all isoforms of CD44, was employed as the primary antibody for 30 min. Internal lymphocytes were used as positive control, and normal gastric tissue as negative control. Also, for negative control, the primary antibody was omitted.

All sections were evaluated by the pathologist who was unaware of the clinical outcome of the patients. Tumors with more than 5% of CD44-positive cancer cells were regarded as positive. The results were reported as positive (cytoplasmic and/or membranous staining) or negative with the percentage of positive cells for each section.

Statistical analysis

The correlation between the CD44 expression status and clinicopathological variables was analyzed using parametric and non-parametric tests run by the statistical software SPSS version 13. Also a log-linear model by the statistical software SAS was used for more detailed analysis of the data. The survival analysis was performed by the Kaplan-Meier test. A P value less than 0.05 was considered statistically significant.

RESULTS

This study included 100 gastric adenocarcinoma cases (74 males and 26 females; male/female ratio: 2.8), with a mean age of 63.3 years (range 26-82 years). Data about stage, grade, histological types and location of tumors are summarized in Table 1. Forty-seven percent of patients were less than 65 years old. The mean age of patients with poorly differentiated tumors was

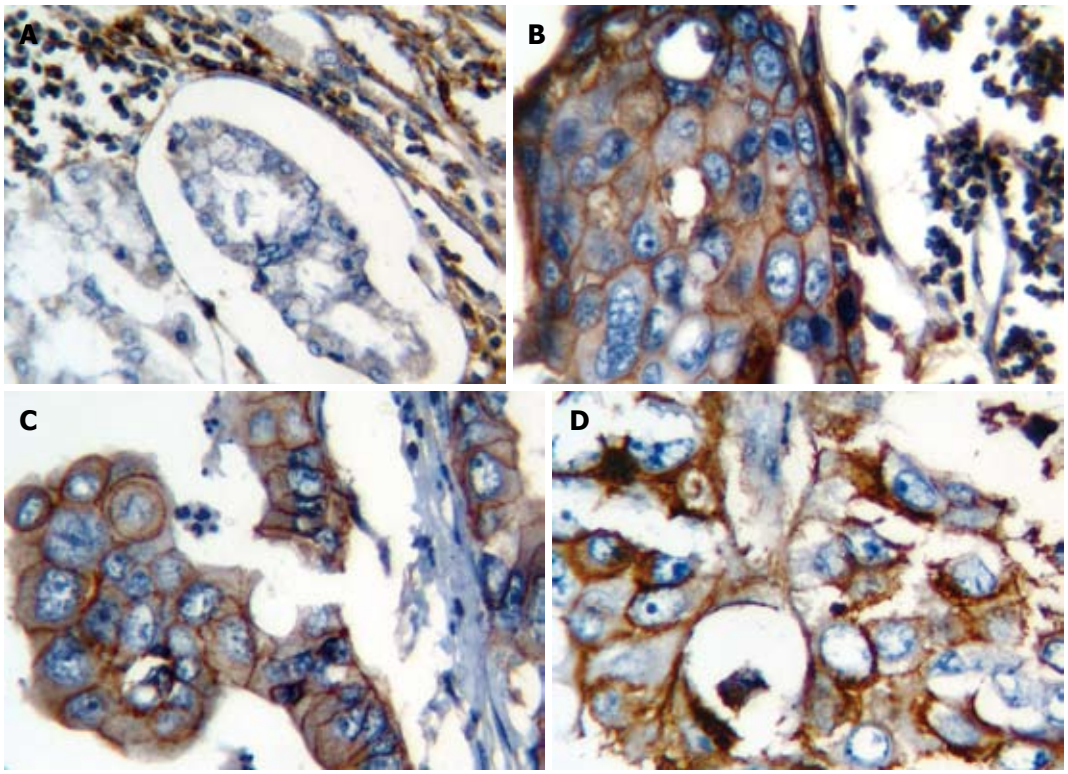

Figure 1 Representative example demonstrating the immunohistochemical staining of CD44 molecule in gastric adenocarcinoma tissue. A: Negative CD44 staining in adenocarcinoma glands and positive staining in the background lymphocytes (× 40); B: Membranous expression of CD44 in carcinoma cells with the stained lymphocytes as internal positive control in the background (× 40); C: Positive membranous staining of CD44 in carcinoma cells (x 40); D: Membranous and cytoplasmic expression of CD44 in carcinoma cells (× 40).

Table 1 Clinicopathological data of all patients

Data	Percentage (%)
Sex	
M	74
F	26
Age (yr)	
< 65	47
> 65	53
Location	
Cardia	37
Body & Fundus	33
Antrum	30
Histology type	
Intestinal	78
Diffuse	22
Grade	
Well-Diff	54
Mod-Diff	17
Poor-Diff	29
T	
T1	0
T2	5
T3	84
T4	11
N	
N0	16
N1	51
N2	30
N3	2
M	
M0	98
M1	2
Stage	
1	3
2	13
3	71
4	13

Diff: Differentiated; Mod: Moderately; Poor: Poorly; M: Male; F: Female.

significantly lower than other patients (55 years *vs* 65 years, $P = 0.019$). Stage Ⅳ disease was significantly more common among patients younger than 65 years ($P = 0.02$). All stage I cases were observed among patients older than 65 years. The diffuse-type cancer was two times higher among the patients younger than 65 years ($P = 0.021$).

Sixty-five percent of patients were CD44-positive and 35% were CD44-negative. CD44 staining was not detected in any adjacent normal gastric mucosa (Figure 1). Of 65 CD44-positive cases, 52 showed CD44 staining only on tumor cell surface membrane, while 13 showed both in cytoplasm and membrane.

In addition, a significant difference in CD44 expression was seen between the two histological subtypes: intestinal-type showed a significantly higher CD44 positivity (71%) compared to diffuse-type (42%) ($P = 0.002$). Moreover, poorly differentiated carcinomas showed a significantly less CD44 positivity, indicating a significant relation between CD44 expression, and the grade of tumor ($P = 0.014$; log linear, $P = 0.004$). Patients with stage 4 cancer expressed CD44 more than other stages, although this was not statistically significant. Other studied variables (sex, age and location of tumor) did not show any significant correlation with the expression of CD44.

Follow-up data were gathered for 71 patients. The median follow-up time was 6 mo. The overall survival was 16 mo. Regarding the results of survival analysis, there was no correlation between sex, age, location, type and the grade of tumor with prognosis. Poor outcome was seen among the patients with higher stage tumors (stages Ⅲ and Ⅳ, $P = 0.034$) compared to those with lower stages. In addition, the patients with CD44-positive tumors showed a poor prognosis (Log Rank, $P = 0.008$,

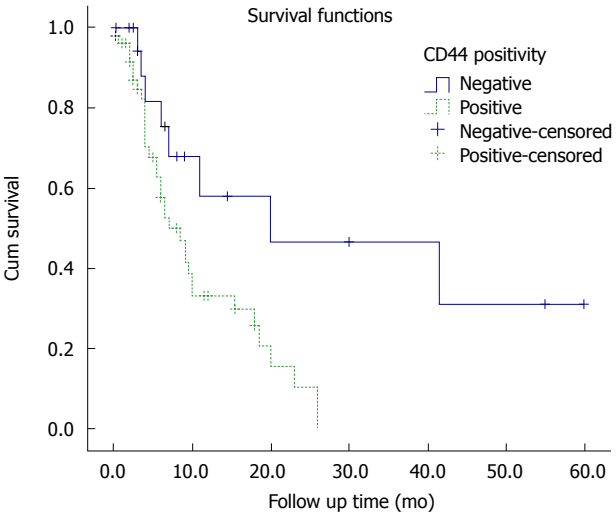

Figure 2 Kaplan-Meier curves of overall survival for CD44-positive and -negative gastric cancer cases.

Figure 2). Among patients with lower stage tumors, CD44 expression affected the prognosis regardless of tumor stage ($P = 0.040$). When analyzing the correlation of CD44 positivity, and intestinal/diffuse-type tumors separately, the results showed that CD44 only affected survival time in intestinal-type ($P = 0.001$), but not the diffuse-type tumors ($P = 0.7$).

DISCUSSION

The present study examined the expression of CD44 as an adhesion molecule in primary tumors by utilizing the IHC technique in 100 patients with gastric adenocarcinoma who had undergone surgery as the first step of management. The results showed that the expression rate of CD44 in tumoral tissue reached 65% in contrast to no expression of this molecule in the adjacent normal mucosa. CD44 was more commonly expressed in the intestinal-type tumor than in diffuse-type tumor, indicating that CD44 expression was related to the histological subtype of tumor, which is in agreement with previous studies[15,16]. In addition, a correlation was found between CD44 expression and tumor grade. But, no correlations between CD44 and other clinicopathological parameters were observed. Survival analysis showed that the grade and the histological type of gastric adenocarcinoma did not significantly affect survival. On the other hand, patients with higher stage adenocarcinoma showed a poor prognosis. CD44 was shown to affect survival, even more significantly than, and independently from the stage of the tumor. Moreover, CD44 positivity appeared to provide a poor prognosis only in patients with the intestinal-type gastric adenocarcinoma.

Since 1993 when Hieder *et al*[17] reported the expression of CD44 variants in gastric cancer, this adhesive molecule was recognized as a molecular marker related to the clinicopathological aspects of gastric cancer. CD44 is a member of a widely expressed family of adhesion receptors. This molecule was first described as a lymphocyte-homing receptor; however, it is expressed on a wide variety of cell types including mature T and B-cells[9,18-20]. Metastasizing tumor cells and recirculating (activated) lymphocytes share several properties, including motility and invasive behavior, an analogy which prompted the hypothesis that malignant cells might use molecules like CD44 for metastasizing[9]. *CD44* gene is located on chromosome 11p12-13, having at least 20 exons, of which ten are expressed in hematopoietic cells or the standard form of the gene. Other exons can be alternatively spliced to make up a wide variety of CD44 splice variants which have been found in various types of human malignancies, and have been considered markers in tumor progression and metastasis[15].

It has been supposed that the evaluation of the CD44 isoforms expression by the IHC method in cases of non-Hodgkin lymphoma, colon and renal cell carcinomas, as well as neuroblastomas may be a useful diagnostic parameter indicating invasive processes[21]. In 1994, Yokozaki *et al*[22] indicated that the detection of CD44 transcription variants can serve as a powerful tool for the diagnosis of gastric cancer. Many studies have indicated that the generation of CD44 splice variants, like V5 and V6, might be linked closely to gastric carcinoma tumorigenesis and differentiation, suggesting that these isoforms can be used as an indicator of tumor progression in the biopsies of patients with gastric carcinoma[23-28]. Moreover, it has been shown that patients with an over-expression of CD44 have a higher lymph node metastatic rate and invasion[27].

It is possible that different results among studies reflect the use of diverse antibodies having subtle variations in specificity. The problem is complicated more by the existence of numerous CD44 isoforms, which may have remarkable homology in their antigenic repertoire, thus increasing the possibility of cross-reactivity between the antibodies. Another reason for such discrepancies is probably the comparison of results having different techniques[9].

The correlation between clinicopathological parameters and CD44 expression in the tumor is known to be different between intestinal and diffuse types of gastric carcinoma[29,30]. A study showed that the intestinal-type was more frequently CD44s- and CD44v6-positive than the diffuse-type tumor[15], although the reactivity to these two antibodies did not correlate with histopathological and clinical prognostic factors in intestinal-type carcinoma[31]. More recently, Yamaguchi *et al*[16] found that the expression of CD44v6 protein was significantly higher in differentiated adenocarcinoma than in diffuse-type carcinoma. On the other hand, Saito *et al*[29] observed that CD44v6 appeared to play an important role in the invasion and metastasis of diffuse-type gastric carcinoma, but not in that of intestinal-type gastric carcinoma, and also demonstrated a significant correlation between CD44v6 expression and poor prognosis of diffuse-type gastric carcinoma.

The results of this study are consistent with previous findings, demonstrating that CD44 is mostly expressed

in the intestinal-type gastric cancer, and its expression is associated with poor prognosis. Thus, it can be concluded that the expression of CD44 is related to the phenotype of gastric malignancy, and may serve as a useful indicator of tumor metastasis, and may have a potential significance in diagnosing gastric cancer.

The mortality associated with gastric carcinoma is almost entirely caused by a subsequent metastatic disease. In fact, the prognostic assessment of gastric carcinoma still relies mainly on TNM staging, but the wide individual variability in prognosis is observed even in the same stages. The accurate prediction of the metastatic potential of the primary tumor, and hence the probable existence of undetected metastases, would be a critical factor in the management of patients with gastric carcinoma[14]. Our results emphasize that expression of CD44 is related to the prognosis of intestinal-type gastric cancer.

In conclusion, this study demonstrated that detection of CD44 protein in routinely fixed gastric carcinoma tissue by the IHC method can be used, along with other established parameters, to assess prognostic outcome, and particularly, to identify patients with a poor short-term prognosis. Furthermore, this suggests that, in the future, assessment of CD44 expression may guide the clinician in delineating a subset of patients with biologically unfavorable tumors who may profit from more intense post-operative adjuvant therapy.

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COMMENTS

Background

Gastric cancer is the 2nd most common cause of cancer-related death in the world. The mortality associated with gastric carcinoma is almost entirely caused by a subsequent metastatic disease. The accurate prediction of the metastatic potential of the primary tumor would be a critical factor in the management of patients with gastric carcinoma. Metastasis is characterized by a loss of adhesion that allows cancer cells to invade and leave the site of origin, subsequently adhering to other sites such as lymph nodes, liver, or peritoneum. Cluster of differentiation 44 (CD44), as an important glycoprotein involved in cellular adhesion, is considered a determinant of metastatic and invasive potential in different malignancies. There are different and even contradictory reports considering the role of this adhesive molecule in gastric carcinogenesis and metastasis.

Research frontiers

While there was no expression in surrounding normal tissue, CD44 expression rate in gastric adenocarcinoma was up to 65%, considering that the intestinal-type tumor expressed this marker, more common than diffuse-type tumor. Beside the prognostic effect of stage, CD44 was shown to affect survival, even more significantly than and independently from the stage of the tumor and this was more pronounced in intestinal-type.

Innovations and breakthroughs

For the first time in Iranian patients, this study demonstrated that detection of the CD44 protein in routinely fixed gastric carcinoma tissue by the IHC method can be used, along with other established parameters, to assess prognostic outcome, and particularly, to identify patients with a poor short-term prognosis.

Applications

In the future, assessment of CD44 expression may guide the clinician in delineating a subset of patients with biologically unfavorable tumors who may benefit from more intense postoperative adjuvant therapy.

Peer review

The results showed that the over-expression of cell adhesion molecule CD44 is correlated with a poor prognosis in patients with the intestinal-type gastric adenocarcinoma. CD44 can, therefore, be utilized as a prognostic marker for this group of patients.

REFERENCES

1 **Henson DE**, Dittus C, Younes M, Nguyen H, Albores-Saavedra J. Differential trends in the intestinal and diffuse types of gastric carcinoma in the United States, 1973-2000: increase in the signet ring cell type. *Arch Pathol Lab Med* 2004; **128**: 765-770

2 **Abbaszadegan MR**, Moaven O, Sima HR, Ghafarzadegan K, A'rabi A, Forghani MN, Raziee HR, Mashhadinejad A, Jafarzadeh M, Esmaili-Shandiz E, Dadkhah E. p16 promoter hypermethylation: a useful serum marker for early detection of gastric cancer. *World J Gastroenterol* 2008; **14**: 2055-2060

3 **Taghavi N**, Nasrollahzadeh D, Merat S, Yazdanbod A, Hormazdi M, Sotoudeh M, Semnani S, Eslami F, Marjani HA, Fahimi S, Khademi H, Malekzadeh R. Epidemiology of upper gastrointestinal cancers in Iran: a sub site analysis of 761 cases. *World J Gastroenterol* 2007; **13**: 5367-5370

4 **Yaghoobi M**, Rakhshani N, Sadr F, Bijarchi R, Joshaghani Y, Mohammadkhani A, Attari A, Akbari MR, Hormazdi M, Malekzadeh R. Hereditary risk factors for the development of gastric cancer in younger patients. *BMC Gastroenterol* 2004; **4**: 28

5 **Panani AD**. Cytogenetic and molecular aspects of gastric cancer: clinical implications. *Cancer Lett* 2008; **266**: 99-115

6 **Kountouras J**, Zavos C, Chatzopoulos D, Katsinelos P. New aspects of Helicobacter pylori infection involvement in gastric oncogenesis. *J Surg Res* 2008; **146**: 149-158

7 **Tamura G**. Alterations of tumor suppressor and tumor-related genes in the development and progression of gastric cancer. *World J Gastroenterol* 2006; **12**: 192-198

8 **Jothy S**. CD44 and its partners in metastasis. *Clin Exp Metastasis* 2003; **20**: 195-201

9 **Zavrides HN**, Zizi-Sermpetzoglou A, Panousopoulos D, Athanasas G, Elemenoglou I, Peros G. Prognostic evaluation of CD44 expression in correlation with bcl-2 and p53 in colorectal cancer. *Folia Histochem Cytobiol* 2005; **43**: 31-36

10 **Wang DR**, Chen GY, Liu XL, Miao Y, Xia JG, Zhu LH, Tang D. CD44v6 in peripheral blood and bone marrow of patients with gastric cancer as micro-metastasis. *World J Gastroenterol* 2006; **12**: 36-42

11 **Sneath RJ**, Mangham DC. The normal structure and function of CD44 and its role in neoplasia. *Mol Pathol* 1998; **51**: 191-200

12 **Kajita M**, Itoh Y, Chiba T, Mori H, Okada A, Kinoh H, Seiki M. Membrane-type 1 matrix metalloproteinase cleaves CD44 and promotes cell migration. *J Cell Biol* 2001; **153**: 893-904

13 **Setala L**, Lipponen P, Tammi R, Tammi M, Eskelinen M, Alhava E, Kosma VM. Expression of CD44 and its variant isoform v3 has no prognostic value in gastric cancer. *Histopathology* 2001; **38**: 13-20

14 **Yoo CH**, Noh SH, Kim H, Lee HY, Min JS. Prognostic significance of CD44 and nm23 expression in patients with stage II and stage IIIA gastric carcinoma. *J Surg Oncol* 1999; **71**: 22-28

15 **Dammrich J**, Vollmers HP, Heider KH, Muller-Hermelink HK. Importance of different CD44v6 expression in human gastric intestinal and diffuse type cancers for metastatic lymphogenic spreading. *J Mol Med* 1995; **73**: 395-401

16 **Yamaguchi A**, Goi T, Yu J, Hirono Y, Ishida M, Iida A, Kimura T, Takeuchi K, Katayama K, Hirose K. Expression

of CD44v6 in advanced gastric cancer and its relationship to hematogenous metastasis and long-term prognosis. *J Surg Oncol* 2002; **79**: 230-235
17 **Heider KH**, Dammrich J, Skroch-Angel P, Muller-Hermelink HK, Vollmers HP, Herrlich P, Ponta H. Differential expression of CD44 splice variants in intestinal- and diffuse-type human gastric carcinomas and normal gastric mucosa. *Cancer Res* 1993; **53**: 4197-4203
18 **Pure E**, Cuff CA. A crucial role for CD44 in inflammation. *Trends Mol Med* 2001; **7**: 213-221
19 **Yoo CH**, Noh SH. The Serum Assay of Soluble CD44 Standard, CD44 Variant 5, and CD44 Variant 6 in Patients with Gastric Cancer. *Cancer Res Treat* 2003; **35**: 3-8
20 **Marhaba R**, Zoller M. CD44 in cancer progression: adhesion, migration and growth regulation. *J Mol Histol* 2004; **35**: 211-231
21 **Gunthert U**, Stauder R, Mayer B, Terpe HJ, Finke L, Friedrichs K. Are CD44 variant isoforms involved in human tumour progression? *Cancer Surv* 1995; **24**: 19-42
22 **Yokozaki H**, Ito R, Nakayama H, Kuniyasu H, Taniyama K, Tahara E. Expression of CD44 abnormal transcripts in human gastric carcinomas. *Cancer Lett* 1994; **83**: 229-234
23 **Stock M**, Otto F. Gene deregulation in gastric cancer. *Gene* 2005; **360**: 1-19
24 **Chen JQ**, Zhan WH, He YL, Peng JS, Wang JP, Cai SR, Ma JP. Expression of heparanase gene, CD44v6, MMP-7 and nm23 protein and their relationship with the invasion and metastasis of gastric carcinomas. *World J Gastroenterol* 2004; **10**: 776-782
25 **Hsieh HF**, Yu JC, Ho LI, Chiu SC, Harn HJ. Molecular studies into the role of CD44 variants in metastasis in gastric cancer. *Mol Pathol* 1999; **52**: 25-28
26 **Joo M**, Lee HK, Kang YK. Expression of E-cadherin, beta-catenin, CD44s and CD44v6 in gastric adenocarcinoma: relationship with lymph node metastasis. *Anticancer Res* 2003; **23**: 1581-1588
27 **Liu YJ**, Yan PS, Li J, Jia JF. Expression and significance of CD44s, CD44v6, and nm23 mRNA in human cancer. *World J Gastroenterol* 2005; **11**: 6601-6606
28 **Chen GY**, Wang DR. The expression and clinical significance of CD44v in human gastric cancers. *World J Gastroenterol* 2000; **6**: 125-127
29 **Saito H**, Tsujitani S, Katano K, Ikeguchi M, Maeta M, Kaibara N. Serum concentration of CD44 variant 6 and its relation to prognosis in patients with gastric carcinoma. *Cancer* 1998; **83**: 1094-1101
30 **Castella EM**, Ariza A, Pellicer I, Fernandez-Vasalo A, Ojanguren I. Differential expression of CD44v6 in metastases of intestinal and diffuse types of gastric carcinoma. *J Clin Pathol* 1998; **51**: 134-137
31 **Hong RL**, Lee WJ, Shun CT, Chu JS, Chen YC. Expression of CD44 and its clinical implication in diffuse-type and intestinal-type gastric adenocarcinomas. *Oncology* 1995; **52**: 334-339

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RAPID COMMUNICATION

Continuous regional arterial infusion therapy with gabexate mesilate for severe acute pancreatitis

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Abstract

AIM: To evaluate the efficacy of continuous regional arterial infusion therapy (CRAI) with gabexate mesilate and antibiotics for severe acute pancreatitis (SAP).

METHODS: We conducted a prospective study on patients who developed SAP with or without CRAI. Out of 18 patients fulfilled clinical diagnostic criteria for SAP in Japan, 9 patients underwent CRAI, while 9 patients underwent conventional systemic protease inhibitor and antibiotics therapy (non-CRAI). CRAI was initiated within 72 h of the onset of pancreatitis. Gabexate mesilate (2400 mg/d) was continuously administered for 3 to 5 d. The clinical outcome including serum inflammation-related parameters were examined.

RESULTS: The duration of abdominal pain in the CRAI group was 1.9 ± 0.26 d, whereas that in the non-CRAI group was 4.3 ± 0.50. The duration of SIRS in the CRAI group was 2.2 ± 0.22 d, whereas that in the non-CRAI group was 3.2 ± 0.28. Abdominal pain and SIRS disappeared significantly in a short period of time after the initiation of CRAI using gabexate mesilate. The average length of hospitalization significantly differed between the CRAI and non-CRAI groups, 53.3 ± 7.9 d and 87.4 ± 13.9 d, respectively. During the first two weeks, levels of serum CRP and the IL6/IL10 ratio in the CRAI group tended to have a rapid decrease compared to those in the non-CRAI group.

CONCLUSION: The present results suggest that CRAI using gabexate mesilate was effective against SAP.

Key words: Severe acute pancreatitis; Arterial infusion; Gabexate mesilate; Antibiotics

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INTRODUCTION

Severe acute pancreatitis (SAP) remains a lethal disease. It is defined as an inflammatory process of the pancreas with possible peripancreatic tissue, and multi-organ involvement inducing multi-organ dysfunction syndrome (MODS) with an increased mortality rate[1,2]. Continuous regional arterial infusion (CRAI) with protease inhibitor nafamostat mesilate and antibiotics has been proven to be effective as an initial therapy in Japan[2]. However, evidence supporting the benefit of CRAI in treating acute pancreatitis is insufficient, and its advisability according to the JPN guidelines for the management

of acute pancreatitis is classed as "Recommendation C"[3]. In the statement, it was described that CRAI with protease inhibitors and antibiotics may possibly reduce the mortality rate and incidence of infectious complications in necrotizing pancreatitis. Actually, until now, most cases have been treated with the protease inhibitor nafamostat mesilate. Here, we performed CRAI using gabexate mesilate to treat SAP, and investigated the clinical benefits including serum inflammation-related parameters such as cytokines and chemokines.

MATERIALS AND METHODS

Patients

The severity of acute pancreatitis was assessed within 48 h of admission according to the diagnostic criteria for the diagnosis of acute pancreatitis by the Research Committee for Intractable Diseases of the Pancreas in Japan by Ministry of Health, Labour and Welfare Japan (Tables 1 and 2)[4-6]. A total of 18 patients fulfilling clinical diagnostic criteria for SAP at six participating institutions were selected for the present study. Nine patients underwent CRAI (CRAI group), while 9 patients underwent conventional systemic protease inhibitor and antibiotics therapy (non-CRAI group). Each institution made the decision to perform CRAI or non-CRAI therapy, so the present study was not a randomized controlled trial. Clinical features of both groups were shown in Table 3. All 9 patients in the CRAI group were men, average age 48.0 ± 13.4 years (mean ± SD). The cause of SAP was alcohol (n = 5), gallstone (n = 1), hyperlipidemia (n = 1), post-endoscopic retrograde cholangiopancreatography (ERCP, n = 1), or unknown (n = 1). On the other hand, 4 of the 9 patients in the non-CRAI group were male and 5 were female (average age of group, 59.9 ± 15.1 years; mean ± SD). Regarding age at onset, no significant difference was observed between CRAI group and non-CRAI group (P = 0.0979). The causes of SAP patients in the non-CRAI group were gallstones (n = 4), alcohol (n = 3), post-ERCP (n = 1), or unknown (n = 1). All patients in both groups were diagnosed as stage 2 SAP. CRAI was initiated within 72 h of the onset of pancreatitis. A 5-Fr shepherd's catheter was placed in either the celiac artery (including the splenic and gastro-duodenal arteries) or in the supra-mesenteric artery, and gabexate mesilate (2400 mg/d) was continuously administered for 3-5 d. Antibiotics were administered every 12 h (panipenem in 5 patients, meropenem in 2 patients, imipenem in 1 patient, and piperacillin in 1 patient). Catheters were placed in the superior mesenteric, celiac, splenic, and gastroduodenal arteries of 3, 3, 2, and 1 patient, respectively. Complications in one patient comprised thrombosis of the superior mesenteric artery, and warfarin was administered. Carbapenem antibiotics were administered to all patients in the non-CRAI group.

Measured parameters

The duration of abdominal pain and of systemic inflammatory response syndrome (SIRS) as well as the length of hospitalization were recorded. As biochemical markers of pancreatitis, the levels of serum pancreatic amylase (P-amylase), the white blood counts (WBC), and C-reactive protein (CRP) were examined on day 0 (onset of pancreatitis), day 1, day 3, day 7, and day 14. ELISAs were performed to determine serum IL-6, IL-8, IL-10, TNF-α, and MCP-1 concentrations on day 0 (onset of pancreatitis), day 1, day 3, day 7, and 14. Samples were

Table 1 Criteria for grading the severity of acute pancreatitis in Japan[4]

Prognostic factors	Clinical signs	Laboratory data
Prognostic factor Ⅰ (2 points for each positive factor)	Shock Respiratory failure Mental disturbance Severe infection Hemorrhagic diathesis	BE ⩽ -3 mmol/L Ht ⩽ 30% (after hydration) BUN ⩾ 40 mg/dL or creatinine ⩾ 2.0 mg/dL
Prognostic factor Ⅱ (1 points for each positive factor)		PaO_2 ⩽ 60 mmHg (room air) FBS ⩾ 200 mg/dL Total protein ⩽ 60 g/L LDH ⩾ 700 IU/L Ca ⩽ 7.5 mg/dL Prothrombin time ⩾ 15 s Platelet count ⩽ $1 \times 10^5/mm^3$ CT grade Ⅳ or Ⅴ
Prognostic factor Ⅲ	SIRS score ⩾ 3 (2 points) Age ⩾ 70 yr (1 point)	

BE: Base excess; Ht: Hematocrit; BUN: Blood urea nitrogen; FBS: Fasting blood sugar; LDH: Lactate dehydrogenase; SIRS: Systemic inflammatory response syndrome. CT grade Ⅳ or Ⅴ: Presence of diffuse and uneven density in the pancreatic parenchyma or the presence of inflammatory changes extending beyond the border of the pancreas. Severity score: Sum of the points for the positive prognostic factors is defined as the severity score. Standardized criteria: Severe, presence of more than one prognostic factor Ⅰ, and/or the presence of more than two prognostic factor Ⅱ (severity score ⩾ 2 points); Moderate, presence of one prognostic factor Ⅱ (severity score = 1 point); Mild, acute pancreatitis without prognostic factor Ⅰ or Ⅱ (severity = 0 point).

Table 2 Stage classification of acute pancreatitis and mortality rate in 2003 in Japan[4]

Stage	Severity score	Severity	No. of patients (%)	Died	Mortality rate (%)
Stage 0	0 point	Mild	943 (53.3)	1	0.1
Stage 1	1 point	Moderate	280 (15.8)	2	0.7
Stage 2	2-8 points	Severe Ⅰ	455 (25.7)	17	3.7
Stage 3	9-14 points	Severe Ⅱ	63 (3.6)	16	25.4
Stage 4	⩾ 15 points	Most severe	27 (1.5)	16	59.3
Total			1786 (100)	52	2.9

In 2004, nationwide survey of patients with acute pancreatitis in Japan who visited the hospitals in the year 2003 (from January 1 to December 31) was performed by stratified random sampling method. From the first survey, the total number of patients treated for acute pancreatitis in Japan in the year 2003 was estimated as 35300 (95% confidence interval, 30500-40000). Clinical records of 1768 patients with acute pancreatitis were obtained in the second survey for analysis of etiology and outcome. Number of patients who died of acute pancreatitis or related complications.

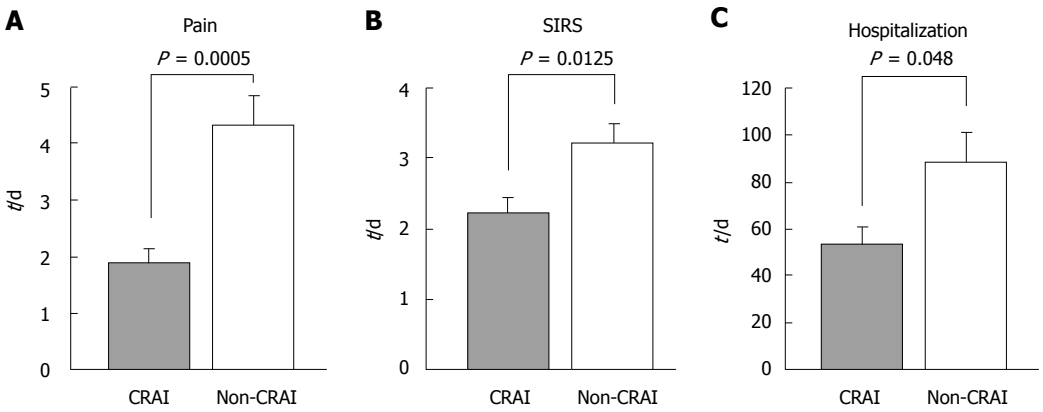

Figure 1 Changes in clinical parameters. The duration of abdominal pain (A) and of systemic inflammatory response syndrome (SIRS) (B) as well as the length of hospitalization (C) were investigated. Grey columns represent data for continuous regional arterial infusion using gabexate mesilate with antibiotics (CRAI-group) and white columns for non-CRAI group. Values are expressed as mean ± SE.

Table 3 The clinical features of patients

	CRAI group (n = 9)	Non-CRAI group (n = 9)
Mean age	48.0 ± 13.4	59.8 ± 15.1
Gender (male/female)	9/0	4/5
Cause of pancreatitis		
Alcoholic	5	3
Biliary	1	4
Hyperlipidemia	1	0
post-ERCP	1	1
Idiopathic	1	1
Severity score		
Mean score (range)	4.0 (2-7)	3.6 (2-7)

determined with commercially available kits according to the manufacturer's instructions for human IL-6, human IL-8, human IL-10, human TNF-α, and human MCP-1 (Biosource, Camarillo, CA, USA).

Statistical analysis

Results are expressed as mean ± SE. We analyzed the duration of abdominal pain, SIRS as well as the length of hospitalization using the proportional hazard model. Serum pancreatic enzymes, inflammation-related parameters, cytokines and chemokines were analyzed using the non-parametric Mann-Whitney U test. P values < 0.05 were considered significant. Pearson's correlation analysis was used to calculate correlations between the data.

RESULTS

Duration of abdominal pain, SIRS, and hospitalization

The duration of abdominal pain in the CRAI group was 1.9 ± 0.26 d (range, 1-3), whereas the duration in the non-CRAI group was 4.3 ± 0.50 (range, 3-8). Abdominal pain disappeared significantly in a short period of time after the initiation of CRAI with the protease inhibitor (P = 0.0005, Figure 1A). Similarly, SIRS disappeared significantly and shortly after the initiation of CRAI (P = 0.0125, Figure 1B). The duration of SIRS in the CRAI group was 2.2 ± 0.22 d (range, 1-3), whereas the duration in the non-CRAI group was 3.2 ± 0.28 (range, 2-4). The average length of hospitalization significantly differed between both groups, 53.3 ± 7.9 and 87.4 ± 13.9 d for the CRAI and non-CRAI, respectively. Patients in the CRAI group discharged significantly in a short period of time after the initiation of CRAI with gabexate mesilate (P = 0.048, Figure 1C).

Changes in serum inflammation-related parameters

P-amylase and WBC quickly decreased, with no significant differences between the groups (Figure 2A and B). During the first two weeks of therapy, levels of serum CRP in the CRAI group rapidly decreased (Figure 2C). IL-6 and IL-10 in the CRAI group rapidly decreased in the same manner as the IL-6/IL-10 ratio (Figure 2D). On the other hand, both CRP and IL-6/IL-10 in the non-CRAI group tended to decrease slowly with a 2-d delay in peak values compared to those in the CRAI group, with no significant differences between the groups. Levels of serum IL-8, TNF-α, and MCP-1 over time did not significantly differ between the two groups (data not shown).

DISCUSSION

Protease inhibitors are widely applied to treat acute pancreatitis in Japan; but since randomized controlled trials (RCTs) are difficult to conduct on patients with acute pancreatitis, only five RCTs have been examined gabexate mesilate[7-11]. The results of a meta-analysis of 4 among 5 trials were negative, and indicated that gabexate mesilate does not lower rates of surgical intervention or mortality. One of the reason was considered as follows; the protease inhibitors used to treat acute necrotizing pancreatitis cannot easily reach the pancreas when administered intravenously, and, because of ischemia or impaired microcirculation, they hardly penetrate into pancreatic tissue[12,13]. However, Chen *et al*[11] conducted an RCT and reported that continuous intravenous administration of high doses of gabexate mesilate (2400 mg/d) decreased the incidence of complications and mortality. On the other hand, since Takeda *et al*

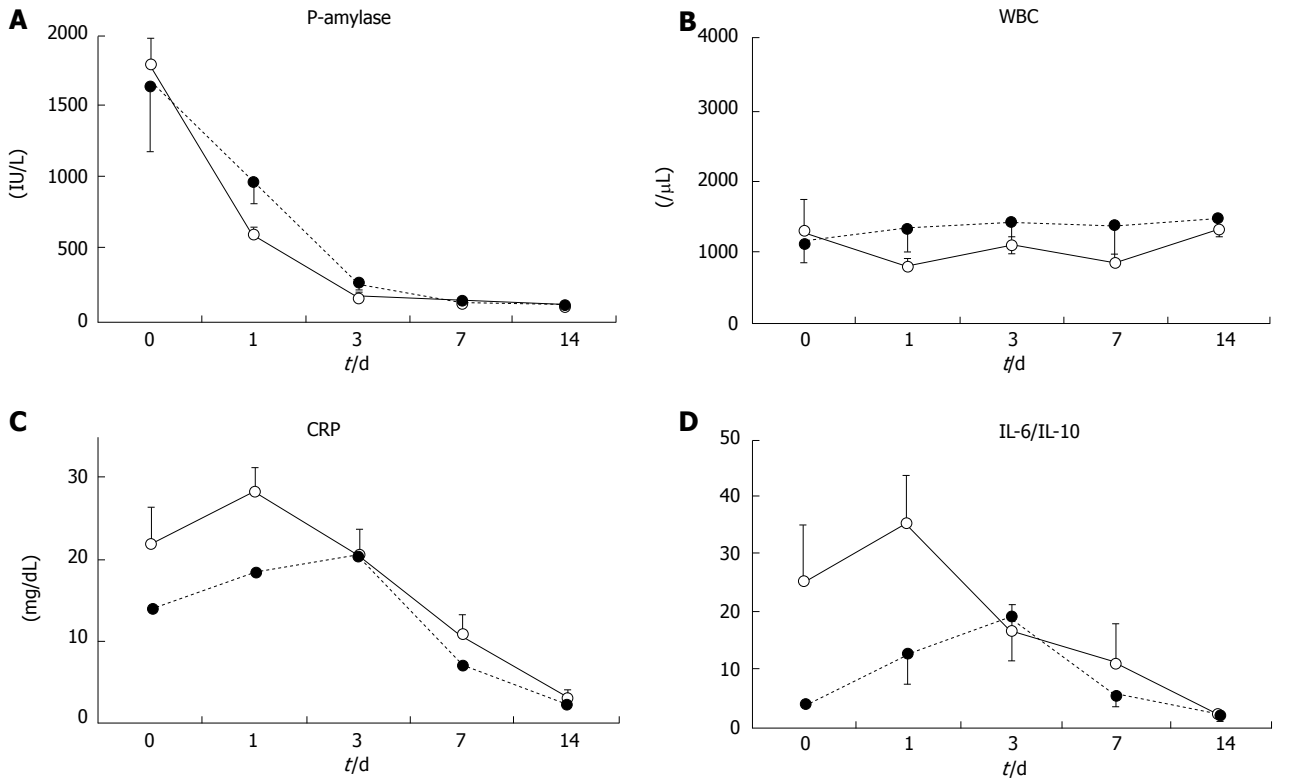

Figure 2 Changes in serum inflammation-related parameters. The levels of serum pancreatic amylase (P-amylase) (**A**), the white blood counts (WBC) (**B**), C-reactive protein (CRP) (**C**, and IL-6/IL-10 ratio **D**) were examined on days 0 (onset of pancreatitis), 1, 3, 7, and 14. Straight lines give data for continuous regional arterial infusion using gabexate mesilate with antibiotics (CRAI-group) and dotted lines for non-CRAI group. Values are expressed as mean ± SE. No significant differences between the groups were observed.

described arterial infusion with a protease inhibitor together with an antibiotic in Japan, severe acute pancreatitis has been treated by CRAI with nafamostat mesilate, and whereas an RCT has not been conducted, the usefulness of CRAI has been documented[14-17]. This strategy suppresses early inflammation and infection in pancreatic tissue, which controls subsequent systemic inflammation. The level of protease inhibitor in pancreatic tissues after CRAI using nafamostat was 5-fold higher than that delivered by intravenous injection, and trypsin activities in pancreatic tissues are significantly suppressed by CRAI[18]. On the other hand, the level of protease inhibitor in pancreatic tissues after CRAI using gabexate mesilate was 32-fold higher than that delivered by intravenous injection[19]. However, until now, CRAI using gabexate mesilate has not been examined sufficiently. In the present study, therefore, we investigated the usefulness of CRAI using gabexate mesilate for patients with SAP. The reasons for using gabexate mesilate were as follows: (1) Gabexate mesilate is the only intravenous protease inhibitor that has been proven effective in an RCT[11,20]. (2) Gabexate mesilate has a higher anticoagulant capacity than nafamostat mesilate[21]. (3) Gabexate mesilate induces less hyperkalemia even at high doses compared to nafamostat mesilate[22]. (4) In Japan, most studies on CRAI have used nafamostat mesilate, and more needs to be understood about gabexate mesilate.

All patients in this study had stage 2 pancreatitis, and since the severity was relatively mild, the patients were discharged in good health without requiring surgical intervention. The duration of pain, SIRS, and hospitalization was shorter for the CRAI group than the non-CRAI group. Previous studies of CRAI evaluated the mortality rates and surgical intervention in lethal SAP; but the present study suggested that CRAI is also effective against relatively milder forms of non-lethal SAP.

Blood cytokines and chemokines play important roles in the progression of severe acute pancreatitis. Local release of the proinflammatory cytokines, IL-18, TNF-α, and IL-1 upregulates IL-6. Levels of anti-inflammatory cytokines such as IL-10 also increase to maintain homeostasis. Excessive proinflammatory responses advance SIRS, and activated neutrophils and endothelial cells damage multiple organs. Ohmoto *et al*[23] reported that, during the healing process of acute pancreatitis, the IL-10/IL-6 ratio initially decreased, but increased as the pancreatitis improved. Put another way, IL-6/IL-10 ratio reveals an increase in a more severe stage of acute pancreatitis. We found here that IL-6 and IL-10 levels quickly increased and then decreased with therapy. The changes in the IL-6/IL-10 ratio were the same as those in CRP, but the ratio tended to decrease 2 d earlier in the CRAI group than in the non-CRAI group. These findings suggested that CRAI using gabexate mesilate effectively treats acute pancreatitis regarding biochemical features. On the other hand, changes in other

proinflammatory cytokines such as IL-8 and TNF-α were not significant. However, among patients with relatively mild stage 2 SAP in the present study, the release of these cytokines in tissues was insufficient to increase and reflect in their blood concentrations.

Essentially, a large-scale RCT should be necessary to verify the effects of CRAI; but to conduct such a study on patients with highly lethal SAP seems to be unethical in Japan. A future RCT might consider enrolling patients with stage 2 pancreatitis that is relatively mild and less fatal than in the present study. In conclusion, the present results suggest that CRAI using gabexate mesilate was effective against SAP in terms of yielding clinical benefits for patients with SAP.

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COMMENTS

Background

Severe acute pancreatitis (SAP) remains a lethal disease. Protease inhibitors are widely applied to treat acute pancreatitis in Japan; but the protease inhibitors used to treat acute necrotizing pancreatitis cannot easily reach the pancreas when administered intravenously, and, because of ischemia or impaired microcirculation, they hardly penetrate into pancreatic tissue. Recently, continuous regional arterial infusion (CRAI) with the protease inhibitor nafamostat mesilate and antibiotics has proven effective as an initial therapy. CRAI has been applied to treat SAP, but the evidence of its value is still scarce.

Research frontiers

The article focuses on the efficacy of CRAI using gabexate mesilate and antibodies for SAP.

Innovations and breakthroughs

The present study shows the efficacy of CRAI using gabexate mesilate for SAP, and the clinical benefits and sequential changes in serum inflammation-related parameters such as cytokines and chemokines. Abdominal pain and SIRS disappeared significantly in a short period of time after the initiation of CRAI with a protease inhibitor compared to non-CRAI. The average length of hospitalization significantly decreased with CRAI and patients discharged significantly in a shorter period of time after the initiation of CRAI with gabexate mesilate compared to non-CRAI.

Applications

CRAI using gabexate mesilate was shown to be effective against SAP in terms of clinical benefits for patients with SAP, and thus may provide a new strategy of treatment for SAP.

Peer review

Effect of continuous regional arterial infusion therapy with gabexate and antibiotics for SAP is very interesting clinical research. Known that SAP may have high mortality, some new modalities of therapy which improve prognosis of patients are welcome.

REFERENCES

1 **Bradley EL 3rd**. A clinically based classification system for acute pancreatitis. Summary of the International Symposium on Acute Pancreatitis, Atlanta, Ga, September 11 through 13, 1992. *Arch Surg* 1993; **128**: 586-590
2 **Al Mofleh IA**. Severe acute pancreatitis: pathogenetic aspects and prognostic factors. *World J Gastroenterol* 2008; **14**: 675-684
3 **Takeda K**, Takada T, Kawarada Y, Hirata K, Mayumi T, Yoshida M, Sekimoto M, Hirota M, Kimura Y, Isaji S, Koizumi M, Otsuki M, Matsuno S. JPN Guidelines for the management of acute pancreatitis: medical management of acute pancreatitis. *J Hepatobiliary Pancreat Surg* 2006; **13**: 42-47
4 **Otsuki M**, Hirota M, Arata S, Koizumi M, Kawa S, Kamisawa T, Takeda K, Mayumi T, Kitagawa M, Ito T, Inui K, Shimosegawa T, Tanaka S, Kataoka K, Saisho H, Okazaki K, Kuroda Y, Sawabu N, Takeyama Y. Consensus of primary care in acute pancreatitis in Japan. *World J Gastroenterol* 2006; **12**: 3314-3323
5 **Koizumi M**, Takada T, Kawarada Y, Hirata K, Mayumi T, Yoshida M, Sekimoto M, Hirota M, Kimura Y, Takeda K, Isaji S, Otsuki M, Matsuno S. JPN Guidelines for the management of acute pancreatitis: diagnostic criteria for acute pancreatitis. *J Hepatobiliary Pancreat Surg* 2006; **13**: 25-32
6 **Ogawa M**, Hirota M, Hayakawa T, Matsuno S, Watanabe S, Atomi Y, Otsuki M, Kashima K, Koizumi M, Harada H, Yamamoto M, Nishimori I. Development and use of a new staging system for severe acute pancreatitis based on a nationwide survey in Japan. *Pancreas* 2002; **25**: 325-330
7 **Valderrama R**, Perez-Mateo M, Navarro S, Vazquez N, Sanjose L, Adrian MJ, Estruch J. Multicenter double-blind trial of gabexate mesylate (FOY) in unselected patients with acute pancreatitis. *Digestion* 1992; **51**: 65-70
8 **Yang CY**, Chang-Chien CS, Liaw YF. Controlled trial of protease inhibitor gabexelate mesilate (FOY) in the treatment of acute pancreatitis. *Pancreas* 1987; **2**: 698-700
9 **Buchler M**, Malfertheiner P, Uhl W, Scholmerich J, Stockmann F, Adler G, Gaus W, Rolle K, Beger HG. Gabexate mesilate in human acute pancreatitis. German Pancreatitis Study Group. *Gastroenterology* 1993; **104**: 1165-1170
10 **Messori A**, Rampazzo R, Scroccaro G, Olivato R, Bassi C, Falconi M, Pederzoli P, Martini N. Effectiveness of gabexate mesilate in acute pancreatitis. A metaanalysis. *Dig Dis Sci* 1995; **40**: 734-738
11 **Chen HM**, Chen JC, Hwang TL, Jan YY, Chen MF. Prospective and randomized study of gabexate mesilate for the treatment of severe acute pancreatitis with organ dysfunction. *Hepatogastroenterology* 2000; **47**: 1147-1150
12 **Inoue K**, Hirota M, Kimura Y, Kuwata K, Ohmuraya M, Ogawa M. Further evidence for endothelin as an important mediator of pancreatic and intestinal ischemia in severe acute pancreatitis. *Pancreas* 2003; **26**: 218-223
13 **Takeda K**, Mikami Y, Fukuyama S, Egawa S, Sunamura M, Ishibashi T, Sato A, Masamune A, Matsuno S. Pancreatic ischemia associated with vasospasm in the early phase of human acute necrotizing pancreatitis. *Pancreas* 2005; **30**: 40-49
14 **Takeda K**, Matsuno S, Sunamura M, Kakugawa Y. Continuous regional arterial infusion of protease inhibitor and antibiotics in acute necrotizing pancreatitis. *Am J Surg* 1996; **171**: 394-398
15 **Anai H**, Sakaguchi H, Uchida H, Matsuo N, Tanaka T, Yoshioka T, Ohishi H, Murao Y, Miyamoto S. Continuous arterial infusion therapy for severe acute pancreatitis: correlation between CT arteriography and therapeutic effect. *J Vasc Interv Radiol* 1999; **10**: 1335-1342
16 **Imaizumi H**, Kida M, Nishimaki H, Okuno J, Kataoka Y, Kida Y, Soma K, Saigenji K. Efficacy of continuous regional arterial infusion of a protease inhibitor and antibiotic for severe acute pancreatitis in patients admitted to an intensive care unit. *Pancreas* 2004; **28**: 369-373
17 **Takeda K**, Matsuno S, Ogawa M, Watanabe S, Atomi Y. Continuous regional arterial infusion (CRAI) therapy reduces the mortality rate of acute necrotizing pancreatitis: results of a cooperative survey in Japan. *J Hepatobiliary Pancreat Surg* 2001; **8**: 216-220
18 **Kakugawa Y**, Takeda K, Sunamura M, Kawaguchi S, Kobari M, Matsuno S. [Effect of continuous arterial infusion of protease inhibitor on experimental acute pancreatitis

induced by closed duodenal loop obstruction] *Nippon Shokakibyo Gakkai Zasshi* 1990; **87**: 1444-1450
19 **Satoh H**, Harada M, Tashiro S, Shiroya T, Imawaka H, Machii K. The effect of continuous arterial infusion of gabexate mesilate (FOY-007) on experimental acute pancreatitis. *J Med Invest* 2004; **51**: 186-193
20 **Pederzoli P**, Cavallini G, Falconi M, Bassi C. Gabexate mesilate vs aprotinin in human acute pancreatitis (GA. ME.P.A.). A prospective, randomized, double-blind multicenter study. *Int J Pancreatol* 1993; **14**: 117-124
21 **Takahashi Y**, Shibata A. The comparative study of nafamostat mesilate (FUT-175), gabexate mesilate (FOY) and heparin on anticoagulant and antifibrinolytic action. *Jpn J Clin Exp Med* 1988; **65**: 127-134
22 **Muto S**, Imai M, Asano Y. Effect of nafamostat mesilate on Na+ and K+ transport properties in the rabbit cortical collecting duct. *Br J Pharmacol* 1993; **109**: 673-678
23 **Ohmoto K**, Yamamoto S. Serum interleukin-6 and interleukin-10 in patients with acute pancreatitis: clinical implications. *Hepatogastroenterology* 2005; **52**: 990-994

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RAPID COMMUNICATION

Chronic gastrointestinal symptoms and quality of life in the Korean population

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Abstract

AIM: To evaluate the prevalence of chronic gastrointestinal symptoms and their impact on health-related quality of life (HRQOL) in the Korean population.

METHODS: A cross-sectional survey, using a reliable and valid Rome Ⅱ based questionnaire, was performed on randomly selected residents, between 18 and 69 years in age. All respondents were interviewed at their homes or offices by a team of interviewers. The impact of chronic gastrointestinal symptoms on HRQOL was assessed using the Korean version of the 36-item Short-Form general health survey (SF-36).

RESULTS: Of the 1807 eligible subjects, 1417 (78.4%: male 762; female 655) were surveyed. Out of the respondents, 18.6% exhibited at least one chronic gastrointestinal symptom. The prevalence of gastroesophageal reflux disease (GERD), defined as heartburn and/or acid regurgitation experienced at least weekly, was 3.5% (95% CI, 2.6-4.5). The prevalence of uninvestigated dyspepsia, irritable bowel syndrome (IBS) and chronic constipation based on Rome Ⅱ criteria were 11.7% (95% CI, 10.1-13.5), 2.2% (95% CI, 1.5-3.1), and 2.6% (95% CI, 1.8-3.5) respectively. Compared with subjects without chronic gastrointestinal symptoms (n = 1153), those with GERD (n = 50), uninvestigated dyspepsia (n = 166) and IBS (n = 31) had significantly worse scores on most domains of the SF-36 scales.

CONCLUSION: The prevalence of GERD, uninvestigated dyspepsia and IBS were 3.5%, 11.7% and 2.2% respectively, in the Korean population. The health-related quality of life was significantly impaired in subjects with GERD, uninvestigated dyspepsia and IBS in this community.

Key words: Chronic gastrointestinal symptom; Gastroesophageal reflux disease; Dyspepsia; Irritable bowel syndrome; Qaulity of life

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INTRODUCTION

Functional gastrointestinal disorders (FGIDs) are highly prevalent in different geographic populations and cause a variety of gastrointestinal symptoms that greatly inconvenience the affected individuals[1,2]. According to a household survey in the United States, 69% of those questioned reported as having at least one of twenty functional gastrointestinal symptoms in the preceding three months[1]. In other population-based surveys, the prevalence of non-ulcer dyspepsia was approximately 30% and irritable bowel syndrome was 15%[2,3]. The prevalence of functional gastrointestinal disorders has been reported to differ based on the geographical region and race. Although the prevalence of such disorders in

Koreans is thought to be different from that in the Westerners, there has been no randomly sampled population-based study on the prevalence of chronic gastrointestinal symptoms in the Korean population.

The health-related quality of life (HRQOL) is a patient-focused concept, referring to an impairment of functional status (physical or mental) and the sense of well-being. As such, it is an important measure of the impact of chronic diseases. Several studies have shown that the HRQOL is significantly reduced in patients with chronic gastrointestinal symptoms in referral settings, compared with the general population, as well as in patients with other chronic conditions, such as GERD and asthma. However, there is a lack of evidence to support a decrease in the HRQOL in the general population having chronic GI symptoms[4,5].

The aims of the present study were to evaluate the prevalence of chronic gastrointestinal symptoms according to the Rome Ⅱ criteria, and to determine whether the HRQOL is impaired in subjects having chronic gastrointestinal symptoms in the Korean community.

MATERIALS AND METHODS

Subjects

The study was carried out in Asan-si, Chungchungnam-do Province from January 3, 2000 to February 28, 2001. Asan-Si is a small city located in the middle of South Korea. The population comprises approximately 180 000 people, and is a combination of urban and farming community. Sociodemographic factors including age, sex, educational background and economic level of Asan-si are similar to the overall Korean population, based on the information obtained from the Korean National Statistical Office (http://www.nso.go.kr). We assumed a symptom prevalence of 40%; thus at least 1024 interviews were required to achieve the required precision of ± 3% with 95% confidence. Therefore, the target sample size was 1400. A cross-sectional survey was performed on randomly selected Asan-si residents, between 18 and 69 years of age. A lengthy consultation was made with the regional health office (RHO) to prepare the survey. For instance, the general regional information provided by the RHO helped to organize visits and complete other administrative activities. Ten out of Asan-si's 17 districts, with population size over 500 were randomly selected. Within each of these districts, a total of 200 households from every third street were chosen. The size, gender and age of all household members were obtained from the RHO database before the visit. Gender and age were used to stratify the households and one person in each household was selected by random sampling, before a list of selected individuals was compiled in each stratum. The regional health office checked the eligibility of the selected individuals by examining their medical records, followed by a telephone interview. Exclusion criteria included pregnant or lactating women, history of surgery of the GI tract including partial resection of the stomach and/or resection of the small/large intestine (subjects with an appendectomy were included), major psychosis, mental retardation or dementia, significant illness that may render them unable to complete the questionnaire, or had other nationalities. The revised list was used for actual household visit after excluding the ineligible individuals. Since only housewives and elderly individuals would be available during the day, we pre-scheduled the time and order of the house visits with the help of the regional health office.

Questionnaire

We developed a bowel symptom questionnaire to identify chronic gastrointestinal symptoms in adults, based on the Rome Ⅱ criteria. The complete questionnaire was 22 pages long and included 130 questions, of which 103 were used to diagnose gastroesophageal reflux and chronic gastrointestinal symptoms. The bowel symptoms questionnaire was modified from the Mayo version of Bowel Symptoms Questionnaire[6] and several items were adopted from the Rome Ⅱ criteria. The validation process consisted of forward and backward translation as well as confirming the patient's ability to understand. However, we did not perform test-retest reliability, because this was not a self-reported questionnaire, and trained interviewers visited homes and helped the subjects to complete the questionnaire. During the survey, a face-to-face interview was conducted to help describe certain parts of the Bowel Symptoms Questionnaire. Specifically, heartburn is not a term in the Korean language; therefore the symptoms had to be verbally described and a diagram indicating the location of the burning sensation was added to the questionnaire in order to help ensure that the subjects fully comprehended the medical conditions under investigation. The remaining 27 questions enquired about the sociodemographic characteristics, self-reported height and weight, physician visits, past illnesses, social habits (smoking, alcohol, and medication use), impact on everyday life, possible anxiety induced by the problem, as well as lifestyle changes that may have been relevant.

HRQOL was assessed by the Korean version of SF-36 (SF-36-K). We previously assessed the reliability, discriminant validity and concurrent validity of the SF-36-K before conducting this study. The SF-36-K was fully applicable and well understood by Korean patients, as well as healthy subjects. The reliability was assessed by using the test-retest method, and the internal consistency method. The test-retest method showed high correlation between the two tests. Cronbach's correlation alpha of all 8 subscales of the SF-36-K was > 0.73 (range, 0.73-0.96). Discriminant validity was supported by comparing the SF-36 score in 179 healthy subjects and 44 patients with gastrointestinal disorders. All 8 domains of the SF-36 were well correlated with subscores of the WHO Quality of Life Scale-K and the Psychological Well-Being Index. The SF-36 is a widely used general health profile questionnaire with 36 questions comprising eight scales: physical functioning (e.g. walking, lifting), role functioning-physical (limitations in ability to perform usual activities), role functioning-emotional (impact of emotional problems on work or

daily activities), social functioning (impact of health or emotional problems on social activities), bodily pain (level of bodily pain or discomfort), mental health (anxiety, depression, sense of psychological well-being), vitality (energy level or fatigue), and general health perceptions (global evaluations of health). The SF-36 is scored from 0 to 100 with higher scores indicating a better HRQOL.

Survey design

We conducted a cross-sectional survey of gastrointestinal symptoms and their impact on the quality of life (QOL) in Korean subjects in cooperation with the public health center. Before the survey, the questionnaire was explained to the health-care personnel of the Public Health Center to outline the purpose of the study and to request their participation. All subjects were interviewed in person at their homes or offices by a team of interviewers, all trained by the same physician (C.M.G.). The consistency and completeness of the completed questionnaire was checked after each interview.

Statistics

The 1807 eligible individuals interviewed can be considered as a representative sample of the Asan-si population. We calculated the prevalence of GERD and chronic gastrointestinal symptoms according to the Rome Ⅱ criteria. The prevalence is presented in percentages with 95% exact confidence intervals (CI). Comparisons among groups were performed by the χ^2 test or Fisher's exact test for categorical data and *t*-test for continuous data. The association between prevalence rate and age in each group was tested by logistic regression model. Differences of SF-36 sub-scale scores between groups were estimated by ANCOVA model with adjustment of covariates. Pair-wise differences between groups without chronic GI symptoms and groups with chronic GI symptoms (GERD, UD and IBS) were tested by Bonferroni adjusted *t*-test. Gender, age, economic level and education variables were used for adjustment. A multiple regression model was performed for SF-36 sub-scale scores for individuals who had chronic GI symptoms. Gender, age, smoking, religion, education, economic level, physician visit and overlapping symptom variables were used as predictors. Statistical analyses were performed using SAS (SAS, Cary, NC, USA).

RESULTS

Response rate and subject characteristics

Among the randomly selected 2024 subjects, a total of 217 were not eligible to participate in the study. One hundred and twenty individuals were no longer living in Asan-si and 97 could not be interviewed due to physical or mental disorders. Out of the 1807 eligible subjects, 314 could not be contacted after three attempts, and 76 refused to participate. A total of 1417 (78.4%) of the 1807 eligible subjects returned the completed survey. Of these respondents, 762 were male (53.8%) and 655 were female (46.2%), with a mean age of 44 years. The demographic and socioeconomic features of the respondents are shown in Table 1.

Table 1 Characteristics of the study population *n* (%)

Variable	Male (*n* = 762)	Female (*n* = 655)	Total (*n* = 1417)
Age			
18-29	163 (11.5)	151 (10.7)	314 (22.2)
30-39	132 (9.3)	110 (7.8)	242 (17.1)
40-49	169 (11.9)	146 (10.3)	315 (22.2)
50-59	150 (10.6)	116 (8.2)	266 (18.8)
60-69	148 (10.4)	132 (9.3)	280 (19.7)
Median age (yr)	45	44	44
Mean age (yr)	44.1 ± 14.4	43.7 ± 15.1	43.9 ±14.8
BMI (kg/m^2)	22.8	22.0	22.4
Ever tobacco smoker (%)	67.8[a]	4.0	38.3
Alcohol use (> 75 g/wk) (%)	36.4[a]	2.6	20.7
Marital status (single)[1] (%)	26.5	28.7	27.5
Employed (%)	85.4[a]	45.6	67.0
Presence of religion (%)	50.5[b]	65.0	57.2
High school graduate (%)	57.0[a]	49.2	53.4
Economic status (%)			
High	6.4	6.3	6.4
Middle	76.1	79.8	77.8
Low	17.5	13.9	15.8

[1]Marital status was divided into single or married/living as a couple. Single status was extended to include unmarried persons, divorced individuals and those with a deceased spouse [a]$P < 0.01$, [b]$P < 0.001$ compared to female.

Prevalence of chronic gastrointestinal symptoms

The prevalence of weekly episodes of heartburn and acid regurgitation was 2.0% (95% CI, 1.2-2.7) and 2.0% (95% CI, 1.3-2.8) respectively. The prevalence of GERD, defined as heartburn and/or acid regurgitation experienced at least weekly, was 3.5% (95% CI, 2.6-4.5). The prevalence of specific chronic gastrointestinal symptoms, according to the Rome Ⅱ criteria is summarized in Table 2. At least one chronic gastrointestinal symptom was present in 18.6% of the 1417 respondents. The most prevalent chronic gastrointestinal symptom was uninvestigated dyspepsia (11.7%; 95% CI, 10.1-13.5). According to the subtypes of dyspepsia, dysmotility-like dyspepsia was the most prevalent (69.9%), followed by ulcer-like dyspepsia (28.3%) and non-specific dyspepsia (1.8%). Thirty one subjects (2.2%) fulfilled the Rome Ⅱ criteria for the diagnosis of IBS (Table 2). Of these, 13 subjects (42%) were classified as diarrhea-predominant, and 12 (39%) constipation-predominant IBS. The remaining 6 subjects (19%) fell into the alternating IBS subgroup. There were no differences in the overall prevalence of IBS based on gender; however, compared to male subjects, females reported more frequent constipation-predominant IBS (IBS-C). Females also reported more frequent chronic constipation. There were no gender-based differences in the prevalence of the other chronic gastrointestinal symptoms (Table 2).

Age-specific prevalence of GERD, uninvestigated dyspepsia, and IBS are shown in Figure 1, and the odds ratio with 95% confidence interval are shown in Table 3. The prevalence of GERD and dyspepsia showed significant differences between different age groups (logistic regression, $P < 0.01$).

Physician visit and medication use

In the present population-based study, 50.5% of the

Table 2 Prevalence of chronic gastrointestinal symptoms, according to the Rome Ⅱ criteria

Chronic gastrointestinal symptoms	Respondent (*n* = 1417) *n*	% (95% exact CI)	Male (*n* = 762) %	Female (*n* = 655) %
Globus	7	0.5 (0.2-1.0)	0.4	0.7
Chronic dysphagia	7	0.5 (0.2-1.0)	0.3	0.7
Rumination	0	0.0 (0.0-0.3)	0	0
Chronic chest pain	21	1.5 (0.9-2.3)	1.6	1.4
Chronic heartburn	24	1.7 (1.1-2.5)	1.8	1.6
Uninvestigated dyspepsia	166	11.7 (10.1-13.5)	10.8	12.8
IBS	31	2.2 (1.5-3.1)	1.8	2.6
IBS-D	13	0.9 (0.5-1.6)	1	0.8
IBS-C	12	0.8 (0.4-1.5)	0.1	1.7[a]
IBS-A	6	0.4 (0.2-0.9)	0.7	0.2
Chronic bloating	57	4.0 (3.1-5.2)	2.9	5.3
Chronic constipation	37	2.6 (1.8-3.6)	0.5	5.0[a]
Chronic diarrhea	11	0.8 (0.4-1.4)	0.8	0.8
Chronic incontinence	18	1.3 (0.8-2.0)	1.6	1.1

IBS: Irritable bowel syndrome; IBD-D: Diarrhea-predominant IBS; IBS-C: Constipation- predominant IBS; IBS-A: Alternating constipation and diarrhea IBS. [a]$P < 0.05$ (Fisher's exact test) compared to male.

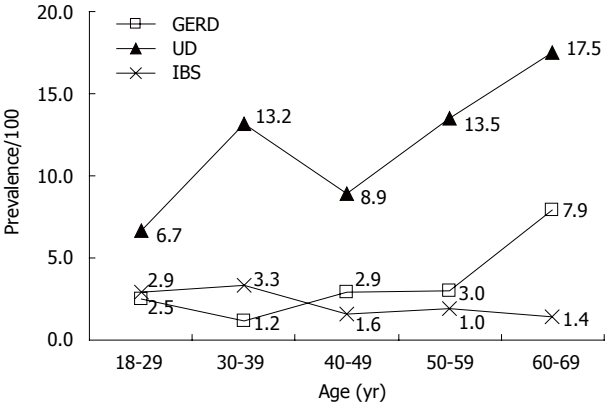

Figure 1 Age specific prevalence rate (per 100) of GERD, UD and IBS in Asan-si, Korea. GERD: Gastroesophageal reflux disease; UD: Uninvestigated dyspepsia; IBS: Irritable bowel syndrome.

subjects surveyed had experienced at least one gastrointestinal symptom in the previous year, and 10.9% had reported visiting a physician due to their gastrointestinal symptoms. With regard to the use of medications, 5.7% of the respondents took non-steroidal anti-inflammatory drugs (NSAIDs), and 5.5% had taken anti-acid agents or antacids in the past year. More women took NSAIDs and constipation medications (Table 4).

Impact of chronic gastrointestinal symptoms on health-related quality of life

Of the 1417 respondents, 1153 individuals did not experience any chronic GI symptoms, while 198 subjects had features suggestive of GERD, UD or IBS, or a combination of these symptoms. There was no significant difference between the two groups with respect to gender, smoking, martial status and BMI ($P > 0.05$). However, age, education level and the number of physician visits were statistically different ($P < 0.001$). The mean age of subjects with chronic GI symptoms and subjects without chronic GI symptoms was 47.8 ± 14.9 and 43.0 ± 14.6, respectively. Subjects with chronic GI symptoms had a lower education level, and also visited a physician more frequently for gastrointestinal symptoms than subjects without chronic GI symptoms. SF-36 subscale scores were calculated for four groups: (1) subjects without chronic gastrointestinal symptoms, (2) subjects with GERD, (3) subjects with uninvestigated dyspepsia, and (4) subjects with IBS. The adjusted mean scores for gender, age, education and economic level, as well as the accompanying *P*-values for the eight domains of the SF-36 are summarized in Table 5. Compared with those not experiencing chronic gastrointestinal symptom, subjects with GERD exhibited significantly worse scores on all except two domains (social-functioning and role-emotional). Subjects with uninvestigated dyspepsia and IBS had significantly worse scores on all domains compared with those not having chronic gastrointestinal symptoms (Table 5). Multiple regression analysis of the association between the HRQOL and covariates was performed on subjects with chronic GI symptoms (n = 198) (Table 6). All covariates shows VIF smaller than 10, thus no covariates were excluded as the cause of multi-

Table 3 Association between age and chronic gastrointestinal symptoms

Age	GERD	UD	IBS
18-29	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)
30-39	0.48 (0.13-1.82)	2.12 (1.19-3.77)	1.15 (0.44-3.04)
40-49	1.14 (0.44-3.00)	1.39 (0.77-2.50)	0.56 (0.18-1.68)
50-59	1.23 (0.46-3.33)	2.28 (1.30-4.02)	0.68 (0.22-2.04)
60-69	3.30 (1.44-7.54)	3.00 (1.75 -5.14)	0.50 (0.15-1.63)

Values are shown as odds ratio and 95% confidence interval. GERD, gastroesophageal reflux disease; UD, uninvestigated dyspepsia; IBS, irritable bowel syndrome.

Table 4 Details of physician visits and use of medications *n* (%)

Variables	Men (*n* = 762)	Women (*n* = 655)	Total (*n* = 1417)
Experience (%) of any GI symptoms	366 (48.0)	351 (53.6)[a]	50.50
Experience (%) of visiting a physician due to GI symptoms	80 (10.5)	75 (11.5)	155 (10.9)
1-2/yr	42 (5.5)	47 (7.2)	89 (6.3)
3-5/yr	12 (1.6)	12 (1.8)	24 (1.7)
6-10/yr	8 (1.0)	4 (0.6)	12 (0.8)
> 10/yr	18 (2.4)	12 (1.8)	30 (2.1)
Use of medications			
NSAIDs	27 (3.5)	54 (8.2)[b]	81 (5.7)
Antacids	35 (4.6)	28 (4.3)	63 (4.5)
H_2RA	11 (1.4)	4 (0.6)	15 (1.1)
Medications for constipation	17 (2.2)	49 (7.5)[b]	66 (4.7)
Antihypertensive medications	17 (2.2)	18 (2.7)	35 (2.5)

[a]$P < 0.05$, [b]$P < 0.01$ compared to male.

Table 5 Comparison of SF-36 subscales between subjects without chronic GI symptoms, and those with GERD, UD and IBS

SF-36 Subscale	Subjects without CGIS (*n* = 1153)	GERD (*n* = 50)	UD (*n* = 166)	IBS (*n* = 31)
Physical functioning	87.6 ± 5.1	76.0 ± 6.2[b]	81.7 ± 5.4[b]	82.0 ± 66.8[a]
Role physical	80.1 ± 10.3	64.9 ± 12.4[b]	68.1 ± 10.9[b]	67.3 ± 13.7[a]
Bodily pain	89.3 ± 6.7	74.6 ± 8.1[b]	76.5 ± 7.1[b]	77.7 ± 8.9[b]
General health	68.8 ± 7.0	49.3 ± 8.5[b]	52.7 ± 7.5[b]	50.0 ± 9.4[b]
Vitality	57.6 ± 7.5	50.0 ± 9.0[a]	51.0 ± 7.9[b]	45.9 ± 10.0[b]
Social functioning	92.0 ± 4.9	88.3 ± 5.9	86.2 ± 5.2[b]	86.7 ± 6.6[a]
Role emotional	86.2 ± 10.4	80.7 ± 12.5	77.7 ± 11.0[b]	71.7 ± 13.8[b]
Mental health	77.7 ± 6.2	67.9 ± 7.5[b]	71.6 ± 6.6[b]	66.1 ± 8.3[b]

Note: Age, gender, education level and economic level adjusted mean and 95% confidence interval of SF-36 subscale. CGIS: Chronic GI symptom; GERD: Gastroesophageal reflux disease; UD: Uninvestigated dyspepsia; IBS: Irritable bowel syndrome. Comparisons were performed between GERD *vs* no CGIS, dyspepsia *vs* no CGIS, and IBS *vs* no CGIS. *P* value was adjusted by Bonferroni method. [a]$P < 0.05$ compared to subjects without chronic gastrointestinal symptom; [b]$P < 0.01$ compared to subjects without chronic gastrointestinal symptom.

Table 6 Multiple regression results: estimated coefficient of predictors of each SF-36 subscale domain

Variable	PF	RP	BP	GH	VT	SF	RE	MH
Female sex	-4.6	-9.6	-4.7	-5.3[b]	-4.3[a]	-3.6	-8.2	-1.8
Age[1]	-5.7[c]	-3.2	-1.3	-4.6[b]	-3.2[a]	-1.4	-1.2	-3.5[b]
Ever smoking	2.5	3	2	-2.6	2.3	2.4	3	1.3
Religion	4.1	9.7	-0.1	1.3	6.5[a]	-3.5	-3.5	2.3
< high school education	-6.7[a]	-5.8	-3	2.6	3.1	0.4	3.8	4.2
Low economic class	-2.8	-14.0[a]	-5.8	-3.3	-0.2	-3.9	-17.3	-0.2
Number of physician visit[2]	0.6	-4.8[a]	-4.8[c]	-4.1[b]	-1.9	-0.9	-4.8[a]	-3.5[c]
Presence of overlapping symptoms	-4.7	-3.5	-8.3[a]	-6.9	1.6	-3.9	-5.5	-8.3[b]
R^2	0.375	0.134	0.165	0.19	0.09	0.09	0.124	0.177

PF: Physical function; RP: Role-physical; BP: Bodily pain; GH: General health; VT: Vitality; SF: Social function; RE: Role-emotion; MH: Mental health. [1]Age variables are categorized by 10 yr intervals; [2]Number of physician visits for gastrointestinal symptoms: 0/yr(0), 1-2/yr(1), 3-5/yr(2), 6-10/yr(3), > 10/yr(4). [a]$P < 0.05$; [b]$P < 0.01$; [c]$P < 0.001$.

collinearity. The results of overall *F*-test were significant for all models ($P < 0.05$). Female gender, old age, a low level of education (< high school education), a low economic class, number of physician visits within the past year, and overlapping chronic gastrointestinal symptoms were associated with reduction in the SF-36 scales.

DISCUSSION

The present population-based study describes the prevalence of chronic gastrointestinal symptoms and their impact on the HRQOL in the Korean population.

Heartburn and acid regurgitation are specific symptoms of GERD, and a diagnosis of GERD can be made on the basis of these symptoms alone without further diagnostic tests[7]. When defined as "at least weekly heartburn and/or acid regurgitation", the prevalence of GERD in the West ranges between 10% and 20%, whereas in Asia the prevalence is reported to be < 5%[8]. In the present study, the prevalence of GERD was found to be 3.5% which is much lower than that in Western countries. According to two previous population studies in Korea, the prevalence of GERD was 5% and 7.1%, respectively[9,10]. It is unclear why the prevalence of GERD is lower in Korea compared to the West. Differences in the intake of dietary fat, body build, genetic factors, and the prevalence of *H pylori* infection are possible contributing factors[11]. The prevalence of dyspepsia has been shown to vary considerably between different populations. Although our data may represent the presence of valid epidemiological differences, it is also possible that the varying definitions used in different population-based studies may have contributed to this discrepancy. In the present study, the prevalence of uninvestigated dyspepsia by Rome Ⅱ criteria was 11.7%. These results are similar to a study from Taiwan, which reported prevalence of functional dyspepsia (FD) by the Rome Ⅱ criteria as 11.8%[12]. Population-based studies in Australia and Mexico have reported prevalence rates of dyspepsia by Rome Ⅱ criteria of 24.4%[13] and 8.0%[14], respectively. Based on Rome Ⅱ criteria, a patient presenting with upper abdominal pain or discomfort that is exclusively relieved by defecation and/or is associated with a change in the bowel pattern is defined as IBS rather than dyspepsia plus IBS[15]. Accordingly, many of the previously Rome I-defined dyspepsia subjects should be reclassified as IBS, rather than IBS with dyspepsia. Furthermore, patients with predominant heartburn should be excluded. Although, the present study could not demonstrate the prevalence of dyspepsia defined by Rome I criteria, the prevalence of dyspepsia by Rome Ⅱ criteria was lower compared to our previous study using the Rome I criteria (11.7% *vs* 15.5%)[9]. Reduction in prevalence has also been reported in other studies that directly compared the prevalence of dyspepsia using Rome I and Rome Ⅱ criteria[12,13,16]. Several individuals who did not meet Rome Ⅱ criteria for dyspepsia were taking medications such as antacids, prokinetic agents and digestives for a long time. If medication alone was indicative of dyspepsia, our study indicates that its prevalence would be 16%. It is also possible that some subjects had GERD and not dyspepsia, or there was an overlap. One of these factors may also account for the low prevalence of GERD in the Korean population. Over-the-counter medications may explain the underestimation of the prevalence of other functional GI disorders. Conversely, a number of drugs can theoretically induce bowel symptoms. However, data supporting the role of drugs, aside from NSAIDs, in the development of bowel symptoms in the general population are lacking[17,18].

The prevalence of IBS in Asian population-based studies has generally been lower compared to in the

West, regardless of the criteria applied. The prevalence of IBS by Rome Ⅱ criteria has been reported to vary from 4.7% to 25% in the West and from 3.7% to 19.1% in Asia[19]. In the present study, the prevalence of IBS by Rome Ⅱ criteria was 2.2%. In addition, the overall prevalence of IBS was similar among men and women; however, the prevalence of constipation-predominant IBS was higher in women. There is typically a significant female predominance with respect to hospital visits by IBS patients in Western countries, but this trend is not consistent with community studies and has been attributed to gender differences in health care utilization[19]. In a recent systematic review of 13 studies on IBS, based on Rome Ⅱ criteria, 7 studies found a higher prevalence of IBS in females and 4 studies found no gender difference, as in the present study[19]. A possible explanation for the lower female to male ratio in IBS in Koreans may be that men encounter more socioeconomic problems, causing increased stress and, as a consequence, an increased level of IBS[20].

In the present study, we also examined the impact of GERD, and two common chronic gastrointestinal symptoms (uninvestigated dyspepsia and IBS) on the HRQOL. Although a number of studies suggest that the HRQOL is significantly reduced in patients with chronic gastrointestinal symptoms in a referral setting, the data are conflicting[21], and very few studies have evaluated the impact of chronic gastrointestinal symptoms on HRQOL in the general population. In the present study, we observed that the quality of life was significantly impaired in subjects with GERD, uninvestigated dyspepsia and IBS. These findings are consistent with previous studies[5,22-24].

The present study showed that old age, female gender, the number of physician visits per year and presence of overlapping symptoms were associated with a negative impact on several domains of the SF-36. In general, old age was associated with a less favorable assessment of their personal health, pessimistic health appraisal, social isolation and unemployment[25]. Few studies have investigated whether women and men with chronic gastrointestinal symptoms differ with respect to the HRQOL measures. In a study based on referral center and primary care patients, Simren *et al*[26] observed that women with IBS had lower HRQOL compared to men with IBS. In another study, Lee *et al*[27] also found that women with IBS reported lower HRQOL scores. In the present study, a greater number of hospital visits was associated with a poorer HRQOL, which is in agreement with a US population-based study[28]. Fifty five percents of subjects with IBS had sought health care in the past year, and subjects with IBS had significantly lower IBS-QOL scores in the mental health and social functioning domains[28].

The strengths of the present study were inclusion of a random population sample, and the use of personal interviews with the subjects. As a result, we obtained a high response rate (78.4%), avoided a significant response bias, and had a negligible number of missing values. Since the interviewers associated with this study were trained before the commencement of contact with the subjects, a uniform survey was possible due to the fact that the interviewers could explain each aspect of the questionnaire fully, especially to subjects who were old, had low education level, or those who could not understand certain items. Sociodemographic factors including age, gender, educational background and economic level of the individuals in Asan-si were similar to the Korean population, based on the information obtained from the Korean National Statistical Office. Asan-si is a combined urban and farming community. We selected 10 out of the 17 districts of Asan-si. Five districts were randomly chosen in urban areas and the remaining five were selected in rural areas, which may have limited the generalization of the study. However, no differences were found between the demographic factors and the prevalence of GI symptoms in the 10 districts, and between rural and urban areas.

In summary, we evaluated the prevalence of chronic gastrointestinal symptoms in the Korean general population and demonstrated a significant impact of chronic gastrointestinal symptoms on the HRQOL. Dyspepsia was found to be the most common chronic gastrointestinal symptom, and the prevalence of GERD and IBS was lower compared to in the West. The presence of chronic gastrointestinal symptoms was found to have a negative impact on the HRQOL. This negative impact was greater in females, the elderly, individuals of lower economic class, and in subjects with higher number of physician visits, and overlapping symptoms.

REFERENCES

1 **Drossman DA**, Li Z, Andruzzi E, Temple RD, Talley NJ, Thompson WG, Whitehead WE, Janssens J, Funch-Jensen P, Corazziari E. U.S. householder survey of functional gastrointestinal disorders. Prevalence, sociodemography, and health impact. *Dig Dis Sci* 1993; **38**: 1569-1580

2 **Agreus L**, Svardsudd K, Nyren O, Tibblin G. Irritable bowel syndrome and dyspepsia in the general population: overlap and lack of stability over time. *Gastroenterology* 1995; **109**: 671-680

3 **Jones RH**, Lydeard SE, Hobbs FD, Kenkre JE, Williams EI, Jones SJ, Repper JA, Caldow JL, Dunwoodie WM, Bottomley JM. Dyspepsia in England and Scotland. *Gut* 1990; **31**: 401-405

4 **Chang L**. Review article: epidemiology and quality of life in functional gastrointestinal disorders. *Aliment Pharmacol Ther* 2004; **20** Suppl 7: 31-39

5 **Halder SL**, Locke GR 3rd, Talley NJ, Fett SL, Zinsmeister AR, Melton LJ 3rd. Impact of functional gastrointestinal disorders on health-related quality of life: a population-based case-control study. *Aliment Pharmacol Ther* 2004; **19**: 233-242

6 **Talley NJ**, Phillips SF, Wiltgen CM, Zinsmeister AR, Melton LJ 3rd. Assessment of functional gastrointestinal disease: the bowel disease questionnaire. *Mayo Clin Proc* 1990; **65**: 1456-1479

7 **Locke GR 3rd**, Talley NJ, Fett SL, Zinsmeister AR, Melton LJ 3rd. Prevalence and clinical spectrum of gastroesophageal reflux: a population-based study in Olmsted County, Minnesota. *Gastroenterology* 1997; **112**: 1448-1456

8 **Dent J**, El-Serag HB, Wallander MA, Johansson S. Epidemiology of gastro-oesophageal reflux disease: a systematic review. *Gut* 2005; **54**: 710-717

9 **Choo KY**, Choi MG, Choi H, Lee DS, Kim JI, Kim SS, Bhang CS, Park SH, Kim JK, Han SW, Choi KY, Chung IS, Chung KW, Sun HS. The prevalence of gastrointestinal symptoms in a rural community in Korea. *Kor J Neurogastroenterol Motil* 2000; **6**: 31-43

10 **Yang SY**, Lee OY, Bak YT, Jun DW, Lee SP, Lee SH, Park GT, Yoon BC, Choi HS, Hahm JS, Lee MH, Lee DH. Prevalence of gastroesophageal reflux disease symptoms and uninvestigated dyspepsia in Korea: a population-based study. *Dig Dis Sci* 2008; **53**: 188-193

11 **Cho YS**, Choi MG, Jeong JJ, Chung WC, Lee IS, Kim SW, Han SW, Choi KY, Chung IS. Prevalence and clinical spectrum of gastroesophageal reflux: a population-based study in Asan-si, Korea. *Am J Gastroenterol* 2005; **100**: 747-753

12 **Lu CL**, Lang HC, Chang FY, Chen CY, Luo JC, Wang SS, Lee SD. Prevalence and health/social impacts of functional dyspepsia in Taiwan: a study based on the Rome criteria questionnaire survey assisted by endoscopic exclusion among a physical check-up population. *Scand J Gastroenterol* 2005; **40**: 402-411

13 **Westbrook JI**, Talley NJ. Empiric clustering of dyspepsia into symptom subgroups: a population-based study. *Scand J Gastroenterol* 2002; **37**: 917-923

14 **Schmulson M**, Ortiz O, Santiago-Lomeli M, Gutierrez-Reyes G, Gutierrez-Ruiz MC, Robles-Diaz G, Morgan D. Frequency of functional bowel disorders among healthy volunteers in Mexico City. *Dig Dis* 2006; **24**: 342-347

15 **Talley NJ**, Stanghellini V, Heading RC, Koch KL, Malagelada JR, Tytgat GN. Functional gastroduodenal disorders. *Gut* 1999; **45** Suppl 2: II37- II42

16 **Thompson WG**, Irvine EJ, Pare P, Ferrazzi S, Rance L. Functional gastrointestinal disorders in Canada: first population-based survey using Rome II criteria with suggestions for improving the questionnaire. *Dig Dis Sci* 2002; **47**: 225-235

17 **Bytzer P**, Hallas J. Drug-induced symptoms of functional dyspepsia and nausea. A symmetry analysis of one million prescriptions. *Aliment Pharmacol Ther* 2000; **14**: 1479-1484

18 **Ofman JJ**, Maclean CH, Straus WL, Morton SC, Berger ML, Roth EA, Shekelle PG. Meta-analysis of dyspepsia and nonsteroidal antiinflammatory drugs. *Arthritis Rheum* 2003; **49**: 508-518

19 **Kang JY**. Systematic review: the influence of geography and ethnicity in irritable bowel syndrome. *Aliment Pharmacol Ther* 2005; **21**: 663-676

20 **Han SH**, Lee OY, Bae SC, Lee SH, Chang YK, Yang SY, Yoon BC, Choi HS, Hahm JS, Lee MH, Lee DH, Kim TH. Prevalence of irritable bowel syndrome in Korea: population-based survey using the Rome II criteria. *J Gastroenterol Hepatol* 2006; **21**: 1687-1692

21 **El-Serag HB**, Olden K, Bjorkman D. Health-related quality of life among persons with irritable bowel syndrome: a systematic review. *Aliment Pharmacol Ther* 2002; **16**: 1171-1185

22 **Koloski NA**, Talley NJ, Boyce PM. The impact of functional gastrointestinal disorders on quality of life. *Am J Gastroenterol* 2000; **95**: 67-71

23 **Xiong LS**, Chen MH, Chen HX, Xu AG, Wang WA, Hu PJ. A population-based epidemiologic study of irritable bowel syndrome in South China: stratified randomized study by cluster sampling. *Aliment Pharmacol Ther* 2004; **19**: 1217-1224

24 **Chen M**, Xiong L, Chen H, Xu A, He L, Hu P. Prevalence, risk factors and impact of gastroesophageal reflux disease symptoms: a population-based study in South China. *Scand J Gastroenterol* 2005; **40**: 759-767

25 **Sobhonslidsuk A**, Silpakit C, Kongsakon R, Satitpornkul P, Sripetch C, Khanthavit A. Factors influencing health-related quality of life in chronic liver disease. *World J Gastroenterol* 2006; **12**: 7786-7791

26 **Simren M**, Abrahamsson H, Svedlund J, Bjornsson ES. Quality of life in patients with irritable bowel syndrome seen in referral centers versus primary care: the impact of gender and predominant bowel pattern. *Scand J Gastroenterol* 2001; **36**: 545-552

27 **Lee OY**, Mayer EA, Schmulson M, Chang L, Naliboff B. Gender-related differences in IBS symptoms. *Am J Gastroenterol* 2001; **96**: 2184-2193

28 **Williams RE**, Black CL, Kim HY, Andrews EB, Mangel AW, Buda JJ, Cook SF. Determinants of healthcare-seeking behaviour among subjects with irritable bowel syndrome. *Aliment Pharmacol Ther* 2006; **23**: 1667-1675

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RAPID COMMUNICATION

Effects of n-3 polyunsaturated fatty acids from seal oils on nonalcoholic fatty liver disease associated with hyperlipidemia

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Abstract

AIM: To investigate the efficacy and safety of n-3 polyunsaturated fatty acids (PUFA) from seal oils for patients with nonalcoholic fatty liver disease (NAFLD) associated with hyperlipidemia.

METHODS: One hundred and forty-four patients with NAFLD associated with hyperlipidemia were included in the 24-wk, randomized, controlled trial. The patients were randomized into two groups. Group A (n = 72) received recommended diet and 2 g n-3 PUFA from seal oils, three times a day. Group B (n = 72) received recommended diet and 2 g placebo, three times a day. Primary endpoints were fatty liver assessed by symptom scores, liver alanine aminotransferase (ALT) and serum lipid levels after 8, 12, 16, and 24 wk. Hepatic fat infiltration was detected by ultrasonography at weeks 12 and 24 after treatment.

RESULTS: A total of 134 patients (66 in group A, 68 in group B) were included in the study except for 10 patients who were excluded from the study. After 24 wk of treatment, no change was observed in body weight, fasting blood glucose (FBG), renal function and blood cells of these patients. Total symptom scores, ALT and triglyceride (TG) levels decreased more significantly in group A than in group B ($P < 0.05$). As expected, there was a tendency toward improvement in aspartate aminotransferase (AST), γ-glutamyltranspeptidase (GGT), and total cholesterol (TCHO) and high-density lipoprotein (HDL) cholesterol levels ($P < 0.05$) after administration in the two groups. However, no significant differences were found between the two groups. The values of low-density lipoprotein (LDL) were significantly improved in group A ($P < 0.05$), but no significant change was found in group B at different time points and after a 24-wk treatment. After treatment, complete fatty liver regression was observed in 19.70% (13/66) of the patients, and an overall reduction was found in 53.03% (35/66) of the patients in group A. In contrast, in group B, only five patients (7.35%, 5/68) achieved complete fatty liver regression (P = 0.04), whereas 24 patients (35.29%, 24/68) had a certain improvement in fatty liver (P = 0.04). No serious adverse events occurred in all the patients who completed the treatment.

CONCLUSION: Our results indicate that n-3 PUFA from seal oils is safe and efficacious for patients with NAFLD associated with hyperlipidemia and can improve their total symptom scores, ALT, serum lipid levels and normalization of ultrasonographic evidence. Further study is needed to confirm these results.

Key words: Nonalcoholic fatty liver disease; Polyunsaturated fatty acids; Seal oil; Hyperlipidemia; Therapy

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INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) represents a

spectrum of conditions characterized by an excessive accumulation of hepatic fat in the absence of alcohol consumption[1]. In Western countries and some regions of China, the prevalence of nonalcoholic steatohepatitis (NASH) and NAFLD is 1%-5% and 15%-39%, respectively[2]. In most of patients, NAFLD follows a relatively benign course and remains stable for years[1]. However, it was recently reported that many cases of cryptogenic liver cirrhosis may be related to unrecognized NASH[3]. Thus, treatment should be reserved for patients at risk of developing severe liver diseases. Medical therapy for NAFLD and NASH has been disappointing to date[4]. Although a number of treatment modalities are available, they cannot prevent the progression of early liver disease to its advanced stage, and the only recommended therapies are dietary modification and weight loss[1,5]. It has been shown that n-3 polyunsaturated fatty acids (PUFA) is effective on NAFLD[6-8]. In this study, we evaluated the efficacy and safety of n-3 PUFA from seal oils in ameliorating serum lipids and liver enzymes in patients with NAFLD associated with hyperlipidemia.

MATERIALS AND METHODS

Patients

One hundred and forty-four patients with NAFLD associated with mixed dyslipidemia were studied as outpatients in the Tonggji Hospital, Tongji University, from September 2006 to June 2008. Written informed consent was obtained from all patients and the study was approved by the Ethics Committee in Tonggji Hospital of Tongji University.

The inclusion criteria were as follows: age between 18 and 65 years, lack of excessive alcohol ingestion confirmed by careful questioning by the primary physician and dietitians (consumption of less than 70 g alcohol in female and 140 g in male per week), elevated serum alanine aminotransferase (ALT) and/or aspartate aminotransferase (AST) above the normal limit, but under 5 times the upper limit of the normal range for $\geqslant$ 6 mo before the study. Diagnosis of dyslipidemia was based on the presence of one or more of the following findings: fasting serum total cholesterol (TCHO) > 5.7 mmol/L, serum triglyceride (TG) > 1.8 mmol/L and high-density lipoprotein cholesterol (HDL-C) < 0.8 mmol/L, fatty liver diagnosed by abdominal ultrasonography, and ability to give informed consent. Exclusion criteria included overuse of alcohol, viral hepatitis, hemochromatosis, Wilson's disease, autoimmune hepatitis, α-1 antitrypsin deficiency, primary sclerosing cholangitis or primary biliary cirrhosis; history of any other hepatic, gastrointestinal, renal, cardiovascular, neurological or hematological disorders, psychiatric disorder which might impair the ability of patients to provide written informed consent; as well as pregnancy, breastfeeding, or lack of effective birth control in women at child-bearing age. In addition, patients were excluded if they were on any medications that could influence liver function during the observation period or involved in any clinical trial before the study.

Study design

All the patients meeting the criteria for enrollment agreed to participate in the study. The patients were randomized into two groups to receive a 24-wk treatment. Group A (n = 72) received recommended diet and 2 g n-3 PUFA from seal oils (Shanghai Hengsheng Biology & Medicine CO. Ltd, Shanghai, China), three times a day. Group B (n = 72) received recommended diet and 2 g placebo (Shanghai Hengsheng Biology & Medicine CO. Ltd, Shanghai, China), three times a day. Recommended diet was composed of 50% carbohydrates, 20% protein and 30% fat in accordance with the American Heart Association diet[9]. All obese and overweight patients were advised to lose their weight with a restriction of daily caloric intake to 25-30 kcal/kg per day[9]. All medications the patients received during the 24-wk treatment period were recorded. At the time of enrolment and when the study was completed, body temperature, body mass index (BMI), blood pressure and heart rate were detected and liver ultrasonography was performed. Laboratory tests included serum ALT, AST, γ-glutamyltranspeptidase (GGT), TG, TCHO, HDL-C, LDL-C, FBS, and complete blood cell counts.

During the 24-wk treatment period, total symptom scores, liver enzymes and fasting lipids were monitored at weeks 8, 12, 16, and 24. Hepatic fat infiltration was detected by upper abdominal ultrasonography at weeks 12 and 24. Symptoms included liver discomfort or pain, weakness, abdominal distention, and nausea. The severity of each clinical symptom was scored using a 4-point scale as follows: 0 score = asymptomatic, 1 score = mild, 2 scores = moderate, 3 scores = severe. All patients were investigated after 12 h fasting and underwent ultrasonography for liver steatosis. Ultrasound scans were performed by a trained operator who was blind to the treatment of participants. The severity of steatosis was also scored using a 4-point scale as follows[10]: grade 0 = normal echogenicity, grade 1 = slight, grade 2 = moderate, grade 3 = severe.

Statistical analysis

The data were presented as mean ± SD and analyzed using SPSS11.5 for Windows (SPSS, Chicago, IL, USA). Statistical analysis for baseline characteristics of the study groups was performed using χ^2 test and t-test. Student's t-test and Wilcoxon signed rank test were used to evaluate the changes in biochemical parameters before and after treatment. $P < 0.05$ was considered statistically significant.

RESULTS

Characteristics of the patients

Of the 144 patients enrolled in this study, 134 completed the protocol and were included in the analysis. The baseline clinical and demographic data about the two

Table 1 Baseline characteristics of groups A and B (mean ± SD)

	Group A (n = 66)	Group B (n = 68)	P vaule
Age (yr)	45.00 ± 10.91	44.03 ± 11.30	0.74
Sex ratio (male/female)	47/19	50/18	0.76
Body height (cm)	169.07 ± 7.96	169.71 ± 7.89	0.88
Weight (kg)	75.71 ± 10.99	75.16 ± 11.33	0.93
BMI (kg/m^2)	26.37 ± 3.12	25.96 ± 2.70	0.57
HR (/min)	75.57 ± 6.24	75.89 ± 6.58	0.32
Duration of NAFLD (mo)	22.32 ± 38.82	13.65 ± 20.00	0.55
SBP (mmHg)	126.59 ± 10.97	125.89 ± 9.87	0.58
DBP (mmHg)	82.26 ± 7.50	81.59 ± 8.02	0.81
HB (g/L)	143.31 ± 16.49	145.53 ± 15.52	0.58
RBC (× 10^{12}/L)	4.71 ± 0.57	4.76 ± 0.61	0.84
WBC (× 10^9/L)	6.16 ± 1.58	6.52 ± 1.45	0.18
Platelet (× 10^9/L)	205.79 ± 49.43	195.78 ± 53.33	0.53
BUN (mmol/L)	5.30 ± 1.67	5.19 ± 1.57	0.63
Creatinine (μmol/L)	72.56 ± 15.27	76.69 ± 15.35	0.2
FBG (mmol/L)	5.89 ± 1.20	5.46 ± 1.82	0.43
Total symptom scores	1.87 ± 1.18	1.79 ± 0.45	0.23
Steatosis degree 0/1/2/3 (%)	0/30/56/14	0/37/48/15	0.63

BMI: Body mass index; HR: Heart rate; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; HB: Haematoglobin; RBC: Red blood cells; WBC: White blood cells; BUN: Blood urea nitrogen; FBG: Fasting blood glucose.

groups are shown in Table 1. Patients in the two groups were matched for age, sex, body height and weight, BMI, HR, course of disease, blood pressure, and blood tests including transaminase and lipid concentrations. The ultrasound stages of steatosis were also paired (P = 0.63). No patient was classified as grade 0. In group A, 37% of the patients were classified as grade1, 48% as grade 2, and 15% as grade 3, respectively. In group B, 30% of the patients were classified as grade 1, 56% as grade 2, and 14% as grade 3, respectively.

Findings in the two groups before and after treatment

No significant difference was observed in dietary compliance, BMI, blood pressure, HR, HB, RBC, WBC, platelet, serum BUN and creatinine(Cr) between the two groups. At the end of a 24-wk treatment period, a significant improvement in several liver and lipid parameters was observed between the two groups. In particular, total symptom scores, ALT and TG levels decreased more significantly (P < 0.01) in Group A (total symptom score = 1.87 ± 1.18 to 0.42 ± 0.72, ALT = 62.79 ± 35.92 U/L to 39.27 ± 18.94 U/L, TG= 3.94 ± 2.69 mmol/L to 2.08 ± 1.03 mmol/L) than in Group B (total symptom score = 1.87 ± 1.18 to 0.42 ± 0.72, ALT = 79.76 ± 50.59U/L to 42.32 ± 22.23U/L, TG = 3.80 ± 2.85 mmol/L to 2.33 ± 1.42 mmol/L) (Table 2). As compared to the pretreatment values, total symptom score, ALT and TG levels at weeks 8, 12, and 16 decreased significantly in the two groups after treatment (P < 0.01).

As expected, there was a tendency toward improvement in AST, GGT, TCHO, and HDL levels (P < 0.05) in the two groups after treatment. However, no significant difference was observed in the two groups.

Table 2 Liver enzymes, lipid parameters and ultrasound findings before and after treatment in groups A and B (mean ± SD)

Variables	Group A (n = 66)	Group B (n = 68)
At baseline		
Total symptom scores	1.87 ± 1.18	1.79 ± 1.45
Serum ALT (U/L)	62.79 ± 35.92	79.76 ± 50.59
Serum AST (U/L)	38.13 ± 20.99	50.09 ± 39.05
Serum GGT (U/L)	72.53 ± 52.48	79.96 ± 5.27
Serum TCHO (mmol/L)	6.30 ± 0.83	5.91 ± 1.16
Serum TG (mmol/L)	3.94 ± 2.69	3.80 ± 2.85
Serum HDL-C (mmol/L)	1.01 ± 0.24	1.05 ± 0.33
Serum LDL-C (mmol/L)	3.26 ± 0.98	3.19 ± 0.92
Steatosis degree 0/1/2/3 (%)	0/30/56/14	0/37/48/15
At week 8		
Total symptom scores	1.00 ± 0.96^{b,c}	1.23 ± 1.21^b
Serum ALT (U/L)	50.81 ± 35.24^{b,c}	61.09 ± 40.30^b
Serum AST (U/L)	35.25 ± 21.20^a	39.84 ± 23.55^a
Serum GGT (U/L)	57.82 ± 50.22^a	79.97 ± 87.16
Serum TCHO (mmol/L)	5.81 ± 0.81^{a,c}	5.90 ± 0.97^a
Serum TG (mmol/L)	2.86 ± 1.49^b	3.17 ± 2.80^b
Serum HDL-C (mmol/L)	1.04 ± 0.19	1.13 ± 0.24
Serum LDL-C (mmol/L)	3.16 ± 0.80^a	3.16 ± 0.85
At week 12		
Total symptom scores	0.66 ± 0.87^{b,d}	0.97 ± 1.10^b
Serum ALT (U/L)	47.48 ± 33.30^{b,c}	55.17 ± 43.15^b
Serum AST (U/L)	30.69 ± 16.80^a	37.26 ± 19.57^a
Serum GGT (U/L)	46.94 ± 35.38^b	72.87 ± 73.30^a
Serum TCHO (mmol/L)	5.66 ± 1.18^a	5.74 ± 1.14^a
Serum TG (mmol/L)	2.47 ± 1.75^{a,c}	2.80 ± 2.57^a
Serum HDL-C (mmol/L)	1.12 ± 0.24^a	1.15 ± 0.28^a
Serum LDL-C (mmol/L)	3.10 ± 0.98^a	3.14 ± 0.85
Steatosis degree 0/1/2/3 (%)	9/64/21/6^a	9/59/26/6^a
At week 16		
Total symptom scores	0.40 ± 0.60^{b,d}	0.77 ± 1.07^b
Serum ALT (U/L)	45.06 ± 34.23^{b,c}	44.13 ± 33.15^b
Serum AST (U/L)	30.18 ± 15.40^a	32.03 ± 16.51^a
Serum GGT (U/L)	44.34 ± 39.50^b	62.86 ± 78.00^a
Serum TCHO (mmol/L)	5.69 ± 0.99^a	5.68 ± 0.99^a
Serum TG (mmol/L)	2.26 ± 1.26^{b,d}	2.64 ± 2.90^b
Serum HDL-C (mmol/L)	1.13 ± 0.22^a	1.22 ± 0.28^a
Serum LDL-C (mmol/L)	3.12 ± 0.83^a	3.12 ± 0.87
At week 24		
Total symptom scores	0.42 ± 0.72^{b,d}	0.53 ± 0.97^b
Serum ALT (U/L)	39.27 ± 18.94^{b,d}	42.32 ± 22.23^b
Serum AST (U/L)	30.45 ± 12.67^a	30.25 ± 14.21^a
Serum GGT (U/L)	42.47 ± 26.84^b	58.43 ± 36.21^b
Serum TCHO (mmol/L)	5.08 ± 0.76^a	5.21 ± 1.22^a
Serum TG (mmol/L)	2.08 ± 1.03^{b,d}	2.33 ± 1.42^b
Serum HDL-C (mmol/L)	1.25 ± 0.25^a	1.20 ± 0.21^a
Serum LDL-C (mmol/L)	3.12 ± 0.84^a	3.11 ± 0.78
Steatosis degree 0/1/2/3 (%)	20/64/12/4^{b,d}	7/51/36/6^a

TCHO: Total cholesterol; TG: Triglycerides; HDL-C: High density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol. ^aP < 0.05, ^bP < 0.01 *vs* baseline; ^cP < 0.05, ^dP < 0.01 *vs* group B.

Significant improvements were found in serum LDL-C levels of group A (P < 0.05), but not found in group B after treatment.

Ultrasonography showed a normal liver echopattern at the end of treatment in 19.70% (13/66) of the patients, and an overall reduction in 53.03% (35/66) of the patients in group A. In contrast, only five patients (7.35%, 5/68) achieved complete regression (P = 0.04), whereas 24 patients (35.29%, 24/68) had a certain reduction (P = 0.04) in group B. No change was

observed in the remaining 64.71% of patients.

Gastrointestinal complaints of increased fecal frequency, epigastria, and defecation were occasionally noted in 8 of the 134 patients; but these adverse effects were not significantly different in the two groups. The patients recovered when they completed the treatment. Most of the patients who completed the treatment had no adverse events, indicating that they can tolerate the treatment. No severe adverse event was observed.

DISCUSSION

NAFLD is a chronic disease with multiple consequences. The spectrum of this disease ranges from simple steatosis to NASH, which may lead to liver fibrosis and cirrhosis[11-13]. It has also been well established that NAFLD is intimately related to various clinical and biological markers of the insulin resistance syndrome[14-16]. The pathogenesis of NASH is multifactorial, including insulin resistance, excessive intracellular fatty acids, oxidant stress, mitochondrial dysfunction and innate immunity[17]. However, the pathogenesis of NAFLD/NASH is yet to be clearly elucidated. Since the most prevailing general theory is the "two-hit" hypothesis proposed by Day and James in 1998[16], most treatment modalities should be focused on improving the "two-hit" hypothesis or insulin resistance[18].

Currently, therapeutic options are limited. The present "gold standard" for NAFLD is weight reduction, or more precisely, a reduction in central obesity so as to reverse insulin resistance[19-21]. Standard practice advocates weight loss and exercise. Such "lifestyle adjustment" or anti-obesity measures (including bariatric surgery when required) can improve insulin sensitivity with only a modest weight loss (2-8 kg)[22-24], which is difficult for most patients to achieve.

It was reported that dietary supplementation with fatty acids, such as fish and fish oils, can improve NAFLD associated with hyperlipidemia by modifying the function of platelets and leukocytes[25-26]. Suggested modes of action are through their modulation of eicosanoid synthesis and reduction in plasma TG concentration. The fat composition of seal oils differs significantly from that of fish. In marine mammals, eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3) are found mainly at the sn-1 and sn-3 positions of TG, whereas in fish, these fatty acids are positioned in sn-2, which may display the better effects on NAFLD associated with hyperlipidemia than fish oils.

Increased fat intake with an excessive amount of n-6 fatty acids can promote NAFLD. However, n-3 PUFA can ameliorate experimental NAFLD[27]. It was reported that n-3 PUFA from different animals is effective against NAFLD[28-30]. In this study, we evaluated the efficacy and safety of n-3 fatty acids from seal oils in 144 patients with NAFLD associated with hyperlipidemia. The results showed that treatment of NAFLD patients with hyperlipidemia with n-3 PUFA from seal oils significantly reduced their total symptom score, ALT and lipid levels, and normalized ultrasonographic evidence compared to treatment with the recommended diet alone.

As expected, there was a tendency toward improvement in AST, GGT, TC, and HDL levels ($P < 0.05$) in the two groups after treatment. However, no significant difference was seen in the two groups. Spadaro *et al*[29] also reported that serum GGT, HDL, ALT, and lipid levels are decreased after treatment. In the present study, the values of LDL were significantly improved in group A ($P < 0.05$), but not in group B at different time points and after a 24-wk treatment period. Ultrasonography showed complete fatty liver regression in 19.70% (13/66) of the patients, and an overall reduction in 53.03% (35/66) of the patients in group A. In contrast, only five patients (7.35%, 5/68) achieved complete regression ($P = 0.04$), whereas 24 patients (35.29%, 24/68) had a certain reduction ($P = 0.04$) in group B. No change was observed in the remaining 64.71% of patients.

Tanaka *et al*[31] reported that treatment with EPA, one of the major components of n-3 PUFA, seems to be safe and efficacious for patients with NASH, largely due to its anti-inflammatory and anti-oxidative properties. On the other hand, n-3 PUFA could reduce VLDL production, resulting in decreased serum triglyceride levels[32-34]. These findings are consistent with our findings, such as improvement in total symptom score, ALT and lipid levels and normalization of ultrasonographic evidence in patients with NAFLD associated with hyperlipidemia. Improvement in serum biochemistry parameters was also observed in group B, indicating that restricted diet and exercise can reverse insulin resistance at a certain extent[35-37]. In the present study, the two drugs (placebo and seal oils) appeared to be safe and effective in patients with NAFLD associated with hyperlipidemia and no severe side effects were observed during treatment.

The gold standard for diagnosis of NAFLD is liver biopsy, but it is not frequently performed in NAFLD patients due to its low acceptance rate[38]. In our study, ultrasonography was performed to detect and monitor changes in the liver since it is sensitive, cheap, invasive and easy to perform. However, lack of histological findings is a major drawback of this investigation.

In conclusion, treatment of NAFLD associated with hyperlipidemia with PUFA from seal oils seems to be safe and efficacious, and can improve the total symptom score, ALT and lipid levels and normalization of ultrasonographic evidence. Further study is needed to confirm these results.

COMMENTS

Background

Recent reports suggest that many cases of cryptogenic liver cirrhosis may be related to unrecognized nonalcoholic steatohepatitis (NASH); however, medical therapy for nonalcoholic fatty liver disease (NAFLD) and NASH has been disappointing to date. The only recommended therapies are dietary modification and weight loss. N-3 polyunsaturated fatty acids (PUFA) seems to be efficacious on treating NAFLD from animal and some small samples human studies.

Research frontiers

The present "gold standard" for treatment of NAFLD is a reduction in central obesity so as to reverse insulin resistance. Several small samples randomized trails have suggested n-3 PUFA from different animals were effective in the treatment of NAFLD; we explored whether seal oil is efficacious and safe in large samples NAFLD patients.

Innovations and breakthroughs

Total symptom scores in NAFLD patients, and large samples were observed besides biochemical indicators and ultrasonography in this study. Seal oils n-3 PUFA can improve liver enzyme, serum lipid levels and normalization of ultrasonographic evidence.

Applications

Seal oils PUFA administration seems to be safe and efficacious for patients with NAFLD associated with hyperlipidemia as well as the "lifestyle adjustment" or anti-obesity measures.

Terminology

NAFLD refers to the presence of hepatic steatosis not associated with a significant intake of ethanol. Insulin resistance is central to the pathogenesis of NAFLD; thus obesity, diabetes, and the metabolic syndrome are frequently associated with the disease.

Peer review

This is an interesting study. Further details need to be given as to the precise ultrasound scoring system that was used to assess the resolution of hepatic steatosis. Was there an objective scoring system used or was this all subjective? The paper is otherwise well written and merits publication.

REFERENCES

1 **Angulo P**. Nonalcoholic fatty liver disease. *N Engl J Med* 2002; **346**: 1221-1231

2 **Zhou YJ**, Li YY, Nie YQ, Ma JX, Lu LG, Shi SL, Chen MH, Hu PJ. Prevalence of fatty liver disease and its risk factors in the population of South China. *World J Gastroenterol* 2007; **13**: 6419-6424

3 **Nagata K**, Suzuki H, Sakaguchi S. Common pathogenic mechanism in development progression of liver injury caused by non-alcoholic or alcoholic steatohepatitis. *J Toxicol Sci* 2007; **32**: 453-468

4 **Moscatiello S**, Marzocchi R, Villanova N, Bugianesi E, Marchesini G. Which treatment for nonalcoholic fatty liver disease? *Mini Rev Med Chem* 2008; **8**: 767-775

5 **Okita M**, Hayashi M, Sasagawa T, Takagi K, Suzuki K, Kinoyama S, Ito T, Yamada G. Effect of a moderately energy-restricted diet on obese patients with fatty liver. *Nutrition* 2001; **17**: 542-547

6 **Hatzitolios A**, Savopoulos C, Lazaraki G, Sidiropoulos I, Haritanti P, Lefkopoulos A, Karagiannopoulou G, Tzioufa V, Dimitrios K. Efficacy of omega-3 fatty acids, atorvastatin and orlistat in non-alcoholic fatty liver disease with dyslipidemia. *Indian J Gastroenterol* 2004; **23**: 131-134

7 **Song BJ**, Moon KH, Olsson NU, Salem N Jr. Prevention of alcoholic fatty liver and mitochondrial dysfunction in the rat by long-chain polyunsaturated fatty acids. *J Hepatol* 2008; **49**: 262-273

8 **Svegliati-Baroni G**, Candelaresi C, Saccomanno S, Ferretti G, Bachetti T, Marzioni M, De Minicis S, Nobili L, Salzano R, Omenetti A, Pacetti D, Sigmund S, Benedetti A, Casini A. A model of insulin resistance and nonalcoholic steatohepatitis in rats: role of peroxisome proliferator-activated receptor-alpha and n-3 polyunsaturated fatty acid treatment on liver injury. *Am J Pathol* 2006; **169**: 846-860

9 **Zelber-Sagi S**, Nitzan-Kaluski D, Goldsmith R, Webb M, Blendis L, Halpern Z, Oren R. Long term nutritional intake and the risk for non-alcoholic fatty liver disease (NAFLD): a population based study. *J Hepatol* 2007; **47**: 711-717

10 **Graif M**, Yanuka M, Baraz M, Blank A, Moshkovitz M, Kessler A, Gilat T, Weiss J, Walach E, Amazeen P, Irving CS. Quantitative estimation of attenuation in ultrasound video images: correlation with histology in diffuse liver disease. *Invest Radiol* 2000; **35**: 319-324

11 **Cortez-Pinto H**, Jesus L, Barros H, Lopes C, Moura MC, Camilo ME. How different is the dietary pattern in non-alcoholic steatohepatitis patients? *Clin Nutr* 2006; **25**: 816-823

12 **Oliveira CP**, Coelho AM, Barbeiro HV, Lima VM, Soriano F, Ribeiro C, Molan NA, Alves VA, Souza HP, Machado MC, Carrilho FJ. Liver mitochondrial dysfunction and oxidative stress in the pathogenesis of experimental nonalcoholic fatty liver disease. *Braz J Med Biol Res* 2006; **39**: 189-194

13 **Alwayn IP**, Gura K, Nose V, Zausche B, Javid P, Garza J, Verbesey J, Voss S, Ollero M, Andersson C, Bistrian B, Folkman J, Puder M. Omega-3 fatty acid supplementation prevents hepatic steatosis in a murine model of nonalcoholic fatty liver disease. *Pediatr Res* 2005; **57**: 445-452

14 **Mendez-Sanchez N**, Arrese M, Zamora-Valdes D, Uribe M. Treating nonalcoholic fatty liver disease. *Liver Int* 2007; **27**: 1157-1165

15 **Harrison SA**, Kadakia S, Lang KA, Schenker S. Nonalcoholic steatohepatitis: what we know in the new millennium. *Am J Gastroenterol* 2002; **97**: 2714-2724

16 **Day CP**, James OF. Steatohepatitis: a tale of two "hits"? *Gastroenterology* 1998; **114**: 842-845

17 **Ma X**, Li Z. Pathogenesis of nonalcoholic steatohepatitis (NASH). *Chin J Dig Dis* 2006; **7**: 7-11

18 **Preiss D**, Sattar N. Non-alcoholic fatty liver disease: an overview of prevalence, diagnosis, pathogenesis and treatment considerations. *Clin Sci* (Lond) 2008; **115**: 141-150

19 **Larter CZ**, Farrell GC. Insulin resistance, adiponectin, cytokines in NASH: Which is the best target to treat? *J Hepatol* 2006; **44**: 253-261

20 **Palasciano G**, Moschetta A, Palmieri VO, Grattagliano I, Iacobellis G, Portincasa P. Non-alcoholic fatty liver disease in the metabolic syndrome. *Curr Pharm Des* 2007; **13**: 2193-2198

21 **Boppidi H**, Daram SR. Nonalcoholic fatty liver disease: hepatic manifestation of obesity and the metabolic syndrome. *Postgrad Med* 2008; **120**: E01-E07

22 **Oh MK**, Winn J, Poordad F. Review article: diagnosis and treatment of non-alcoholic fatty liver disease. *Aliment Pharmacol Ther* 2008; **28**: 503-522

23 **Schreuder TC**, Verwer BJ, van Nieuwkerk CM, Mulder CJ. Nonalcoholic fatty liver disease: An overview of current insights in pathogenesis, diagnosis and treatment. *World J Gastroenterol* 2008; **14**: 2474-2486

24 **Krasnoff JB**, Painter PL, Wallace JP, Bass NM, Merriman RB. Health-related fitness and physical activity in patients with nonalcoholic fatty liver disease. *Hepatology* 2008; **47**: 1158-1166

25 **Vognild E**, Elvevoll EO, Brox J, Olsen RL, Barstad H, Aursand M, Osterud B. Effects of dietary marine oils and olive oil on fatty acid composition, platelet membrane fluidity, platelet responses, and serum lipids in healthy humans. *Lipids* 1998; **33**: 427-436

26 **Puri P**, Baillie RA, Wiest MM, Mirshahi F, Choudhury J, Cheung O, Sargeant C, Contos MJ, Sanyal AJ. A lipidomic analysis of nonalcoholic fatty liver disease. *Hepatology* 2007; **46**: 1081-1090

27 **El-Badry AM**, Graf R, Clavien PA. Omega 3 - Omega 6: What is right for the liver? *J Hepatol* 2007; **47**: 718-725

28 **Allard JP**, Aghdassi E, Mohammed S, Raman M, Avand G, Arendt BM, Jalali P, Kandasamy T, Prayitno N, Sherman M, Guindi M, Ma DW, Heathcote JE. Nutritional assessment and hepatic fatty acid composition in non-alcoholic fatty liver disease (NAFLD): a cross-sectional study. *J Hepatol* 2008; **48**: 300-307

29 **Spadaro L**, Magliocco O, Spampinato D, Piro S, Oliveri C, Alagona C, Papa G, Rabuazzo AM, Purrello F. Effects of n-3 polyunsaturated fatty acids in subjects with nonalcoholic fatty liver disease. *Dig Liver Dis* 2008; **40**: 194-199

30 **Capanni M**, Calella F, Biagini MR, Genise S, Raimondi L, Bedogni G, Svegliati-Baroni G, Sofi F, Milani S, Abbate R, Surrenti C, Casini A. Prolonged n-3 polyunsaturated fatty acid supplementation ameliorates hepatic steatosis in

patients with non-alcoholic fatty liver disease: a pilot study. *Aliment Pharmacol Ther* 2006; **23**: 1143-1151

31 **Tanaka N**, Sano K, Horiuchi A, Tanaka E, Kiyosawa K, Aoyama T. Highly purified eicosapentaenoic acid treatment improves nonalcoholic steatohepatitis. *J Clin Gastroenterol* 2008; **42**: 413-418

32 **Murano Y**, Funabashi T, Sekine S, Aoyama T, Takeuchi H. Effect of dietary lard containing higher alpha-linolenic acid on plasma triacylglycerol in rats. *J Oleo Sci* 2007; **56**: 361-367

33 **Vernaglione L**, Cristofano C, Chimienti S. Omega-3 polyunsaturated fatty acids and proxies of cardiovascular disease in hemodialysis: a prospective cohort study. *J Nephrol* 2008; **21**: 99-105

34 **Elizondo A**, Araya J, Rodrigo R, Poniachik J, Csendes A, Maluenda F, Diaz JC, Signorini C, Sgherri C, Comporti M, Videla LA. Polyunsaturated fatty acid pattern in liver and erythrocyte phospholipids from obese patients. *Obesity* (Silver Spring) 2007; **15**: 24-31

35 **Ueno T**, Sugawara H, Sujaku K, Hashimoto O, Tsuji R, Tamaki S, Torimura T, Inuzuka S, Sata M, Tanikawa K. Therapeutic effects of restricted diet and exercise in obese patients with fatty liver. *J Hepatol* 1997; **27**: 103-107

36 **Huang MA**, Greenson JK, Chao C, Anderson L, Peterman D, Jacobson J, Emick D, Lok AS, Conjeevaram HS. One-year intense nutritional counseling results in histological improvement in patients with non-alcoholic steatohepatitis: a pilot study. *Am J Gastroenterol* 2005; **100**: 1072-1081

37 **Harrison SA**, Fincke C, Helinski D, Torgerson S, Hayashi P. A pilot study of orlistat treatment in obese, non-alcoholic steatohepatitis patients. *Aliment Pharmacol Ther* 2004; **20**: 623-628

38 **Wieckowska A**, McCullough AJ, Feldstein AE. Noninvasive diagnosis and monitoring of nonalcoholic steatohepatitis: present and future. *Hepatology* 2007; **46**: 582-589

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RAPID COMMUNICATION

ERCC1 polymorphism, expression and clinical outcome of oxaliplatin-based adjuvant chemotherapy in gastric cancer

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Abstract

AIM: To determine the influence of excision repair cross complementing group 1 (*ERCC1*) codon 118 polymorphism and mRNA level on the clinical outcome of gastric cancer patients treated with oxaliplatin-based adjuvant chemotherapy.

METHODS: Eighty-nine gastric cancer patients treated with oxalipatin-based adjuvant chemotherapy were included in this study. *ERCC1* codon 118 C/T polymorphism was tested by polymerase chain reaction-ligation detection reaction (PCR-LDR) method in peripheral blood lymphocytes of those patients; and the intratumoral *ERCC1* mRNA expression was measured using reverse transcription PCR in 62 patients whose tumor tissue specimens were available.

RESULTS: No significant relationship was found between *ERCC1* codon 118 polymorphism and *ERCC1* mRNA level. The median relapse-free and overall survival period was 20.1 mo and 28.4 mo, respectively. The relapse-free and overall survivals in patients with low levels of *ERCC1* mRNA were significantly longer than those in patients with high levels ($P < 0.05$), while there was no significant association found between *ERCC1* 118 genotypes and the disease prognosis. Multivariate analysis also showed that *ERCC1* mRNA level was a potential predictor for relapse and survival in gastric cancer patients treated with oxaliplatin-based adjuvant chemotherapy ($P < 0.05$).

CONCLUSION: *ERCC1* codon 118 polymorphism has no significant impact on *ERCC1* mRNA expression, and the intratumoral *ERCC1* mRNA level but not codon 118 polymorphism may be a useful predictive parameter for the relapse and survival of gastric cancer patients receiving oxaliplatin-based adjuvant chemotherapy.

Key words: Gastric cancer; Adjuvant chemotherapy; Excision repair cross complementing group 1; Gene polymorphism

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INTRODUCTION

In China, gastric cancer is the leading cause of cancer deaths, accounting for nearly one-fourth of all cancer deaths. Surgery is the primary modality for managing early-stage and locally-advanced disease. However, even after gastrectomy, the majority of patients develop local or distant recurrence[1]. Adjuvant chemotherapy for gastric cancer has been under clinical investigation for more than four decades. Fluoropyrimidines, platinum-drugs and taxanes were shown to be effective in the treatment of gastric cancer. However, the response rates of these drugs or their combinations were less than 50%[2,3]. There is no standard regimen for postoperative treatment at the moment. Having an effective assay to predict the response to a given chemotherapeutic protocol beforehand would greatly enhance the success rate as well as the life quality of the patients.

The nucleotide excision repair (NER) system plays a significant role in repairing a variety of distorting

lesions, including platinum-drug induced DNA adducts. Oxaliplatin is a platinum-based therapeutic agent that has shown anti-tumor activities in gastric cancer. Resistance to oxaliplatin has been attributed to enhanced tolerance and repair of DNA damage through the NER pathway. As an excision nuclease within the NER pathway, excision repair cross complementing group 1 (*ERCC1*) has been reported to play a major role in the response to platinum-based chemotherapy. Studies have shown that the higher the *ERCC1* expression levels, the less sensitive the tumors to platinum therapies[4-7]. Recently, a single nucleotide polymorphism at codon 118 (C→T) was reported to be associated with altered *ERCC1* mRNA levels[8] and clinical outcome in cancer patients treated with platinum-based chemotherapy[9-12]. However, the results about the relationship among *ERCC1* codon 118 polymorphism, *ERCC1* mRNA level and platinum sensitivity are controversial. In this study, we investigated whether the *ERCC1* codon 118 polymorphism could influence *ERCC1* mRNA expression, and whether the polymorphism, and the intratumoral *ERCC1* mRNA expression have the prognostic value for the gastric cancer patients receiving oxaliplatin-based adjuvant treatment.

MATERIALS AND METHODS

Patients

From June 2001 to March 2006, 89 patients with histologically confirmed gastric cancer were enrolled in this study at the 4th Affiliated Hospital of Suzhou University. Inclusion criteria included: (1) patients without early recurrence or incurable resection, (2) patients receiving no other adjuvant treatment, such as radiotherapy or immunotherapy, and (3) patients with their performance status score of 0-1 and a life expectancy over 6 mo. All those patients received radical surgery, and then were treated with at least four cycles of oxaliplatin-based adjuvant treatment, including 70 with 5-FU/leucovorin/oxaliplatin (FOLFOX4: oxaliplatin 85 mg/m^2 and leucovorin 400 mg/m^2 followed on days 1 and 2 by 5-FU 400 mg/m^2 intravenous (IV) bolus, then 600 mg/m^2 IV over 22-h continuous infusion, and repeated every 2 wk), 9 with 5-FU/leucovorin/oxaliplatin/other regimens (taxanes or hydroxycamptothecin) (paclitaxel 135 mg/m^2 or docetaxol 75 mg/m^2 on day 1, hydroxycamptothecin 8 mg/m^2 on days 1-5; and the usage of 5-FU and leucovorin was the same as that in FOLFOX4). If patients had hematologic toxic effects of -grade 3 or grade 4 or nonhematologic toxic effects of grades 2-4, their daily dose was reduced properly.

Blood samples were collected in EDTA-containing tubes from gastric cancer patients before surgery or chemotherapy, and tumor tissue samples were obtained during surgery, and stored in liquid nitrogen until preparation of RNA extracts. Follow-up of those patients was made at 3-mo intervals after chemotherapy at outpatient clinics or by routine phone calls. This study was approved by the ethics and research committee of our hospital.

Table 1 The sequences of primers and probes

Primers or probes	Sequences (5'-3')	Length of product (bp)
actin-U	AGAAGATGACCCAGATCATGTT	290
actin-L	CTTAATGTCACGCACGATTTCC	
ERCC1-U	TACCACAACCTGCACCCAGACTAC	321
ERCC1-L	CTGACTGTCCGTTTTGTTGACTGA	
ERCC1-118-U	GGTCATCCCTATTGATGGCTTCTG	154
ERCC1-118-L	AGCTCACCTGAGGAACAGGGCACAG	
ERCC1-118-P	p-TTGCGCACGAACTTCAGTACGGGAT GGGACACTAATCGGAGGATTA-FAM	
ERCC1-118-T	CTACGGAG GATTATGAGGAGCTGCGT CGCCAAATTCCCAGGGCACA	92
ERCC1-118-C	CTACGAAATCAGGAGGATTATGAGGA GACGTCGCCAAATTCCCAGGG CACG	97

Genotyping of ERCC1 codon 118 polymorphism

Genomic DNA was isolated from peripheral blood lymphocytes using Axygene genomic DNA purification kit (Axygen Biotechnology, China). The primers and probes are listed in Table 1. Genotyping of *ERCC1* codon 118 was performed using polymerase chain reaction-ligation detection reaction (PCR-LDR) method as described previously[13].

Relative quantitative analysis of ERCC1 mRNA using reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was extracted from 62 tumor tissues using Trizol (Invitrogen Corporation, CA) according to the manufacturer's instructions. The amount of total RNA was estimated by ultraviolet absorbance at 260 nm, and the quality was determined by agarose gel electrophoresis in the presence of formaldehyde. cDNA strand synthesis was performed using a reverse transcription system (Promega Corporation, US).

ERCC1 and an internal reference gene (β-*actin*) cDNA fragments were amplified separately by PCR in triplicates. The PCRs were carried out in a total volume of 25 μL including 2 μL cDNA, 1× PCR buffer, 1.5 mmol/L $MgCl_2$, 0.2 mmol/L dNTPs, 0.5 μmol/L each primer, 1 U hot-start Taq DNA polymerase (QIAgen). Cycling parameters were as follows: 95℃ for 15 min; 35 cycles of 94℃ for 40 s, 52℃ for 30 s, and 72℃ for 30 s; and a final extension step at 72℃ for 10 min. PCR products were analyzed by 2% agarose gel electrophoresis, and ethidium bromide staining following by visualization with ultraviolet illumination using a gel imaging analyzing system. *ERCC1* amplification products were calculated as a ratio of the gray scale of *ERCC1* to that of β-*actin*.

Statistical analysis

Data analysis was performed using SPSS 13.0 for Windows. *ERCC1* levels were categorized into a low and high value using the median concentration as a cut-off point. The relationship between the genotype frequencies, mRNA, levels and clinical characteristics were assessed by χ^2 or Fisher's exact probability tests. The Mann-Whitney *U* test was used to assess the correlation between *ERCC1* genotypes and mRNA levels. Relapse-free survival (RFS) was defined as the

Table 2 Relationship among *ERCC1* genotypes, mRNA expression and clinical characteristics of gastric cancer

Clinical characteristics	*n*	*ERCC1* polymorphism		χ^2	*P*	*ERCC1* mRNA		χ^2	*P*
		C/C (*n* = 45)	C/T + T/T (*n* = 44)			High value (*n* = 31)	Low value (*n* = 31)		
Age (yr)									
⩾ 58	48	25	23	0.096	0.833	18	18	0	1.000
< 58	41	20	21			13	13		
Gender									
Male	66	31	35	1.318	0.334	20	24	0.253	0.402
Female	23	14	9			11	7		
TNM stage									
I - II	19	13	6	3.082	0.079	6	6	0	1.000
III-IV	70	31	39			25	25		
Grading									
G2	45	20	24	0.552	0.527	15	19	1.042	0.444
G3	44	21	20			16	12		

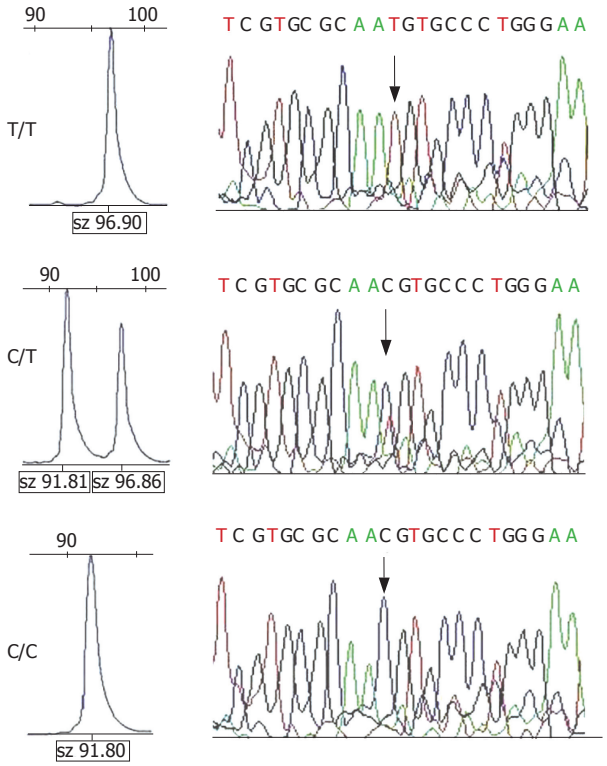

Figure 1 Genotyping results of *ERCC1* codon 118 polymorphism electrophoresis results of PCR-LDR products with different genotypes and its sequencing results. LDR products of *ERCC1* 118 C/C and T/T were 92 and 97 base pairs. The SNP sites are indicated by the arrowhead. The results were completely matched to the corresponding results derived from PCR-LDR.

time interval between the date of surgery and the date of confirmed relapse or the date of last follow-up. Overall survival (OS) was defined as the time between surgery and death. Survival curves were generated by the Kaplan-Meier method, and verified by the log-rank test. Cox proportional hazards regression analysis was used to estimate odds ratios (ORs) and their 95% confidence intervals (CIs), representing the overall relative risk of relapse and death associated with *ERCC1* polymorphism or expression, and to adjust for potential confounding variables. All of the values were two-sided and statistical significance was defined as $P < 0.05$.

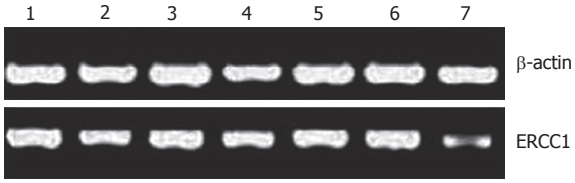

Figure 2 RT-PCR results of *ERCC1* mRNA in gastric cancer tissues.

RESULTS

ERCC1 genotypes and mRNA expression

A total of 89 patients were analyzed. Their demographic and disease characteristics are shown in Table 2. The allelic discrimination data from PCR-LDR assay were confirmed by direct sequencing of representative PCR products (Figure 1). Of the 89 patients, the frequencies of *ERCC1* codon 118 C/C, C/T and T/T were 50.6% (45/89), 42.7% (38/89) and 6.7% (6/89); and the allele frequencies of A and T were 71.9% and 28.1%, respectively. Genotype distribution of *ERCC1* codon 118 was consistent with the Hardy-Weinberg equilibrium among patients ($\chi^2 = 0.288$, $P > 0.05$).

Gastric cancer tissue samples were available in 62 patients. The intratumoral expression of *ERCC1* mRNA in those tissues was tested by semi-quantitative RT-PCR (Figure 2). A marked inter-individual variation in *ERCC1* mRNA expression in the 62 samples was observed: *ERCC1*/β-*actin* ratios ranged from 0.087 to 1.006 with a median value of 0.672. The median value was assigned as the cut-off value to divide those 62 patients into two groups with high or low *ERCC1* mRNA values.

No significant relationship was found between *ERCC1* expression and *ERCC1* codon 118 genotypes (the median *ERCC1* expression was 0.680 for C/C and 0.665 for C/T + T/T; $Z = -0.592$, $P = 0.554$) (Figure 3).

No significant association was found between age, gender, stage or grading and *ERCC1* codon 118 polymorphism or mRNA levels, except that a trend was found between the polymorphism and stage ($P = 0.079$) (Table 2).

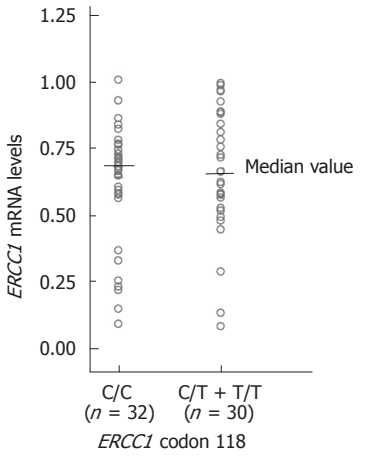

Figure 3 Relationship between *ERCC1* mRNA levels and codon 118 polymorphism.

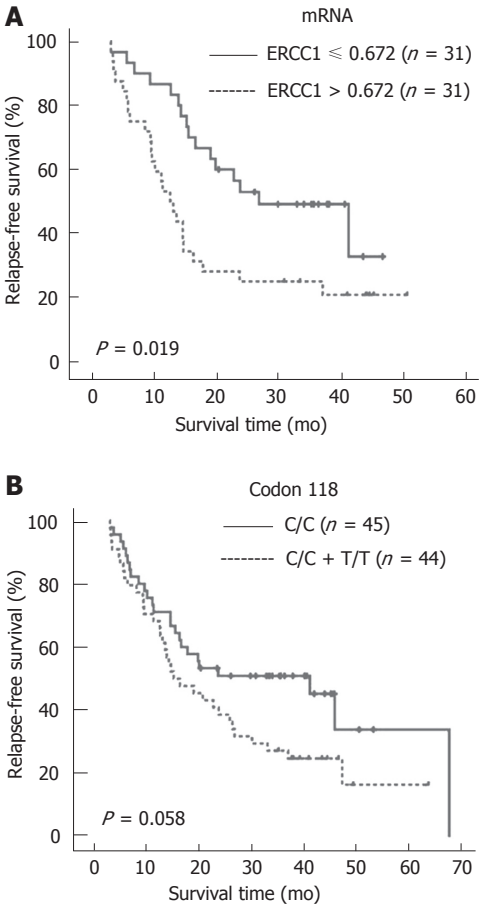

Figure 4 Relapse-free survival curves of gastric cancer patients according to *ERCC1* mRNA expression and *ERCC1* 118 C/T polymorphism relapse-free survival curves according to *ERCC1* mRNA expression (A) or *ERCC1* 118 polymorphism (B). The relapse-free survival in patients with high levels of *ERCC1* mRNA (> 0.672) was significantly poorer than that in patients with low levels (⩽ 0.672) ($P < 0.05$), while there was no significant difference between patients with *ERCC1* 118 C/C and variant genotypes (T/T or C/T).

Associations between ERCC1 polymorphism, mRNA levels and clinical outcome

Patients with the C/C genotype showed a trend towards correlation with prolonged RFS when compared to those with the C/T + T/T genotypes (22.0 mo *vs* 16.5 mo, χ^2 = 3.602, $P = 0.058$, Figure 4A). The median OS was 29.8 (95% CI = 20.9-83.1) mo for the patients with the C/C genotype, and 26.4 (95% CI = 22.1-34.7) mo in those with the C/T or T/T genotype ($\chi^2 = 1.548$, $P = 0.214$) (Figure 5A). The median RFS was 23.8 mo in patients with low *ERCC1* values, but only 13.2 mo in patients with high *ERCC1* levels ($\chi^2 = 5.464$, $P = 0.019$) (Figure 4B). A significant difference in OS also was found between the groups with low *ERCC1* levels and high *ERCC1* levels (29.6 mo *vs* 18.7 mo, $\chi^2 = 4.546$, $P = 0.033$) (Figure 5B).

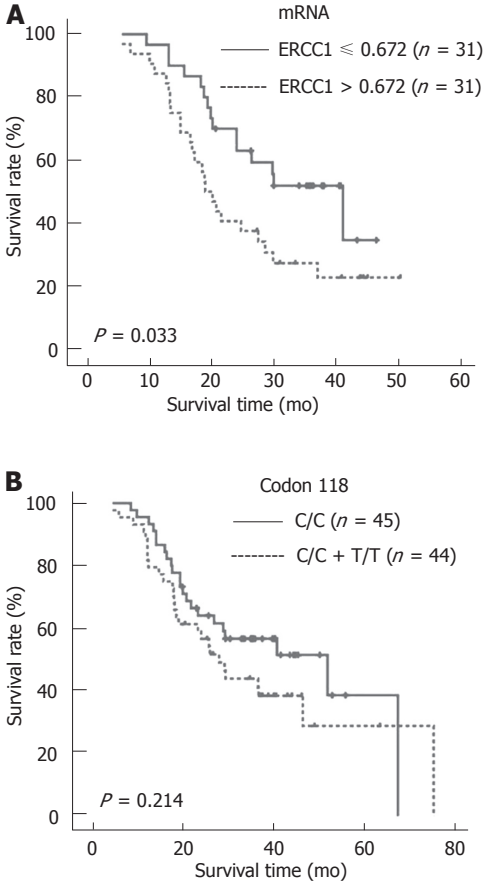

Figure 5 Overall survival curves of gastric cancer patients according to *ERCC1* mRNA expression and *ERCC1* 118 C/T polymorphism survival curves according to *ERCC1* mRNA levels (A) or *ERCC1* 118 polymorphism (B). The overall survival in patients with low level of *ERCC1* mRNA was significantly longer than that in patients with high levels ($P < 0.05$), while there was no significant difference found between patients with *ERCC1* 118 C/C and variant genotypes (T/T or C/T).

Cox multivariate analysis showed that, after adjustment for age, gender, stage and grading, a high *ERCC1* mRNA level appeared to be an independent risk factor for RFS (adjusted OR = 2.493, 95% CI: 1.291-4.814, $P = 0.006$) and OS (adjusted OR = 2.449, 95% CI: 1.264-4.743, $P = 0.008$). No significant association was found between *ERCC1* codon 118 genotypes and RFS (adjusted OR = 1.644, 95% CI = 0.954-2.833, $P = 0.074$) or OS (adjusted OR = 1.310, 95% CI = 0.727-2.358, $P = 0.369$).

DISCUSSION

Optimal chemotherapeutic treatment would allow clinicians to maximize the benefits of cancer

chemotherapy. Successful adjuvant chemotherapy following gastrectomy is crucial for a favorable outcome in gastric cancer. However, few prognostic and predictive markers have been identified to individualize treatment, maximize therapeutic effect. The *ERCC1* expression and codon 118 polymorphism have been reported to influence platinum-based drug sensitivity in advanced or metastatic cancers. The aim of this study was to determine whether *ERCC1* codon 118 polymorphism could influence the intratumoral *ERCC1* mRNA level and predict the clinical outcome of gastric cancer patients treated with oxaliplatin-based adjuvant chemotherapy.

Some studies suggested that impaired DNA repair within the tumor could lead to the decreased removal of platinum-DNA adducts and, therefore, increased clinical response to platinum chemotherapy. *ERCC1* mRNA level has been shown to correlate with nucleotide excision repair capacity. Chinese hamster ovary cells, which do not express a functional ERCC1 protein, are more susceptible to platinum-drugs than the parental cell line with normal ERCC1[4]. So it is naturally expected that the higher the levels of *ERCC1* expression, the less susceptible the tumors to platinum agents. Recently, a synonymous polymorphism at codon 118 converting a common codon usage (AAC) to an infrequent one (AAT), both coding for asparagine, has been associated with reduced mRNA and protein levels[8]. However, the assumed relationship between *ERCC1* codon 118 polymorphism and expression was not always observed[14]. In this study, the *ERCC1* mRNA levels in patients with C/C genotype was higher than that in patients with C/T or T/T genotype; but the difference failed to reach statistical significance ($P > 0.05$), which suggested that the polymorphism may have limited impact on *ERCC1* mRNA levels. In addition, the possibility that *ERCC1* codon 118 polymorphism is in linkage disequilibrium with other *ERCC1* mutations or polymorphisms that directly affect its expression also cannot be ruled out. Other possible reasons may be the relatively small sample size of the present study, and the quantitative method for *ERCC1* mRNA expression used in this study. In the future, using more accurate real-time quantitative RT-PCR assay on the large number of patients may help us to give more persuasive data on the putative association.

Although *ERCC1* codon 118 polymorphism has been extensively studied for its involvement in carcinogenesis[15,16], the predictive value of the polymorphism on platinum chemotherapy has not been studied thoroughly. The functional importance of this polymorphism is still under debate. A limited number of studies suggest that the favorable prognosis seems associated with the T allele[12,17-19]; but controversial results also exist[9-11,20,21]. Viguier *et al*[12] and Martinez-Balibrea *et al*[19] found that colorectal cancer patients with the *ERCC1* 118 T/T genotype were more likely to respond to oxaliplatin-based chemotherapy than carriers of the other genotypes. The favorable effect of T/T genotype also was found in lung cancer[22], pancreatic cancer[23], and ovarian cancer[18] patients treated with platinum-based chemotherapy. However, other studies on lung cancer[10] and colorectal cancer[9,11,20,21] showed opposite results. In addition, several studies demonstrated that no clear association was found between *ERCC1* codon 118 polymorphism and platinum sensitivity[24-26]. In a recent study on advanced gastric cancer treated with fluorouracil/cisplatin palliative chemotherapy, a tendency to higher response rate was found in patients with C allele ($P = 0.09$). In this study, patients with the C/C genotype also showed a trend to prolonged RFS when compared to those with the other genotypes, while no significant relationship was found between *ERCC1* codon 118 genotypes and OS. The small sample size ($n = 89$) of the present study might remain a limitation to clarify the exact role of *ERCC1* codon 118 polymorphism. Other possible reasons for controversial results may include genotyping in normal or tumor tissues, variable doses and schedules of platinum-based therapy, different ethnic populations, variable tumor stage and different kind of cancers.

A limitation of the presented study is that we only analyzed germline genotype. The germline genotypes offer better clinical accessibility and applicability, compared to tumor tissue, which presents difficulties in obtaining and handling samples. To analyze somatic genotype from tumor tissues was not easy. It is difficult to purify malignant cells from miscellaneous normal cells in clinical tumor tissue even using laser microdissection. The classification of a certain gene polymorphism may be hampered in a mixture of normal and malignant cells, which has been clearly illustrated by loss of heterozygosity. The correlation between germline genotype from peripheral blood and tumor tissue should be considered. To the best of our knowledge, there are no related reports on the impact of LOH on *ERCC1* polymorphism in gastric cancer. Hence, the possible influence of LOH on *ERCC1* genotyping should be discussed in the future.

Relative consensus conclusions were obtained regarding the effect of *ERCC1* expression on platinum-drug sensitivity. A multi-centers study on non–small-cell lung cancer found that cisplatin-based adjuvant chemotherapy significantly prolonged survival among patients with ERCC1-negative tumors, but not among patients with ERCC1-positive tumors[5]. In advanced gastric cancer patients treated with 5-FU and oxaliplatin, favorable response rate and survival were also found in patients without ERCC1 protein expression[7]. Other studies showed that the low intratumoral *ERCC1* mRNA expression was associated with favorable clinical outcomes after treatment with platinum-based chemotherapy in lung cancer[27,28], colorectal cancer[29], gastric cancer[30-32], ovarian cancer[33], bladder cancer[34], head and neck cancer[35]. A recent phase Ⅲ trial in non-small-cell lung cancer also demonstrated that assessment of intratumoral *ERCC1* mRNA expression is feasible in the clinical setting and predicts response to cisplatin[36]. However, most of those studies focused on the influence of *ERCC1* expression on the effect of platinum-drug in advanced or metastatic diseases, little was known about its effect on platinum-drug adjuvant chemotherapy. Our

results suggested that low *ERCC1* mRNA level appeared to be an independent prognostic factor for better prognosis, which is consistent with the results observed in advanced gastric cancer[30-32].

In conclusion, *ERCC1* codon 118 polymorphism has no significant effect on *ERCC1* mRNA expression; and the intratumoral *ERCC1* mRNA level, but not *ERCC1* codon 118 polymorphism may be an important prognostic marker for the clinical outcome of gastric cancer patients treated with oxaliplatin-based adjuvant chemotherapy. Detection of the intratumoral *ERCC1* mRNA expression may give meaningful clinical information with respect to the rational choice platinum compound in the treatment of gastric cancer.

COMMENTS

Background

Oxaliplatin is one of the most effective agents against gastric cancer; its efficacy rate differs greatly among patients. Resistance to oxaliplatin has been attributed to enhanced tolerance and repair of DNA damage. Excision repair cross complementing group 1 (ERCC1) has been reported to play a major role in the response to platinum chemotherapy, but little is know about the effect of *ERCC1* codon 118 polymorphism and expression on clinical outcome of oxaliplatin-based adjuvant chemotherapy in gastric cancer.

Research frontiers

ERCC1 has been reported to play a major role in the response to platinum chemotherapy. Studies have shown that the higher the *ERCC1* expression levels, the less sensitive the tumors to platinum therapies. Recently, a single nucleotide polymorphism at codon 118 (C→T) was reported to be associated with altered *ERCC1* mRNA levels and clinical outcome in cancer patients treated with platinum-based chemotherapy. However, the results about the relationship among *ERCC1* codon 118 polymorphism, *ERCC1* mRNA level and platinum sensitivity are controversial.

Innovations and breakthroughs

No significant relationship was found between *ERCC1* codon 118 polymorphism and *ERCC1* mRNA levels. It is found that *ERCC1* mRNA level but not codon 118 polymorphism was a potential indicator in predicting the relapse and survival of gastric cancer patients treated with oxaliplatin-based adjuvant chemotherapy. To our knowledge, it is the first report to study the effect of intratumoral *ERCC1* expression and *ERCC1* codon 118 polymorphism on clinical outcome of oxaliplatin-based adjuvant chemotherapy in Chinese patients with gastric cancer.

Applications

Controlled, prospective clinical trials are required to confirm our results and to establish the advantage of pre-treatment tumor biopsy for *ERCC1* screening, which permits a more rational decision on whether to precede an oxaliplatin-based adjuvant chemotherapy. So patients who are unlikely to respond may spare unnecessary toxicity and can be treated with alternative drugs.

Terminology

Oxaliplatin is a widely applied medicine for chemotherapy of gastrointestinal cancer. ERCC1 is an important enzyme for DNA repair.

Peer review

This is a good study. Over all the study has clinical relevance for LDLT programmes.

REFERENCES

1 **Macdonald JS**. Treatment of localized gastric cancer. *Semin Oncol* 2004; **31**: 566-573
2 **Carrato A**, Gallego-Plazas J, Guillen-Ponce C. Adjuvant therapy of resected gastric cancer is necessary. *Semin Oncol* 2005; **32**: S105-S108
3 **Hejna M**, Wöhrer S, Schmidinger M, Raderer M. Postoperative chemotherapy for gastric cancer. *Oncologist* 2006; **11**: 136-145
4 **Bramson J**, Panasci LC. Effect of ERCC-1 overexpression on sensitivity of Chinese hamster ovary cells to DNA damaging agents. *Cancer Res* 1993; **53**: 3237-3240
5 **Olaussen KA**, Dunant A, Fouret P, Brambilla E, André F, Haddad V, Taranchon E, Filipits M, Pirker R, Popper HH, Stahel R, Sabatier L, Pignon JP, Tursz T, Le Chevalier T, Soria JC. DNA repair by ERCC1 in non-small-cell lung cancer and cisplatin-based adjuvant chemotherapy. *N Engl J Med* 2006; **355**: 983-991
6 **Fujii T**, Toyooka S, Ichimura K, Fujiwara Y, Hotta K, Soh J, Suehisa H, Kobayashi N, Aoe M, Yoshino T, Kiura K, Date H. ERCC1 protein expression predicts the response of cisplatin-based neoadjuvant chemotherapy in non-small-cell lung cancer. *Lung Cancer* 2008; **59**: 377-384
7 **Kwon HC**, Roh MS, Oh SY, Kim SH, Kim MC, Kim JS, Kim HJ. Prognostic value of expression of ERCC1, thymidylate synthase, and glutathione S-transferase P1 for 5-fluorouracil/oxaliplatin chemotherapy in advanced gastric cancer. *Ann Oncol* 2007; **18**: 504-509
8 **Yu JJ**, Mu C, Lee KB, Okamoto A, Reed EL, Bostick-Bruton F, Mitchell KC, Reed E. A nucleotide polymorphism in ERCC1 in human ovarian cancer cell lines and tumor tissues. *Mutat Res* 1997; **382**: 13-20
9 **Stoehlmacher J**, Park DJ, Zhang W, Yang D, Groshen S, Zahedy S, Lenz HJ. A multivariate analysis of genomic polymorphisms: prediction of clinical outcome to 5-FU/oxaliplatin combination chemotherapy in refractory colorectal cancer. *Br J Cancer* 2004; **91**: 344-354
10 **Isla D**, Sarries C, Rosell R, Alonso G, Domine M, Taron M, Lopez-Vivanco G, Camps C, Botia M, Nuñez L, Sanchez-Ronco M, Sanchez JJ, Lopez-Brea M, Barneto I, Paredes A, Medina B, Artal A, Lianes P. Single nucleotide polymorphisms and outcome in docetaxel-cisplatin-treated advanced non-small-cell lung cancer. *Ann Oncol* 2004; **15**: 1194-1203
11 **Park DJ**, Zhang W, Stoehlmacher J, Tsao-Wei D, Groshen S, Gil J, Yun J, Sones E, Mallik N, Lenz HJ. ERCC1 gene polymorphism as a predictor for clinical outcome in advanced colorectal cancer patients treated with platinum-based chemotherapy. *Clin Adv Hematol Oncol* 2003; **1**: 162-166
12 **Viguier J**, Boige V, Miquel C, Pocard M, Giraudeau B, Sabourin JC, Ducreux M, Sarasin A, Praz F. ERCC1 codon 118 polymorphism is a predictive factor for the tumor response to oxaliplatin/5-fluorouracil combination chemotherapy in patients with advanced colorectal cancer. *Clin Cancer Res* 2005; **11**: 6212-6217
13 **Huang ZH**, Hua D, Li LH, Zhu JD. Prognostic role of p53 codon 72 polymorphism in gastric cancer patients treated with fluorouracil-based adjuvant chemotherapy. *J Cancer Res Clin Oncol* 2008; **134**: 1129-1134
14 **Park DJ**, Stoehlmacher J, Zhang W, Tsao-Wei D, Groshen S, Zahedy S, Gil J, Mallik N, Lenz HJ. ERCC1 polymorphism is associated with differential ERCC1 mRNA levels. American Association for Cancer Research's 93rd Annual Meeting. 2002 April 6-10
15 **Zhou W**, Liu G, Park S, Wang Z, Wain JC, Lynch TJ, Su L, Christiani DC. Gene-smoking interaction associations for the ERCC1 polymorphisms in the risk of lung cancer. *Cancer Epidemiol Biomarkers Prev* 2005; **14**: 491-496
16 **Yin J**, Vogel U, Guo L, Ma Y, Wang H. Lack of association between DNA repair gene ERCC1 polymorphism and risk of lung cancer in a Chinese population. *Cancer Genet Cytogenet* 2006; **164**: 66-70
17 **Suk R**, Gurubhagavatula S, Park S, Zhou W, Su L, Lynch TJ, Wain JC, Neuberg D, Liu G, Christiani DC. Polymorphisms in ERCC1 and grade 3 or 4 toxicity in non-small cell lung cancer patients. *Clin Cancer Res* 2005; **11**: 1534-1538
18 **Steffensen KD**, Waldstrøm M, Jeppesen U, Brandslund I, Jakobsen A. Prediction of response to chemotherapy by ERCC1 immunohistochemistry and ERCC1 polymorphism in ovarian cancer. *Int J Gynecol Cancer* 2008; **18**: 702-710
19 **Martinez-Balibrea E**, Abad A, Aranda E, Sastre J, Manzano JL, Díaz-Rubio E, Gómez-España A, Aparicio J, García T, Maestu I, Martínez-Cardús A, Ginés A, Guino E.

Pharmacogenetic approach for capecitabine or 5-fluorouracil selection to be combined with oxaliplatin as first-line chemotherapy in advanced colorectal cancer. *Eur J Cancer* 2008; **44**: 1229-1237

20 **Ruzzo A**, Graziano F, Loupakis F, Rulli E, Canestrari E, Santini D, Catalano V, Ficarelli R, Maltese P, Bisonni R, Masi G, Schiavon G, Giordani P, Giustini L, Falcone A, Tonini G, Silva R, Mattioli R, Floriani I, Magnani M. Pharmacogenetic profiling in patients with advanced colorectal cancer treated with first-line FOLFOX-4 chemotherapy. *J Clin Oncol* 2007; **25**: 1247-1254

21 **Ryu JS**, Hong YC, Han HS, Lee JE, Kim S, Park YM, Kim YC, Hwang TS. Association between polymorphisms of ERCC1 and XPD and survival in non-small-cell lung cancer patients treated with cisplatin combination chemotherapy. *Lung Cancer* 2004; **44**: 311-316

22 **Zhou W**, Gurubhagavatula S, Liu G, Park S, Neuberg DS, Wain JC, Lynch TJ, Su L, Christiani DC. Excision repair cross-complementation group 1 polymorphism predicts overall survival in advanced non-small cell lung cancer patients treated with platinum-based chemotherapy. *Clin Cancer Res* 2004; **10**: 4939-4943

23 **Kamikozuru H**, Kuramochi H, Hayashi K, Nakajima G, Yamamoto M. ERCC1 codon 118 polymorphism is a useful prognostic marker in patients with pancreatic cancer treated with platinum-based chemotherapy. *Int J Oncol* 2008; **32**: 1091-1096

24 **Tibaldi C**, Giovannetti E, Vasile E, Mey V, Laan AC, Nannizzi S, Di Marsico R, Antonuzzo A, Orlandini C, Ricciardi S, Del Tacca M, Peters GJ, Falcone A, Danesi R. Correlation of CDA, ERCC1, and XPD polymorphisms with response and survival in gemcitabine/cisplatin-treated advanced non-small cell lung cancer patients. *Clin Cancer Res* 2008; **14**: 1797-1803

25 **Ruzzo A**, Graziano F, Kawakami K, Watanabe G, Santini D, Catalano V, Bisonni R, Canestrari E, Ficarelli R, Menichetti ET, Mari D, Testa E, Silva R, Vincenzi B, Giordani P, Cascinu S, Giustini L, Tonini G, Magnani M. Pharmacogenetic profiling and clinical outcome of patients with advanced gastric cancer treated with palliative chemotherapy. *J Clin Oncol* 2006; **24**: 1883-1891

26 **Keam B**, Im SA, Han SW, Ham HS, Kim MA, Oh DY, Lee SH, Kim JH, Kim DW, Kim TY, Heo DS, Kim WH, Bang YJ. Modified FOLFOX-6 chemotherapy in advanced gastric cancer: Results of phase II study and comprehensive analysis of polymorphisms as a predictive and prognostic marker. *BMC Cancer* 2008; **8**: 148

27 **Lord RV**, Brabender J, Gandara D, Alberola V, Camps C, Domine M, Cardenal F, Sánchez JM, Gumerlock PH, Tarón M, Sánchez JJ, Danenberg KD, Danenberg PV, Rosell R. Low ERCC1 expression correlates with prolonged survival after cisplatin plus gemcitabine chemotherapy in non-small cell lung cancer. *Clin Cancer Res* 2002; **8**: 2286-2291

28 **Ceppi P**, Volante M, Novello S, Rapa I, Danenberg KD, Danenberg PV, Cambieri A, Selvaggi G, Saviozzi S, Calogero R, Papotti M, Scagliotti GV. ERCC1 and RRM1 gene expressions but not EGFR are predictive of shorter survival in advanced non-small-cell lung cancer treated with cisplatin and gemcitabine. *Ann Oncol* 2006; **17**: 1818-1825

29 **Shirota Y**, Stoehlmacher J, Brabender J, Xiong YP, Uetake H, Danenberg KD, Groshen S, Tsao-Wei DD, Danenberg PV, Lenz HJ. ERCC1 and thymidylate synthase mRNA levels predict survival for colorectal cancer patients receiving combination oxaliplatin and fluorouracil chemotherapy. *J Clin Oncol* 2001; **19**: 4298-4304

30 **Metzger R**, Leichman CG, Danenberg KD, Danenberg PV, Lenz HJ, Hayashi K, Groshen S, Salonga D, Cohen H, Laine L, Crookes P, Silberman H, Baranda J, Konda B, Leichman L. ERCC1 mRNA levels complement thymidylate synthase mRNA levels in predicting response and survival for gastric cancer patients receiving combination cisplatin and fluorouracil chemotherapy. *J Clin Oncol* 1998; **16**: 309-316

31 **Matsubara J**, Nishina T, Yamada Y, Moriwaki T, Shimoda T, Kajiwara T, Nakajima TE, Kato K, Hamaguchi T, Shimada Y, Okayama Y, Oka T, Shirao K. Impacts of excision repair cross-complementing gene 1 (ERCC1), dihydropyrimidine dehydrogenase, and epidermal growth factor receptor on the outcomes of patients with advanced gastric cancer. *Br J Cancer* 2008; **98**: 832-839

32 **Wei J**, Zou Z, Qian X, Ding Y, Xie L, Sanchez JJ, Zhao Y, Feng J, Ling Y, Liu Y, Yu L, Rosell R, Liu B. ERCC1 mRNA levels and survival of advanced gastric cancer patients treated with a modified FOLFOX regimen. *Br J Cancer* 2008; **98**: 1398-1402

33 **Dabholkar M**, Vionnet J, Bostick-Bruton F, Yu JJ, Reed E. Messenger RNA levels of XPAC and ERCC1 in ovarian cancer tissue correlate with response to platinum-based chemotherapy. *J Clin Invest* 1994; **94**: 703-708

34 **Bellmunt J**, Paz-Ares L, Cuello M, Cecere FL, Albiol S, Guillem V, Gallardo E, Carles J, Mendez P, de la Cruz JJ, Taron M, Rosell R, Baselga J. Gene expression of ERCC1 as a novel prognostic marker in advanced bladder cancer patients receiving cisplatin-based chemotherapy. *Ann Oncol* 2007; **18**: 522-528

35 **Handra-Luca A**, Hernandez J, Mountzios G, Taranchon E, Lacau-St-Guily J, Soria JC, Fouret P. Excision repair cross complementation group 1 immunohistochemical expression predicts objective response and cancer-specific survival in patients treated by Cisplatin-based induction chemotherapy for locally advanced head and neck squamous cell carcinoma. *Clin Cancer Res* 2007; **13**: 3855-3859

36 **Cobo M**, Isla D, Massuti B, Montes A, Sanchez JM, Provencio M, Viñolas N, Paz-Ares L, Lopez-Vivanco G, Muñoz MA, Felip E, Alberola V, Camps C, Domine M, Sanchez JJ, Sanchez-Ronco M, Danenberg K, Taron M, Gandara D, Rosell R. Customizing cisplatin based on quantitative excision repair cross-complementing 1 mRNA expression: a phase III trial in non-small-cell lung cancer. *J Clin Oncol* 2007; **25**: 2747-2754

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CASE REPORT

Acalculous cholecystitis due to *Salmonella enteritidis*

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Abstract

Acute acalculous cholecystitis (AAC) is defined as an acute inflammation of the gallbladder in the absence of stones. We herein report a case of a young man who developed AAC after a *Salmonella enteritidis* gastrointestinal infection.

Key words: Cholecystitis; Young adult; Infectious; *Salmonella enteritidis*

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INTRODUCTION

Acute acalculous cholecystitis (AAC) is defined as an acute inflammation of the gallbladder in the absence of stones. Traditionally, it was considered a fatal disease almost exclusively of critical ill patients; however, there are recent reports of cases of AAC affecting less severe patients with good prognosis treated with antibiotics, in the absence of cholecystectomy.

We herein report a case of a young man who developed AAC after a *Salmonella enteritidis* gastrointestinal infection.

CASE REPORT

A 27-year-old man was admitted to hospital with abdominal pain, diarrhoea, persistent vomiting and 38℃ temperature. On physical examination, he was febrile, but in a good state of health. His abdomen was mildly tender to palpation with guarding in his lower right area. Laboratory tests disclosed a white cell count of 5300 × 1000/μL with 50% neutrophils and 32% lymphocytes, and haemoglobin and platelets were normal. The biochemical studies including liver and renal tests, electrolyte panel and coagulation profile, were normal. An abdominal X-ray film showed gas in several loops of a moderately dilated small bowel, and an abdominal sonography disclosed marked mucosal thickening in the right quadrant affecting ileon loops, cecum and ascending colon with small lymph node enlargement; the remainder of the abdominal contents, including the gallbladder were normal. Serology for *Salmonella typhi* H and O, Yersinia and Shigella were negative, as were blood cultures. The coproculture obtained on admission was positive for *Salmonella enteritidis*.

The patient was treated with intravenous fluids, analgesics and antipyretics and became afebrile on the second day; in a week time, the abdominal pain subsided and he was able to restart oral diet so he was discharged from hospital.

The following day he returned to the Emergency Department due to epigastric and right hypochondria pain, nausea and fever. He had no diarrhoea. On physical examination, he presented a temperature of 38℃, a tender upper abdomen, and Murphy's sign. The laboratory tests showed mild normocytic-normochromic anaemia with 7900 × 1000/μL white cells. The biochemical tests were normal. A new abdominal sonography disclosed normal intestinal loops, but his gallbladder was distended and presented a markedly thick wall (7 mm) with no stones, and was surrounded by a little fluid collection.

He was then administered intravenous antibiotics

(ciprofloxacine and metronidazole) and showed no signs of fever on the second day. The abdominal pain slowly subsided. Blood, urine and faeces cultures taken on admission showed negative results. He was discharged 10 d later on oral antibiotics and controlled as an outpatient. A new abdominal ultrasound disclosed a normal gallbladder without lithiasis or sludge.

DISCUSSION

AAC accounts for 5%-14% of all cases of acute cholecystitis[1,2]. Patients tend to be predominantly male and older than 50 years of age.

The pathogenesis of AAC is not well defined as the precise mechanism is unknown to date. It seems that several factors such as ischemia, infection and bile changes are involved. Ryu *et al*[1] found that patients with visceral atherosclerosis may be at increased risk for acute acalculous cholecystitis due to an impaired mucosal resistance. Systemic sepsis with release of mediators and bile stasis with alterations in the chemical composition of bile are another implicated potential pathogenic mechanisms involved. Multiple risk factors such as previous surgery and trauma or burn injury have been associated, but none of them were present in our patient.

However, as in our patient, AAC may also occur from secondary infection of the gallbladder following a systemic infection by bacteria[3], virus[4], parasites[5] or fungi.

AAC due to primary bacterial infection is rare. Several cases have been reported complicating *Salmonella typhi* infection[3,6] and after non-typhoidal salmonellosis[7,8] as well.

During the past two decades, an increase in the number of *Salmonella enteritidis* isolates has been observed even in developed countries[9], and there are also rare complications of this common disease described in medical literature[10] Some of these complications are extra-intestinal such as septic arthritis[11] or meningitis[12], but most of them are intra-abdominal[13] due to blood or lymphatic spread of the gbacteria.

Among the latter, AAC is infrequent and can occur even weeks after the diarrhoea has stopped[13] (our patient was discharged from hospital asymptomatic and developed symptoms 24 h later). The diagnosis is based on clinical symptoms, and ultrasound provides the definite diagnosis.

Salmonella enteritidis can be absent in blood cultures and be cultivated in faeces and bile[8,14]. The bacterium, like any other intestinal pathogen, can not only reach the gallbladder through blood drainage but also directly from the bowel along the bile ducts, as could have been the case in our patient.

Most cases described in literature experienced a bad outcome due to gallbladder gangrene, and perforation[2]. Even with early cholecystectomy in good surgical candidates[2], or cholecystostomy or endoscopy nasobiliary drainage in bad ones[15], the outcomes were bad. However, this has changed as the disease is now described in less severely ill patients with no adverse prognosis factors. In this setting, a 4-6 wk course of broad spectrum antibiotics, as indicated in our patient, is recommended. If symptoms cease and a control ultrasound shows a non-dilated gallbladder with a thin wall, cholecystectomy is not needed.

In conclusion, this case shows that AAC, a rare complication of *Salmonella enteritidis*, can also be present in non-critically ill patients. In this setting, the prognosis is better, cholecystectomy is not always needed and patients treated with a long course of wide spectrum antibiotics can obtain a good prognosis.

REFERENCES

1 **Ryu JK**, Ryu KH, Kim KH. Clinical features of acute acalculous cholecystitis. *J Clin Gastroenterol* 2003; **36**: 166-169
2 **Kalliafas S**, Ziegler DW, Flancbaum L, Choban PS. Acute acalculous cholecystitis: incidence, risk factors, diagnosis, and outcome. *Am Surg* 1998; **64**: 471-475
3 **Avalos ME**, Cerulli MA, Lee RS. Acalculous acute cholecystitis due to Salmonella typhi. *Dig Dis Sci* 1992; **37**: 1772-1775
4 **Basar O**, Kisacik B, Bozdogan E, Yolcu OF, Ertugrul I, Koklu S. An unusual cause of acalculous cholecystitis during pregnancy: hepatitis A virus. *Dig Dis Sci* 2005; **50**: 1532
5 **Anthoine-Milhomme MC**, Chappuy H, Cheron G. Acute acalculous cholecystitis in a child returning from the Ivory Coast. *Pediatr Emerg Care* 2007; **23**: 242-243
6 **Axelrod D**, Karakas SP. Acalculous cholecystitis and abscess as a manifestation of typhoid fever. *Pediatr Radiol* 2007; **37**: 237
7 **Garrido-Benedicto P**, Gonzalez-Reimers E, Santolaria-Fernandez F, Rodriguez-Moreno F. Acute acalculous cholecystitis due to Salmonella. *Dig Dis Sci* 1994; **39**: 442-443
8 **Sese Torres J**, Morlans Molina G, Capdevila Cirera A, Valls Camp X, Herrero Reche A. [Acute alithiasic cholecystitis caused by infectious gastroenteritis] *Med Clin* (Barc) 1985; **84**: 672
9 **Mishu B**, Koehler J, Lee LA, Rodrigue D, Brenner FH, Blake P, Tauxe RV. Outbreaks of Salmonella enteritidis infections in the United States, 1985-1991. *J Infect Dis* 1994; **169**: 547-552
10 **Ochoa J**, Ricarte E, Carrasco M, Simon MA, Cabello J, Yanguela JM. [Complications of acute gastroenteritis caused by Salmonella no typhi] *Rev Esp Enferm Apar Dig* 1989; **75**: 262-266
11 **Meldrum R**, Feinberg JR. Septic arthritis of the ankle due to Salmonella enteritidis: a case report. *South Med J* 2004; **97**: 77-79
12 **Aissaoui Y**, Azendour H, Balkhi H, Haimeur C, Atmani M. [Postoperative meningitis caused by an unusual etiological agent: Salmonella enteritidis] *Neurochirurgie* 2006; **52**: 547-550
13 **Fernandez Rodriguez R**, Moreno Sanchez D, Martinez Fernandez R, Medina Asensio J, Ferrero Collado A. [Enterocolitis caused by Salmonella enteritidis complicated by acute cholecystitis without lithiasis] *Rev Esp Enferm Apar Dig* 1988; **74**: 477-479
14 **Sese J**, Mas J, Pujol R, Capdevila A. [Acute non-calculous Salmonella enteriditis cholecystitis, diagnosis by percutaneous puncture] *Med Clin* (Barc) 1986; **87**: 564-565
15 **Owen CC**, Jain R. Acute Acalculous Cholecystitis. *Curr Treat Options Gastroenterol* 2005; **8**: 99-104

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CASE REPORT

Splenic rupture following colonoscopy

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Abstract

Colonoscopy is a safe and routinely performed diagnostic and therapeutic procedure for different colorectal diseases. Although the most common complications are bleeding and perforation, extracolonic or visceral injuries have also been described. Splenic rupture is a rare complication following colonoscopy, with few cases reported. We report a 60-year-old female who presented to surgical consultation 8 h after a diagnostic colonoscopy. Clinical, laboratory and imaging findings were suggestive for a massive hemoperitoneum. At surgery, an almost complete splenic disruption was evident, and an urgent splenectomy was performed. After an uneventful postoperative period, she was discharged home. Splenic injury following colonoscopy is considered infrequent. Direct trauma and excessive traction of the splenocolic ligament can explain the occurrence of this complication. Many times the diagnosis is delayed because the symptoms are due to colonic insufflation, so the most frequent treatment is an urgent splenectomy. A high index of suspicion needs an early diagnosis and adequate therapy.

Key words: Colonoscopy; Splenic injury; Splenic rupture; Splenectomy

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Guerra JF, San Francisco I, Pimentel F, Ibanez L. Splenic rupture following colonoscopy. *World J Gastroenterol* 2008; 14(41): 6410-6412 Available from: URL: http://www.wjgnet.com/1007-9327/14/6410.asp DOI: http://dx.doi.org/10.3748/wjg.14.6410

INTRODUCTION

Colonoscopy is a safe and routinely performed diagnostic and therapeutic procedure for different large bowel diseases. The most common complications include bleeding (1%) and perforation (0.1%-0.2%), and the chance of complication increases if any therapeutic actions are added, such as polypectomy or dilation[1,2]. Extracolonic or visceral injuries, including pneumothorax, pneumomediastinum, acute appendicitis, retroperitoneal abscess and others, are far less common[3]. Splenic injury is a rare complication of colonoscopy with few cases described; the first one was reported in 1974[4]. Even when some patients with late presentation have been mentioned[5,6], most of them developed symptoms a few hours after colonoscopy, and the majority of them underwent emergency surgery. We report a case of splenic rupture following colonoscopy, treated with urgent splenectomy.

CASE REPORT

A 60-year old female, with no significant medical history, underwent a diagnostic colonoscopy in another center. During the procedure, two rectal polyps (5 mm each) were resected. It was not difficult to reach the ileocecal valve. Endoscopy was performed under intravenous sedation (midazolam, 5 mg iv). She was observed in the recovery room for 2 h and then discharged home. Eight hours later, the patient came to our institution, complaining of diffuse abdominal pain and distension. On examination, she was pale, with a pulse rate of 100 beats per minute and her blood pressure was 103/52 mmHg. She had nonspecific abdominal tenderness, but no peritoneal signs. A colonic perforation after colonoscopy was suspected. The patient was resuscitated with vigorous intravenous fluid administration at the intermediate care unit. Her chest and abdominal X-ray showed no free air (Figure 1). Blood tests showed a hematocrit of 18% (hemoglobin, 6 g/dL). After an adequate haemodynamic stabilization and transfusion of 3 units of packed red cells, an abdominal

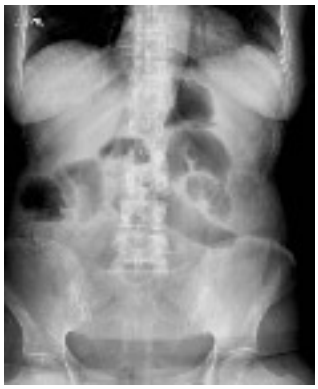

Figure 1 Abdominal X-ray showing no free air.

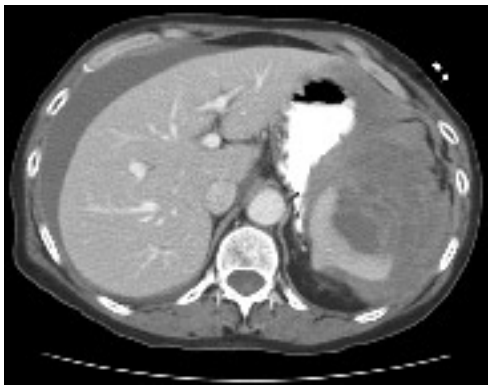

Figure 2 Hemoperitoneum and perisplenic hematoma.

CT scan demonstrated free fluid with a density suggesting blood, and a 12.5 cm × 9.6 cm left subphrenic perisplenic hematoma (Figure 2). No pneumoperitoneum was evident. At laparotomy, about three liters of intraperitoneal blood, and an almost complete splenic disruption were evident, so a splenectomy was performed. There were no perisplenic adhesions. No colon wounds or tears were seen. The postoperative course was uneventful, and she was discharged home on postoperative day 4. Specimen histologic examination revealed nothing but haemorragic parenchyma and inflammatory response, without any underlying splenic disease.

DISCUSSION

Splenic injury following colonoscopy is considered very infrequent. Only 40 cases have been reported[3-15]. To our knowledge, our case is the first case coming from South America. We believe that this is a rare but, most of the times, an under published complication of colonoscopy, so the real incidence might be higher. Although no specific causes have been established, the mechanism of injury may be related to direct trauma or preferently, excessive traction on the splenocolic ligament, which results in evulsion of the splenic capsule and different grades of parenchymal disruption[3,5,7]. A polypectomy or a biopsy was performed in most of reported cases[8]. Splenomegaly, inflammatory bowel disease, pancreatitis and intrabdominal post-surgical adhesions have been mentioned as predisposing factors, due to a suspected decreased mobility between the spleen and colon[8-10]. However, these are not constant findings at surgery[9]. The addition of external abdominal pressure during colonoscopy has also been proposed as a risk factor for the development of this complication[7,8]. Usually, the clinical presentation occurs within the first 24 h after colonoscopy[7,8]; but many times the diagnosis is delayed because the symptoms are attributed to colonic insufflation[3]. Finally, the diagnosis is made in a critically ill patient, with the onset of hypotension and acute anemia. There are also some reported cases with a late presentation (from more than 24 h to 10 d) and mild symptoms[5,6]. CT scan is the imaging modality of choice[11,12], which determines the extent of splenic damage, and demonstration of hemoperitoneum. This information, added to the clinical setting, may help decide on the therapeutic option. In most series, splenectomy is the most frequent treatment of choice[3-9]. Very few cases have been treated with transfusion of hemocomponents, broad spectrum antibiotics and close hemodynamic monitoring[10,13,14]. Another therapeutic action successfully described is splenic artery embolization[15]. The use of this "conservative" treatment must be in direct relation with the hemodynamic status of each case, and in the expertise of a multidisciplinary medical team. In our case, the patient was treated with an urgent splenectomy, and had an uneventful postoperative period.

We believe that this is an unusual and probably under reported complication of colonoscopy. As colonoscopy is performed widely in different centers, the medical team should be aware of the possibility of a splenic injury after colonoscopy and a high level of suspicion needs an early diagnosis and adequate treatment.

REFERENCES

1 **Macrae FA**, Tan KG, Williams CB. Towards safer colonoscopy: a report on the complications of 5000 diagnostic or therapeutic colonoscopies. *Gut* 1983; **24**: 376-383
2 **Schwesinger WH**, Levine BA, Ramos R. Complications in colonoscopy. *Surg Gynecol Obstet* 1979; **148**: 270-281
3 **Espinal EA**, Hoak T, Porter JA, Slezak FA. Splenic rupture from colonoscopy. A report of two cases and review of the literature. *Surg Endosc* 1997; **11**: 71-73
4 **Wherry DC**, Zehner H Jr. Colonoscopy-fiberoptic endoscopic approach to the colon and polypectomy. *Med Ann Dist Columbia* 1974; **43**: 189-192
5 **Taylor FC**, Frankl HD, Riemer KD. Late presentation of splenic trauma after routine colonoscopy. *Am J Gastroenterol* 1989; **84**: 442-443
6 **Merchant AA**, Cheng EH. Delayed splenic rupture after colonoscopy. *Am J Gastroenterol* 1990; **85**: 906-907
7 **Janes SE**, Cowan IA, Dijkstra B. A life threatening complication after colonoscopy. *BMJ* 2005; **330**: 889-890
8 **Ahmed A**, Eller PM, Schiffman FJ. Splenic rupture: an unusual complication of colonoscopy. *Am J Gastroenterol* 1997; **92**: 1201-1204
9 **Al Alawi I**, Gourlay R. Rare complication of colonoscopy. *ANZ J Surg* 2004; **74**: 605-606
10 **Tsoraides SS**, Gupta SK, Estes NC. Splenic rupture after colonoscopy: case report and literature review. *J Trauma* 2007; **62**: 255-257
11 **Zenooz NA**, Win T. Splenic rupture after diagnostic

colonoscopy: a case report. *Emerg Radiol* 2006; **12**: 272-273
12 **Johnson C**, Mader M, Edwards DM, Vesy T. Splenic rupture following colonoscopy: two cases with CT findings. *Emerg Radiol* 2006; **13**: 47-49
13 **Heath B**, Rogers A, Taylor A, Lavergne J. Splenic rupture: an unusual complication of colonoscopy. *Am J Gastroenterol* 1994; **89**: 449-450
14 **Rockey DC**, Weber JR, Wright TL, Wall SD. Splenic injury following colonoscopy. *Gastrointest Endosc* 1990; **36**: 306-309
15 **Stein DF**, Myaing M, Guillaume C. Splenic rupture after colonoscopy treated by splenic artery embolization. *Gastrointest Endosc* 2002; **55**: 946-948

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CASE REPORT

Therapeutic barium enema for bleeding colonic diverticula: Four case series and review of the literature

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Abstract

The prevalence of diverticular diseases of the colon, including severe and persistent bleeding in Eastern countries, has increased in the last decades. The bleeding from colonic diverticula is the most common cause of acute lower gastrointestinal bleeding. Herein, we report four cases of severe and persistent bleeding of colonic diverticular disease that could be treated with a high concentration barium enema. These four cases showed a similar pattern of bleeding whose source could not be identified. Colonoscopy revealed fresh blood in the entire colon and many diverticula were noted throughout the colon. No active bleeding source was identified, but large adherent clots in some diverticula were noted. After endoscopic and angiographic therapies failed, therapeutic barium enema stopped the severe bleeding. These patients remained free of re-bleeding in the follow-up period (range 17-35 mo) after the therapy. We report the four case series of therapeutic barium enema and reviewed the literature pertinent to this procedure.

Key words: Therapeutic barium enema; Colonic diverticula; Diverticular bleeding

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INTRODUCTION

Diverticular hemorrhage is a common cause of lower gastrointestinal bleeding[1-3]. Most colonic diverticula are asymptomatic and remain uncomplicated. However, it was reported that severe diverticular hemorrhage occurs in 3%-5% of patients with diverticula[4,5]. Most cases of diverticular bleeding resolve themselves. However, massive bleeding of diverticula often requires endoscopic or angiographic therapy. As in some cases, the source of bleeding cannot be identified or multiple sites of bleeding are found, endoscopic or angiographic treatment is not so effective for bleeding. Although surgical treatment has been performed for persistent bleeding, the patients are often elderly and, therefore, at a high risk for surgery.

Most of the reported series of therapeutic barium enema are from Western countries. However, the prevalence of diverticular diseases of the colon, including severe and persistent bleeding in the Eastern countries, has increased in the last decades. We present herein four patients with severe and persist bleeding due to colonic diverticular disease that were treated with high concentration barium enemas, and have reviewed the literature pertinent to this procedure.

CASE SERIES

Case 1

A 63-year-old man was hospitalized for a several-day history of painless passage of bright red blood per rectum. He had hypertension and diabetes mellitus, and had been taking medication for hypertension for ten years. He had no history of receiving non-steroidal anti-inflammatory drugs, low-dose aspirin, and anticoagulants. His past history revealed two bleeding episodes from colonic diverticula that resolved on their own. Physical examination revealed no abdominal tenderness. Laboratory tests showed severe anemia.

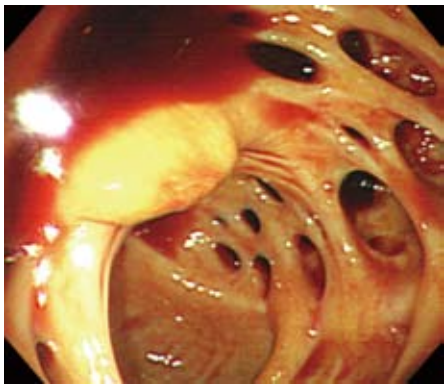

Figure 1 Endoscopic appearance of bleeding diverticula with adherent clots in the ascending colon.

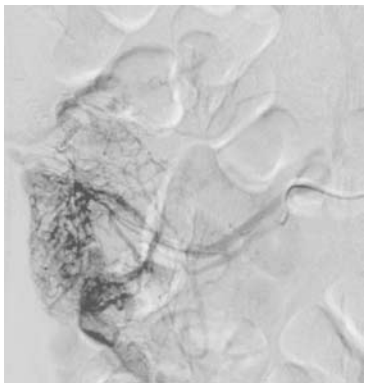

Figure 2 Superior mesenteric arteriogram showing hypervascularity in the ascending colon.

Colonoscopy revealed fresh blood in the entire colon and many diverticula with adherent clots were noted throughout the colon (Figure 1). No active bleeding source was identified; but large adherent clots in some diverticula were noted. Endoscopic placement of metallic clips was done for some diverticula with large adherent clots suspicious of being a bleeding source. On the next day, the patient had severe hematochezia again and required additional transfusion of 4 units of packed red cells. Scintigraphic examination failed to identify the bleeding site. Emergency angiography was performed. Although superior mesenteric arteriography could not reveal any extravasation, hypervascularity was noted on the right side of the colon (Figure 2). Vasopressin infusion was administered; but the patient continued to bleed, and required 4 units of blood. We discussed the situation with the patient and his family, and explained the subsequent treatment modalities, including surgical treatment or optional therapeutic barium enema. After obtaining informed consent, we performed therapeutic barium enema. Barium (concentration: 200%, volume: about 400 mL) including 50 000 units of thrombin was administered per rectum, and the leading edge of the contrast medium was followed up to the ascending colon by fluoroscopy. The enema tip was withdrawn one hour after confirming that the diverticula in the ascending colon were filled with barium (Figure 3). On the next day, we confirmed that multiple diverticula were filled with barium by abdominal X-ray examination. Ten days after the therapy, we also confirmed that the multiple diverticula at the right side of the colon were filled with barium. The patient was discharged without any further bleeding or complications, and surgical treatment was avoided. The patient remained free of re-bleeding more than 3 years after the therapy.

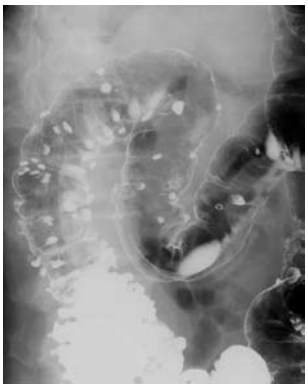

Figure 3 X-ray revealing diverticula in the ascending colon filled with barium.

Case 2

A-67-year old man with diverticula, noted on prior screening colonoscopy, was transferred to our hospital with sudden painless massive rectal bleeding. While in hospital, he developed painless hematochezia and severe anemia requiring transfusion of 2 units of packed red cells. He had hypertension, and had been taking medication for hypertension for five years. He had a history of receiving non-steroidal anti-inflammatory drugs for lumbago. Colonoscopy revealed fresh blood in the entire colon and many diverticula throughout the colon. Endoscopic placement of metallic clips was done for some diverticula suspicious of being a bleeding source. Two days later, the patient had severe hematochezia again and required additional transfusion of 2 units of packed red cells. Emergency angiography could not reveal any extravasation. Therapeutic barium enema was performed and the patient remained free of re-bleeding two years after the therapy.

Case 3

A-76-year old man visited the Emergency Department for a three-day history of diarrhea and painless hematochezia. He had hypertension and had been taking medication for hypertension. Colonoscopy revealed fresh blood throughout the colon and many diverticula in the ascending and sigmoid colon. Endoscopic placement of metallic clips was done for some diverticula suspicious of being a bleeding source. Three days later, the patient began bleeding again, and required transfusion of 2 units of packed red cells. The patient and his family wished to avoid intervention, and agreed to the therapeutic barium enema. Active bleeding was stopped after the therapeutic barium enema. He remained free of re-bleeding 20 mo after the therapy.

Case 4

A-63-year old man was hospitalized for a two-day history

Table 1 Cases undergoing therapeutic barium enema for diverticular hemorrhage

Case	1	2	3	4
Gender	M	M	M	M
Age (yr)	63	67	76	63
Associated diseases	HT,DM	HT	HT	None
NSAID, L-Asp, Anticoagulants	None	NSAID	None	None
Previous bleeding episodes (*n*)	2	2	1	1
Location	Throughout the colon	Throughout the colon	Ascending sigmoid	Ascending
Appearance	AC, FB	FB	FB	AC
Hemorrhagic site	NI	NI	NI	NI
Follow-up period (mo)	35	23	20	17
Recurrent bleeding	None	None	None	None

NI: Not identified; AC: Adherent clot; FB: Fresh blood; HT: Hypertension; DM: Diabetes mellitus; NSAID: Nonsteroidal anti-inflammatory drug; L-Asp: Low-dose aspirin.

of painless passage of bright red blood per rectum. Colonoscopy revealed fresh blood in the entire colon and many diverticula with adherent clots in the ascending colon. Endoscopic placement of metallic clips was done for some diverticula suspicious of being a bleeding source. During the following hospitalization days, the patient experienced 3 further episodes of bleeding and received 3 units of packed red cells. The patient wished to avoid intervention and agreed to receive therapeutic barium enema. There was no evidence of re-bleeding 17 mo after the therapy.

DISCUSSION

The prevalence of diverticular diseases of the colon in the Eastern countries has increased in the last decades, and the increasing prevalence reflects changes in the life-style and eating habits. A previous study on 6849 patients undergoing barium enema examination during an 8-year period (from 1985 to 1992) revealed an increase in the frequency from 10.7% in 1985 to 17.8% in 1992[6] Another study concluded that diverticular diseases of the right colon have increased steadily in Japan, suggesting that diverticulitis and bleeding may continue to increase[7]. As many reports stated, the prevalence of colonic diverticula and their related severe bleeding have increased recently in the Eastern countries including Japan[6-8].

Colonic diverticula are usually asymptomatic. However, in some cases, acute and chronic inflammation, hemorrhage, and perforation develop as complications of this disease. Bleeding from colonic diverticula is the most common cause of acute lower gastrointestinal bleeding[1-3]. It was reported that acute lower intestinal bleeding occurs in up to 3%-5% of colonic diverticula[4,5]. Most cases of diverticular bleeding resolve themselves and diverticular bleeding stops spontaneously in 70%-80% of cases[9]. However, some patients require evaluation by colonoscopy and angiography, or surgical treatment to stop their bleeding. There are some case reports on various techniques of treatment with colonoscopy for diverticular bleeding including heater probes, epinephrine injection therapy, argon plasma coagulation, and endo-clip application[10-16]. Endoscopic treatment can be useful when a source of lower gastrointestinal bleeding is identified. However, when the source of bleeding cannot be identified or multiple sites of bleeding are found, endoscopic treatment is not effective for stopping bleeding. The specific location of bleeding points is very important for therapeutic colonoscopy of bleeding diverticula. Intermittent diverticular hemorrhage can also lead to incomplete endoscopic therapy.

Angiography is recognized as an accurate diagnostic modality for detecting the site of active gastrointestinal bleeding. Mesenteric angiography is indicated when the flow is estimated to be greater than 0.5-1 mL/min, and offers a potential for selective vasopressin infusion or arterial embolization if the bleeding site is identified[16]. However, angiography frequently fails to reveal the source of gastrointestinal hemorrhage. In addition, in p patients with multiple sites of bleeding or intermittent and quiescent bleeding, it is difficult to treat diverticular hemorrhage by angiographic intervention. Furthermore, angiography is an invasive modality with complications such as arterial dissection and occlusion, bowel infarction, myocardial infarction with vasopressin infusion, and renal failure due to contrast medium[1,17].

In our four cases, colonoscopy demonstrated large amounts of fresh blood throughout the colon and many diverticula with adherent blood clots; but it was difficult to identify the active bleeding site, and the specific bleeding points. Endoscopic and angiographic therapies failed to identify the bleeding points, and could not stop bleeding. As an optional therapy, therapeutic barium enema was performed for severe bleeding to avoid surgical treatment. Table 1 summarizes the four cases of persistent and severe diverticular bleeding in whom the active bleeding sites could not be identified and therapeutic barium enema was effective and no re-bleeding was detected. Each case had one or two persistent bleeding episodes previously and underwent repeated endoscopic treatment. However, these patients remained well and had no re-bleeding after barium enema treatment for 17-35 mo. In all cases, the concentration of barium was 200%, the volume was 400 mL, and the enema tip was withdrawn one hour after the therapy (Table 1). In our four cases, although no severe complications of therapeutic barium enema (such as perforation) occurred, we have to take into account the

Table 2 Previous reports on therapeutic barium enema for diverticular hemorrhage

Author, year	*n*	Location	Concentration of barium	Successful cases	Recurrent bleeding
Adams *et al* 1970	28	NR	NR (20%)[1]	26	9
Chorost *et al* 2000	1	TO	20%	1	0
Koperna *et al* 2001	63	NR	NR	53	10
Matsuhashi *et al* 2003	1	AC, TC, SC	200% (with 1 mg of epinephrine)	1	0
Our cases 2008	4	TO AC, SC (2 cases) AC	200%	4	0

[1]Mentioned in the discussion section. TO: Throughout the colon; AC: Ascending colon; NR: Not reported; SC: Sigmoid colon; TC: Transverse colon.

possibility of perforation in patients with diverticulitis.

Some case reports and clinical studies of therapeutic barium enema for diverticular hemorrhage have been reported (Table 2)[8,18-20]. A previous case report[8] presented successful treatment with a high concentration of barium with 1 mg of epinephrine. In that case, epinephrine was added to the solution for vasoconstriction; but the possible adverse effects of epinephrine such as sudden hypertension were not ruled out. Another case report[18] presented successful treatment with a 20% barium sulphate solution at a height of 0.9 m for 5 min. In 1970, Adams *et al*[19] demonstrated that 26 of 28 acute bleeding episodes were arrested by therapeutic barium enema with a 20% concentration of barium. That study also stated that the only single complication was laceration of the rectal mucosa by the enema tube[19]. A previous clinical study[20] evaluated the efficacy of barium enema therapy for severe diverticular bleeding and concluded that therapeutic barium enema is the treatment of choice for the first bleeding episode, while surgical resection should be performed if re-bleeding occurs. In that report, the failure rate of conservative treatment and therapeutic barium enema with consequent re-bleeding was 43.4% and 15.9%, respectively[20]. Furthermore, an investigation suggested that complications develop more often in patients after colonic resection than in those after barium enema therapy, and that the mortality after surgery is significantly higher than that following therapeutic barium enema[20].

Most of the reported cases of therapeutic barium enema were from the Western countries. However, the prevalence of diverticular diseases of the colon in the Eastern countries including Japan has increased recently. From our present experiences, therapeutic barium enema is also effective for right side diverticula which are typically located in the Eastern countries.

It is difficult to clarify the mechanism underlying the effect of therapeutic barium enema. Adams *et al*[19] mentioned two potential factors, namely the pressure by the barium solution producing tamponade of the bleeding vessel, and the direct hemostatic action by the barium sulfate. The effect of barium on bleeding in the gastrointestinal tract is also described in a previous report[21]. That report mentioned that tap-water enema is better, as it contains no anticoagulants, and is more effective in producing clot formation than most barium suspensions.

A clinical study on surgery for complicated colonic diverticula concluded that in patients with multiple bleeding sites or severe ongoing hemorrhage from a source that cannot be localized despite endoscopic and angiographic assessment, subtotal or total colectomy may be imperative[22]. However, in view of the mortality of surgical therapy, optional and non-invasive therapies such as therapeutic barium enema are needed in the cases that require colonic resection.

In conclusion, the prevalence of diverticular diseases of the colon has increased in the last decades not only in the Western countries, but also in the Eastern countries. Barium enema therapy is effective for diverticular hemorrhage when the active bleeding site could not be identified by colonoscopy. When no other therapeutic techniques are available, barium enema therapy may be useful as an optional therapy which may avoid surgical therapy. As far as we know, there are few reports on randomized trials of treatment of colonic diverticular bleeding. Because of the limited number of clinical case series, further randomized controlled trials of treatment are required to clarify the role of therapeutic barium enema in bleeding diverticula.

REFERENCES

1 **Vernava AM 3rd**, Moore BA, Longo WE, Johnson FE. Lower gastrointestinal bleeding. *Dis Colon Rectum* 1997; **40**: 846-858
2 **Longstreth GF**. Epidemiology and outcome of patients hospitalized with acute lower gastrointestinal hemorrhage: a population-based study. *Am J Gastroenterol* 1997; **92**: 419-424
3 **Zuckerman GR**, Prakash C. Acute lower intestinal bleeding. Part II: etiology, therapy, and outcomes. *Gastrointest Endosc* 1999; **49**: 228-238
4 **Stollman NH**, Raskin JB. Diagnosis and management of diverticular disease of the colon in adults. Ad Hoc Practice Parameters Committee of the American College of Gastroenterology. *Am J Gastroenterol* 1999; **94**: 3110-3121
5 **McGuire HH Jr**, Haynes BW Jr. Massive hemorrhage for diverticulosis of the colon: guidelines for therapy based on bleeding patterns observed in fifty cases. *Ann Surg* 1972; **175**: 847-855
6 **Miura S**, Kodaira S, Shatari T, Nishioka M, Hosoda Y, Hisa

TK. Recent trends in diverticulosis of the right colon in Japan: retrospective review in a regional hospital. *Dis Colon Rectum* 2000; **43**: 1383-1389
7 **Nakada I**, Ubukata H, Goto Y, Watanabe Y, Sato S, Tabuchi T, Soma T, Umeda K. Diverticular disease of the colon at a regional general hospital in Japan. *Dis Colon Rectum* 1995; **38**: 755-759
8 **Matsuhashi N**, Akahane M, Nakajima A. Barium impaction therapy for refractory colonic diverticular bleeding. *AJR Am J Roentgenol* 2003; **180**: 490-492
9 **McGuire HH Jr**. Bleeding colonic diverticula. A reappraisal of natural history and management. *Ann Surg* 1994; **220**: 653-656
10 **Prakash C**, Chokshi H, Walden DT, Aliperti G. Endoscopic hemostasis in acute diverticular bleeding. *Endoscopy* 1999; **31**: 460-463
11 **Simpson PW**, Nguyen MH, Lim JK, Soetikno RM. Use of endoclips in the treatment of massive colonic diverticular bleeding. *Gastrointest Endosc* 2004; **59**: 433-437
12 **Jensen DM**, Machicado GA, Jutabha R, Kovacs TO. Urgent colonoscopy for the diagnosis and treatment of severe diverticular hemorrhage. *N Engl J Med* 2000; **342**: 78-82
13 **Mauldin JL**. Therapeutic use of colonoscopy in active diverticular bleeding. *Gastrointest Endosc* 1985; **31**: 290-291
14 **Hokama A**, Uehara T, Nakayoshi T, Uezu Y, Tokuyama K, Kinjo F, Saito A. Utility of endoscopic hemoclipping for colonic diverticular bleeding. *Am J Gastroenterol* 1997; **92**: 543-546
15 **Bloomfeld RS**, Rockey DC, Shetzline MA. Endoscopic therapy of acute diverticular hemorrhage. *Am J Gastroenterol* 2001; **96**: 2367-2372
16 **Ramirez FC**, Johnson DA, Zierer ST, Walker GJ, Sanowski RA. Successful endoscopic hemostasis of bleeding colonic diverticula with epinephrine injection. *Gastrointest Endosc* 1996; **43**: 167-170
17 **Peter DJ**, Dougherty JM. Evaluation of the patient with gastrointestinal bleeding: an evidence based approach. *Emerg Med Clin North Am* 1999; **17**: 239-261, x
18 **Chorost MI**, Fruchter G, Kantor AM, Wu J, Ghosh BC. The therapeutic barium enema revisited. *Clin Radiol* 2001; **56**: 856-858
19 **Adams JT**. Therapeutic barium enema for massive diverticular bleeding. *Arch Surg* 1970; **101**: 457-460
20 **Koperna T**, Kisser M, Reiner G, Schulz F. Diagnosis and treatment of bleeding colonic diverticula. *Hepatogastroenterology* 2001; **48**: 702-705
21 **Miller RE**, Skucas J, Violante MR, Shapiro ME. The effect of barium on blood in the gastrointestinal tract. *Radiology* 1975; **117**: 527-530
22 **Funariu G**, Bintintan V, Seicean R. Urgent surgery for complicated colonic diverticula. *J Gastrointestin Liver Dis* 2006; **15**: 37-40

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CASE REPORT

Polysplenia syndrome with preduodenal portal vein detected in adults

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Abstract

Polysplenia syndrome, defined as the presence of multiple spleens of almost equal volume, is a rare condition involving congenital anomalies in multiple organ systems. We report this anomaly in a 41-year-old female who underwent a left lateral sectionectomy due to recurrent cholangitis and impacted left lateral duct stones. Polysplenia syndrome with preduodenal vein was diagnosed preoperatively by computed tomography (CT) and surgery was done safely. Although the polysplenia syndrome with preduodenal portal vein (PDPV) in adult is rarely encountered, surgeons need to understand the course of the portal vein and exercise caution in approaching the biliary tract.

Key words: Polysplenia; Polysplenia syndrome; Preduodenal portal vein; Intrahepatic duct stones; Congenital anomaly

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INTRODUCTION

Polysplenia syndrome is a rare disease that occurs in patients with two or more spleens of identical sizes and various organ anomalies[1]. Reports indicate that most patients with polysplenia syndrome die before 5 years of age because the disease is often associated with congenital anomalies, such as cardiovascular anomalies[2]. Severe cardiovascular anomalies include interruption of the suprarenal inferior vena cava, atrioventricular septal defects, ipsilateral pulmonary venous drainage, ventricular outflow tract obstruction, dextrocardia and abnormal great vessel relationships[3]. Some patients with polysplenia syndrome have a normal heart or only minor cardiac defects, are often diagnosed incidentally in patients being treated for other disease[4]. However, they may harbor anomalies in abdominal organs or the gastrointestinal tract, one example of which is a preduodenal portal vein (PDPV)[5]. A PDPV can be diagnosed early as duodenal obstruction in infants, but is often found incidentally or during surgery when there are no symptoms[6].

One of the ways to prevent injuries to the hepatic portal system during surgery is to diagnosis polyspenia syndrome accompanied by a PDPV prior to surgery. We treated a patient with polysplenia syndrome, which was diagnosed during a left lateral sectionectomy to treat intrahepatic duct stones, and we report our findings along with related studies.

CASE REPORT

A 41-year-old female presented for evaluation of right upper quadrant pain of 1 mo duration. At a local hospital, she was noted to have polysplenia and left lateral intrahepatic duct stones without intrahepatic duct dilatation of segment 4. She was referred to our institution for further evaluation and treatment. The physical examination revealed no abnormalities. An abdominal computed tomography (CT) showed intrahepatic duct stones with polysplenia and the portal vein was located anteriorly to the duodenum (Figure 1). Laboratory findings revealed

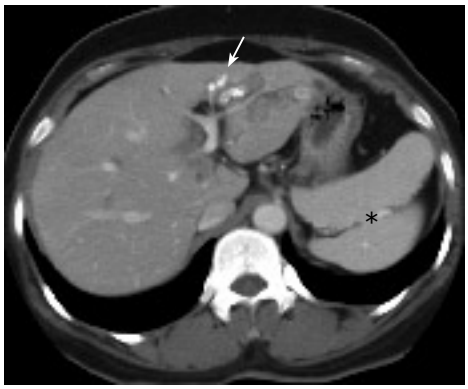

Figure 1 Abdominal computed tomography scan. Polysplenia (asterisks) are present in the left upper quadrant and it shows the left lateral intrahepatic duct stones with atrophy (arrow).

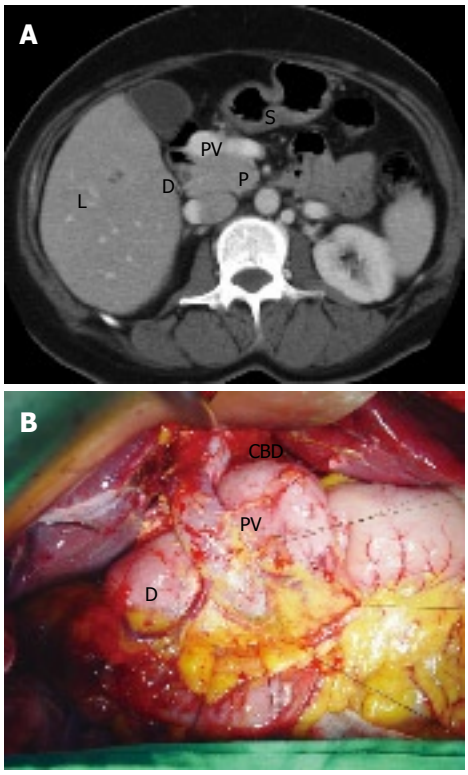

Figure 2 The location and presentation in surgery of the portal vein. A: The portal vein (PV) was located ventrally to the duodenum (D) and pancreas (P), liver (L) and stomach (S) are in the normal position; B: At surgery, the portal vein (PV) was seen running anteriorly across the duodenal first portion (D), the common bile duct (C) was identified left to the portal vein.

elevated levels of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and lactate dehydrogenase. Echocardiography and chest radiography were normal. The diagnosis of intraheptic duct stones with polyspenia syndrome was established.

The surgical procedure for intrahepatic duct stones began with a thorough exploration of the abdomen. The portal vein was detected in front of the first part of the duodenum. The portal vein passed upward, right to the common bile duct, and bifurcated into the left and right portal branches in the porta hepatis (Figure 2). Both the common bile duct and the right hepatic artery were identified to the left of the portal vein. The left hepatic artery originated from the left gastric artery. The presence of two spleens was confirmed on the left side of the upper abdomen, along with the greater curvature of the stomach. A nasogastric tube passed through duodenum without any difficulty. We could not locate the stenotic duodenal region posterior to the portal vein. The shape of the liver and pancreas were normal; the small bowel and colon were normally positioned.

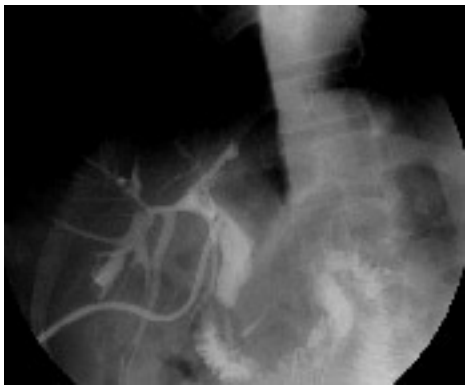

Figure 3 T-tube cholangiography shows variations of right intrahepatic bile duct.

A left lateral sectionectomy with a cholecystectomy was performed safely. An intraoperative choledochoscopy through the segment 3 bile duct was done for removal of the right intrahepatic duct stone, and we confirmed no residual stones existed in the bile duct based on T-tube cholangiography (Figure 3). The postoperative course was uneventful, and the patient was discharged 12 d postoperatively.

DISCUSSION

As the name of the condition implies, polysplenia syndrome refers to patients with two or more spleens. Studies also include descriptions of cases that involve a number of very small spleens, a multilobular spleen with tiny accessory spleen, and an undivided spleen[2,7].

The spleen develops during the 5th embryonic week from the splenic primordia, originating from the dorsal mesogastrium. The initial splenic primordia are then created as incisures on the left side of the dorsal mesogastrium. When the incisures fail to fuse, they create two or more spleens[8]. The blood flow of an embryo makes a transition from symmetric to asymmetric around the 25th d to determine the visceral sidedness, and it has been suggested that this is when cono-truncal anomalies and anomalies of atrioventricular canal occur[9]. At the same time, PDPV also develop. Venous blood is drained from the primitive gut, consisting of two vitelline veins of the yolk sac. The veins implement communication in the liver (cranial communication), behind (middle communication) and in front of (caudal communication) the duodenum. When the caudal and cranial communications are lost during the 9-mm embryo stage, the S-shape portal vein is created. It is believed that the PDPV anomaly is developed during this stage if cranial

and middle communications are lost[10].

Although the exact cause of polysplenia is unknown, studies have suggested that it is caused by various factors including embryogenic, genetic, and teratogenic components[11]. Since splenic anomalies (splenic agenesis, hypogenesis and polysplenia) are often accompanied by anomalies in the cardiovascular tract and other abdominal organs, it can be inferred that the spleen plays a significant role during the early embryonic stage[12].

Reports indicate that polysplenia syndrome occurs in both genders with an identical frequency[5]. Known cardiovascular anomalies include absence or hypoplasia of the suprarenal inferior vena cava (with or without azygos or hemiazygos continuation), levoisomerism of the right bronchial tree, dextrocardia, ventricular septal defects, and the absence of the coronary sinuses, most patients die before 5 years of age due to the accompanying cardiovascular anomalies. Patients without cardiac anomalies may reach adulthood, accounting for 10%-15% of cases of polysplenia. Since most adult patients do not exhibit any symptoms, polysplenia syndrome is often diagnosed incidentally during other procedures[5]. Even adult patients can have anomalies in abdominal organs including visceral heterotaxia with a right-sided stomach, a left-sided or large midline liver, right-sided spleen, malrotation of the intestine, a short pancreas, and anomalies of the inferior vena cava. Cases with only PDPV, as with our patient, are recognized as very minor anomalous cases. Even though intestinal obstruction is often displayed in such cases, there were no anomalies of the digestion system for our patient. First described by Knight HO in 1921, PDPV is a congenital anomaly that involves the portal vein passing in front of the duodenum[13]. A PDPV can be associated with duodenal atresia, stenosis, web, annular pancreas and malrotation, and surgery may be required for treatment[14]. However, since the portal vein is a thin-walled, low-pressure vessel, it is highly unlikely that PDPV alone can cause duodenal obstruction. Patients can survive to reach adulthood without any symptoms, and the anomaly is often found during examinations or surgeries to treat other diseases.

Polysplenia syndrome can be detected relatively easily with diagnostic imaging including abdominal CT and magnetic resonance imaging (MRI)[15]. In our case, polysplenia syndrome was diagnosed incidentally while examining the patient who was complaining of pain in the right upper abdomen and fever. Known causes of atrophy of the lateral segment of the liver in PDPV include selective portal vein obstruction, biliary duct obstruction, partial obstruction of the portal vein associated with distention of the hepatic bile duct, long-standing malnutrition and cachexia, or toxic and vascular influences[14]. However, in the case of our patient, it is likely that the atrophy was caused by intrahepatic duct stones. Reports indicate that most cases of PDPV in adults involve surgery for cholelithiasis, making us believe that PDPV could be a cause for cholelithiasis[14]. When surgery is required, care must be exercised, especially for procedures involving the upper abdomen. If PDPV is not detected prior to surgery, it can cause severe complications, such as hemorrhage and vascular ligation. Such accidents can be prevented by performing careful diagnostic imaging in advance, such as CT, and especially noting the possibility of PDPV in cases of polysplenia syndrome.

REFERENCES

1 **Griffiths JD**, Marshall VC. Torsion of the spleen in the polysplenia syndrome. *Aust N Z J Surg* 1984; **54**: 571-573
2 **Gayer G**, Hertz M, Strauss S, Zissin R. Congenital anomalies of the spleen. *Semin Ultrasound CT MR* 2006; **27**: 358-369
3 **Roguin N**, Hammerman H, Korman S, Riss E. Angiography of azygos continuation of inferior vena cava in situs ambiguus with left isomerism (polysplenia syndrome). *Pediatr Radiol* 1984; **14**: 109-112
4 **Gayer G**, Apter S, Jonas T, Amitai M, Zissin R, Sella T, Weiss P, Hertz M. Polysplenia syndrome detected in adulthood: report of eight cases and review of the literature. *Abdom Imaging* 1999; **24**: 178-184
5 **Peoples WM**, Moller JH, Edwards JE. Polysplenia: a review of 146 cases. *Pediatr Cardiol* 1983; **4**: 129-137
6 **Ooshima I**, Maruyama T, Ootsuki K, Ozaki M. Preduodenal portal vein in the adult. *J Hepatobiliary Pancreat Surg* 1998; **5**: 455-458
7 **Abut E**, Akkaya L, Uysal U, Arman A, Guveli H, Bolukbas C, Kurdas OO. Selective spleen scintigraphy in the diagnosis of polysplenia syndrome. *Br J Radiol* 2004; **77**: 698-700
8 **Gayer G**, Zissin R, Apter S, Atar E, Portnoy O, Itzchak Y. CT findings in congenital anomalies of the spleen. *Br J Radiol* 2001; **74**: 767-772
9 **Miyabara S**, Sugihara H, Kamio A, Oota K, Abe H, Kato S. Atypical polysplenia only with the hepatic segment of inferior vena in a middle-aged. *Acta pathol Jpn* 1984; **34**: 111-116
10 **Muneta S**, Sakai S, Fukuda H, Imamura Y, Matsumoto I. Polysplenia syndrome with various visceral anomalies in an adult: embryological and clinical considerations. *Intern Med* 1992; **31**: 1026-1031
11 **de la Monte SM**, Hutchins GM. Sisters with polysplenia. *Am J Med Genet* 1985; **21**: 171-176
12 **Nakada K**, Kawaguchi F, Wakisaka M, Nakada M, Enami T, Yamate N. Digestive tract disorders associated with asplenia/polysplenia syndrome. *J Pediatr Surg* 1997; **32**: 91-94
13 **Knight HO**. An anomalous portal vein with its surgical dangers. *Ann Surg* 1921; **74**: 697-699
14 **Ishizaki Y**, Tanaka M, Okuyama T. Surgical implications of preduodenal portal vein in the adult. Case report and review of the literature. *Arch Surg* 1994; **129**: 773-775
15 **Kobayashi H**, Kawamoto S, Tamaki T, Konishi J, Togashi K. Polysplenia associated with semiannular pancreas. *Eur Radiol* 2001; **11**: 1639-1641

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CASE REPORT

Splenic inflammatory pseudotumor mimicking angiosarcoma

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Abstract

Splenic tumors are rare. Differentiation of the tumors before operation is of great value regarding the outcome. A case of a 32-year-old man with a splenic inflammatory pseudotumor (IPT) mimicking splenic angiosarcoma is described. The tumor was highly suspected of being splenic angiosarcoma based on radiological findings preoperatively. However, after splenectomy, histopathological examinations revealed splenic IPT. Splenic IPT and angiosarcoma are rare and often pose diagnostic difficulties because the clinical and radiological findings are obscure. Due to large differences in prognosis, we briefly reviewed the clinical, radiological, and pathological features of both of the tumors.

Key words: Splenic inflammatory pseudotumor; Splenic angiosarcoma; Spleen tumor

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INTRODUCTION

Although splenic tumors are rare, differentiation of the tumors before operation is of great value. Among them, splenic inflammatory pseudotumor (IPT) is a benign tumor characterized microscopically by a proliferation of inflammatory cells[1]; but splenic angiosarcoma is a dismal malignancy of vascular origin[2]. We usually differentiate them before operation by imaging studies. In this report, we present a case of splenic IPT mimicking angiosarcoma on radiological findings.

CASE REPORT

A 32-year-old man was a carrier of hepatitis B virus for years with regular follow-up at outpatient clinics. A splenic mass was incidentally detected by sonography. Physical examinations were unremarkable. The patient had no systemic complaints except mild vague discomfort over the epigastric region. Biochemical and hematological investigations were all within normal ranges except for slightly elevated serum glutamate-oxaloacetate transaminase (SGOT). Tumor markers including CEA, AFP, CA-199, and CA-125 were all negative. Sonography of the spleen showed a well-defined encapsulated tumor, 6.3 × 6.1 cm in diameter, with hyperechoic density in the central portion (Figure 1). Non-contrast and contrast abdominal computed tomography (CT) showed a mass over the spleen with high density in the central portion and multiple diffuse low-attenuation nodules in liver parenchyma (Figure 2A and B). Magnetic resonance imaging (MRI) was done for differentiation and revealed a mass lesion around 6 cm in the spleen, which showed partially dense intensity in T2-weighted image (Figure 3A) and peripheral nodule enhancement with gadolinium-contrast filling and pooling in T1 contrast-enhancement dynamic study (Figure 3B-D).

Overall, there were two parts of different signal

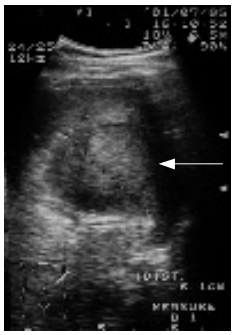

Figure 1 Sonography showed a well-defined encapsulated tumor, 6.3 cm x 6.1 cm in size, over the spleen with hyperechoic density in the central portion (arrow) of the tumor.

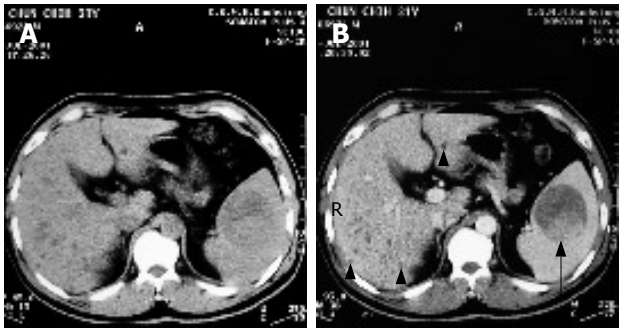

Figure 2 Abdominal CT. A: Non-contrast CT showed a well-defined tumor over the spleen; B:Contrast CT showed partial enhancement of the tumor with high density in the dorsal portion of the tumor (arrow) and multiple diffuse low-attenuation nodules in liver parenchyma (arrowhead).

intensity comprising the tumor at the spleen. The major part of the circumscribed lesion showed persistent low-signal intensity with some stippling enhancement from MRI dynamic contrast-enhanced series, while the minor portion located at the dorsal aspect showed similar signal-intensity change as the normal spleen parenchyma either at pre- or post-contrast phases, which is suggestive of hypervascular lesion, such as angiosarcoma.

Diffuse multiple tiny cystic lesions were noted in bilateral lobes of the liver. Biliary hamartoma was first considered. However, differential diagnosis should have included multiple hepatic cysts, micro-abscesses or even metastases. Therefore, primary splenic angiosarcoma with the possibility of multiple liver metastasis was the first consideration.

Surgical intervention was indicated, and exploratory laparotomy was performed thereafter. During the operation, the spleen was removed smoothly and liver hypertrophy with multiple tiny cystic lesions over the liver surface was noted. There was no evidence of malignancy in the frozen sections examined.

The specimen of spleen measured 11 × 8.5 × 6 cm in size and weighed 240 g (Figure 4A). Grossly, there was a well-circumscribed tumor inside the spleen, measuring 5.5 × 5.0 × 4.0 cm in size (Figure 4B). There were two parts of different appearances comprising the tumor. The major part of the circumscribed lesion showed yellowish appearance with some stippling red spots scattered over the cut surface, while the minor portion located at the dorsal aspect showed similar appearance as the normal spleen parenchyma.

Microscopically, the sections of the specimen were

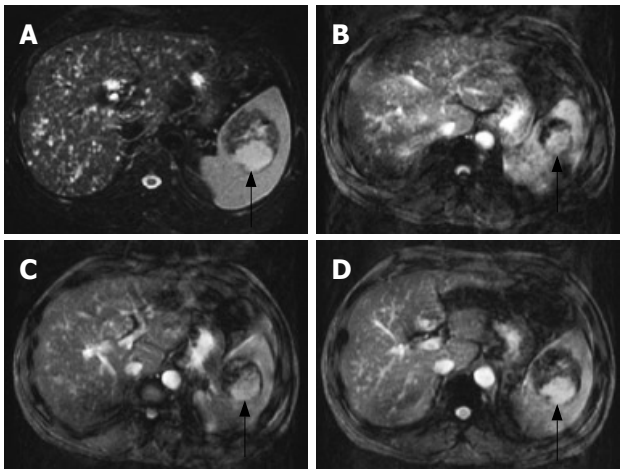

Figure 3 MRI. A: In T2-weighted image, a well-circumscribed tumor (arrow) in the spleen, which showed partially dense intensity; B-D: In T1 contrast-enhancement dynamic study, peripheral nodule enhancement with gadolinium-contrast filling and pooling were noted in the tumor.

composed of fibrosis, focal sclerosis, plump spindle cells and vascular proliferation (Figure 5). An admixture of inflammatory cells included lymphocytes, plasma cells and neutrophils, in which lymphocytes were predominant. The major part of the tumor consisted of more fibrosis and focally sclerotic change, while the minor part consisted of more vascular proliferation and more inflammatory cell infiltration. The sections of the specimen were compatible with the picture of IPT.

The patient's postoperative course was uneventful, and he has been well for 3 years following surgery.

DISCUSSION

The term "inflammatory pseudotumor" was first proposed in 1954, since these lesions are composed of inflammatory cells. Inflammatory pseudotumors have been reported in several anatomic locations, such as orbit, respiratory tract, gastrointestinal tract, and liver[3]. However, splenic involvement is extremely rare. The first 2 cases of splenic inflammatory pseudotumor were reported in 1984. About one-half of the lesions are discovered incidentally during work-up for other malignancies after splenectomy for other conditions such as idiopathic thrombocytopenic purpura, or at autopsy[4]. Clinical symptoms are not specific to the disease, with left upper quadrant or epigastric pain. Splenomegaly is usually present. Laboratory investigations may reveal fever, anemia, hypergammaglobulinemia, thrombocytosis, or hypersplenism; however, more than one-third of the cases reported showed no evidence of any abnormality in laboratory investigations[1]. Other patients have signs of immune thrombocytopenic purpura[4]. However, our patient only had history of hepatitis B with mild vague abdominal discomfort.

There are several radiological modalities suggested for diagnosing splenic IPT, including ultrasonography, CT, and MRI. Ultrasonography usually shows a low echoic mass in most cases[5]. CT is the radiological

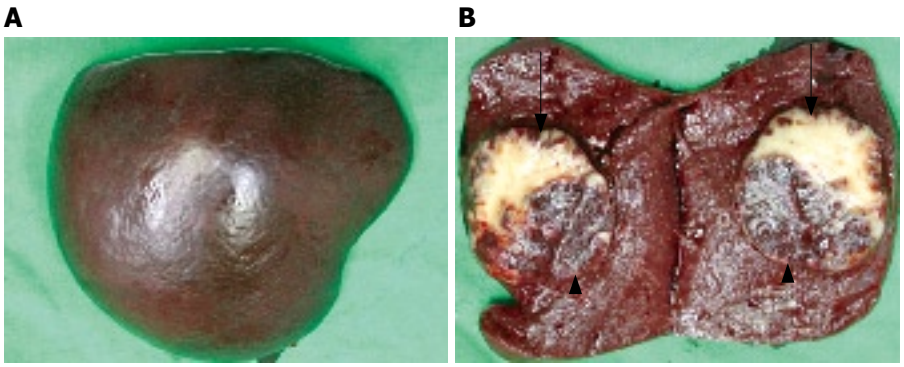

Figure 4 The specimen of spleen. The removed spleen (A) was 11 cm x 8.5 cm x 6 cm in size. A well-circumscribed tumor (B), measuring 5.5 cm x 5 cm x 4 cm, over the central portion of the spleen was noted. There were two parts of different appearances comprising the tumor. The major part of the circumscribed lesion (arrow) showed yellowish appearance with some stippling red spots scattered over the cut surface. While the minor portion (arrowhead) located at dorsal aspect showed similar appearance as the normal spleen parenchyma.

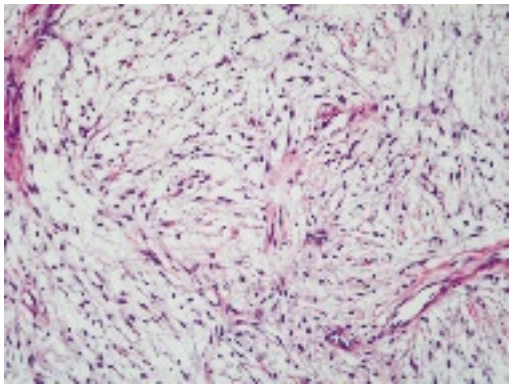

Figure 5 Histologic findings (hematoxylin and eosin stain, original magnification x 200). The sections of the specimen are composed of fibrosis, focal sclerosis, plump spindle cells and vascular proliferation.

test that most often demonstrates the presence of a splenic lesion and usually demonstrates a low-density mass in both the non-enhanced and enhanced modes. Although CT is quite sensitive, this modality is not specific in differentiating between splenic IPT and other malignancies[3]. MRI findings usually show an iso- or low-intensity mass on the T1-weighted images and a low-intensity mass on the T2-weighted images, while also demonstrating a low-intensity in the early phase and a high-intensity mass in the delayed phase of a dynamic study. However, the same criticism can be said about MRI with T1- and T2-weighted imaging for the poor specificity in differentiating pseudotumors from other malignancies[3].

Gross features in all reported cases of splenic IPT are described as a solitary well-circumscribed lesion with a white or tan cut surface that compresses the adjacent splenic parenchyma. Histological examinations of specimens are composed of acute and chronic inflammatory cells infiltrating a stroma of mesenchymal myofibroblastic spindle cells. The inflammatory cells are predominantly plasma cells, mature lymphocytes, and rarely eosinophils.

Etiology and pathogenesis of IPT remain unknown. Some characteristics described support an immunologic disorder[4]. Pathologic features of pseudotumor may be related to the production of mediators in inflammation, and note that interleukin-1 can produce local lesions and systemic manifestations with IPT. In addition, granulomatous inflammation process, focal parenchymal necrosis with hemorrhage, disturbance of blood supply or bacterial or viral infection have all been speculated in the pathogenesis of splenic IPT[6].

If a primary splenic tumor is suspected, splenectomy is required for diagnostic purposes and for therapy. Laparoscopic surgery for benign splenic tumors is considered to be a valid procedure. However, if the splenic lesions have a malignant potential, laparoscopic surgery elevates the risk of either intra-abdominal dissemination or skin metastasis. Therefore, if malignant splenic tumor can not be ruled out, laparoscopic surgery is not suggested[7].

Angiosarcoma is a malignancy of vascular origin, and is characterized by masses of endothelial cells with cellular atypia and anaplasia[2]. Angiosarcoma may occur anywhere in the body, but most often in the skin, soft tissues, breast and liver. Splenic involvement is exceedingly rare. Clinical manifestations include abdominal discomforts, splenomegaly and signs of immune thrombocytopenic purpura[2], which were not specific to the disease. Pathogenesis remains obscure. Prognosis is extremely poor, with a 6-month survival rate of 20%[8]. The tumor commonly metastasizes to the liver, lung, bone, lymph nodes, omentum, or peritoneum.

There are also several radiological modalities suggested for diagnosing splenic angiosarcoma including sonography, CT, MRI and angiography. Unlike splenic pseudotumor, sonographic findings in splenic angiosarcoma show a heterogeneous mass or multiple reflective areas[8,9]. CT may demonstrate hypoattenuating lesions on nonenhanced scans. Areas of high attenuation on noncontrast CT may represent acute hemorrhage or hemosiderin deposits. Contrast enhancement of angiosarcoma may show some enhancements similar to that of hepatic hemangioma[2]. The MRI appearance of splenic hemangioma is similar to that of hemangioma of the liver. The lesion will be hypo- or isointense on T1-weighted MR images and hypertense on T2-weighted MR images. T1-weighted images obtained after contrast administration may demonstrate a difference between the cystic and solid components[2]. The most specific modality to differentiate splenic IPT from angiosarcoma is angiography. Angiographic findings of angiosarcoma show a hypervascular tumor with contrast pooling, and that of splenic IPT usually show avascular or hypovascular tumor[10].

In the present case, sonography showed hyperechoic density in the central portion of the tumor. CT also

showed a high density area and partial enhancement in the central portion of splenic tumor with multiple diffuse low-attenuation nodules in liver. MRI revealed a mass, which showed partial dense intensity in T2-weighted image. In T1 mode with gadolinium-enhancement, peripheral nodule with contrast filling and pooling, which was compatible with a picture of angiosarcoma, was noted. Therefore, the first impression of angiosarcoma with suspected liver metastasis was reasonable preoperatively. The reason why splenic IPT could mimic the picture of angiosarcoma could be explained by the hypervascular component within the minor part of the tumor, which absorbed the contrast medium in radiological examination. Accordingly, the ITP should be kept in mind in the differential diagnosis of splenic space-occupying lesions even if the imaging modality does not favor it.

REFERENCES

1 **Ozkara SK**, Gurbuz Y, Ercin C, Muezzinoglu B, Turkmen M. Inflammatory pseudotumor of the spleen. *Virchows Arch* 2001; **438**: 629-631

2 **Thompson WM**, Levy AD, Aguilera NS, Gorospe L, Abbott RM. Angiosarcoma of the spleen: imaging characteristics in 12 patients. *Radiology* 2005; **235**: 106-115

3 **Chen WH**, Liu TP, Liu CL, Tzen CY. Inflammatory pseudotumor of the spleen. *J Chin Med Assoc* 2004; **67**: 533-536

4 **Hatsuse M**, Murakami S, Haruyama H, Inaba T, Shimazaki C. Inflammatory pseudotumor of the spleen complicated by idiopathic thrombocytopenic purpura. *Ann Hematol* 2005; **84**: 619-620

5 **Hayasaka K**, Soeda S, Hirayama M, Tanaka Y. Inflammatory pseudotumor of the spleen: US and MRI findings. *Radiat Med* 1998; **16**: 47-50

6 **Neuhauser TS**, Derringer GA, Thompson LD, Fanburg-Smith JC, Aguilera NS, Andriko J, Chu WS, Abbondanzo SL. Splenic inflammatory myofibroblastic tumor (inflammatory pseudotumor): a clinicopathologic and immunophenotypic study of 12 cases. *Arch Pathol Lab Med* 2001; **125**: 379-385

7 **Tsugawa K**, Hashizume M, Migou S, Kawanaka H, Sugimachi K, Irie H, Maeda T, Akaboshi K. Laparoscopic splenectomy for an inflammatory pseudotumor of the spleen: operative technique and case report. *Hepatogastroenterology* 1998; **45**: 1887-1891

8 **Hai SA**, Genato R, Gressel I, Khan P. Primary splenic angiosarcoma: case report and literature review. *J Natl Med Assoc* 2000; **92**: 143-146

9 **Ha HK**, Kim HH, Kim BK, Han JK, Choi BI. Primary angiosarcoma of the spleen. CT and MR imaging. *Acta Radiol* 1994; **35**: 455-458

10 **Moriyama S**, Inayoshi A, Kurano R. Inflammatory pseudotumor of the spleen: report of a case. *Surg Today* 2000; **30**: 942-946

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CASE REPORT

Rare pulmonary and cerebral complications after transarterial chemoembolization for hepatocellular carcinoma: A case report

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Author contributions: Zhao H and Lou JY contributed equally to this work; Zhao H, Lou JY, Wang HQ, Fan QQ, Chen XX, observed and treated the patient with rare complications; Zhao H and Lou JY wrote the paper.

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Abstract

We report a rare case of acute pulmonary and cerebral complication after transarterial chemoembolization (TACE) for inoperable hepatocellular carcinoma. The case involved a large tumor and hepatic vein invasion. Nonspecific pulmonary and cerebral symptoms such as acute dyspnoea and transient consciousness loss developed in the patient, a 49-year-old woman, following the TACE due to pulmonary and cerebral oil embolism. The chest and brain conditions of this patient improved after some supportive therapies and nursing interventions. She also subsequently completed the other three procedures of TACE.

Key words: Hepatocellular carcinoma; Chemoembolization; Therapeutic; Pulmonary embolism; Cerebral embolism

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Zhao H, Wang HQ, Fan QQ, Chen XX, Lou JY. Rare pulmonary and cerebral complications after transarterial chemoembolization for hepatocellular carcinoma: A case report. *World J Gastroenterol* 2008; 14(41): 6425-6427 Available from: URL: http://www.wjgnet.com/1007-9327/14/6425.asp DOI: http://dx.doi.org/10.3748/wjg.14.6425

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the leading causes of cancer-related death in China where the majority of HCC patients have underlying hepatic B virus (HBV) infection and cirrhosis, and most cases are unresectable due to late stage and multifocali. Transarterial chemoembolization (TACE) is one of the most common treatment modalities as a palliative and preoperative method for patients with advanced HCC, which can improve the resection rate of HCC and prolong the survival time of patients. HCC has a tendency to invade the portal and hepatic veins, which may result in formation of hepatic arterio-venous shunts. Some rare complications such as remote ectopic embolism can be caused by this kind of abnormal shunts. In this paper, we report a rare pulmonary and cerebral complication of HCC, which is probably associated with hepatic vein invasion.

CASE REPORT

A 49-year-old woman was admitted to the Department of Radiology of the Second Affiliated Hospital of Zhejiang University in October 2004 with right upper quadrant pain and weight loss. She was a hepatitis B virus carrier. Her α-fetoprotein level was 1185.3 ng/mL. Ultrasonography and computed tomography (CT) revealed a 10-cm mass in the posterior segments of the right liver lobe. A 1.5-cm mass was also found in the left lateral segment. These clinical signs indicated that the patient had inoperable HCC and Child-Pugh class A cirrhosis. TACE was offered to the patient. Angiogram demonstrated no obvious hepatic arterio-venous shunt, but multiple smaller masses in both lobes of the liver. An emulsion of oxaliplatin, pirarubicin, hydroxycamptothecin and lipiodol were prepared, 35 mL and 3 mL of the mixture were administered intra-arterially to the right and left hepatic artery, respectively. The patient experienced right upper quadrant pain after TACE and had an uneventful recovery. One month later, a second TACE procedure was performed *via* the right hepatic artery and 40 mL of the mixture was administered. On the next day, she experienced sudden acute dyspnoea and the peripheral oxygen saturation decreased to 90%. The chest X-ray showed some

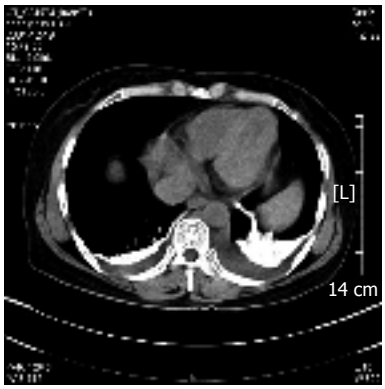

Figure 1 Chest CT scan revealing multiple iodized oil-like high-density materials in parenchyma of the lung.

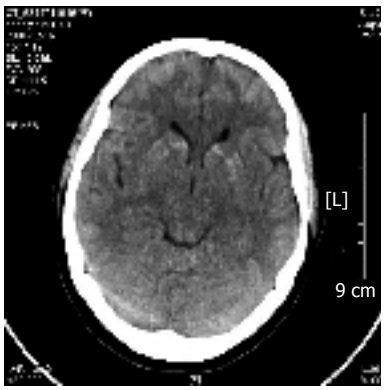

Figure 2 Non-contrast enhanced CT scanning showing multiple disseminated hyper-intense lesions in the brain, consistent with deposition of iodized oil.

increased reticular shadows in the left lung, especially in the lower zones, and a chest CT scan revealed multiple iodized oil-like high-density materials in parenchyma of the lung (Figure 1). After 10 mg dexamethasone i.v. and other supportive therapies were administered, the respiratory symptom was attenuated. Two days later, the patient suffered from a serious headache and transient consciousness loss, accompanying nausea and vomiting followed by confusion, lower extremity weakness. Non-contrast enhanced CT scanning showed multiple disseminated hyper-intense lesions in the brain, consistent with deposition of iodized oil (Figure 2). One week later, her respiratory and neurologic symptoms disappeared completely, and she was discharged. The patient also consequently completed the other three TACE procedures, during which no similar symptoms occurred.

DISCUSSION

TACE has various severe complications, including acute hepatic failure, intrahepatic biloma, pseudoaneurysm formation, and ectopic infarction, which occur in less than 1% of patients. Pulmonary embolism is a rare complication of TACE. Xia *et al*[1] reported a total of 2012 TACE procedures in 1348 patients, but pulmonary embolism occurred only in case. Sporadic cases of cerebral embolism after TACE have also been reported[2-5]. No reports are available on pulmonary embolism accompanying cerebral embolism after TACE. We encountered a rare pulmonary and cerebral complication of HCC, which was probably associated with hepatic vein invasion. The patient had nonspecific respiratory and neurological symptoms, including cough, dyspnoea, headache, transient loss of consciousness, confusion, and weak extremities. Chest X-ray and CT scanning showed some positive findings, indicating deposition of iodized oil, and the diagnosis of pulmonary and cerebral embolism was confirmed clinically.

The underlying mechanisms of pulmonary and cerebral embolism after TACE are still obscure. Hepatic arterio-venous shunt, which is associated with hepatic vein invasion of HCC, may be the reasonable explanation for pulmonary embolism. Vascular abnormalities, referred to as pulmonary arterio-venous shunt, can be found in patients with advanced liver disease[6]. If patients have pulmonary embolism after TACE, the oil emboli may also pass through the pulmonary arterio-venous shunt and enter the systemic circulation. In this case, the patient suffered from pulmonary and cerebral embolism subsequently. Thus, we hypothesize that iodized oil passed through the hepatic arterio-venous shunt, and then traveled to the cerebral artery through intrapulmonary arterio-venous shunt There are also some other hypotheses including intracardiac right-to-left shunt (e.g. patent foramen ovale) and right-to-left shunt *via* the arteriovenous anastomosis between the right inferior phrenic artery (IPA) and the intrapulmonary vasculature. However, there is no evidence to support the theory of intracardiac or IPA shunts in this patient.

Although pulmonary and cerebral embolism or infarctions are rare complications of TACE in patients with HCC, we should be aware of this kind of situations when we observe complications of TACE. When angiogram shows any hepatic arterio-venous shunts, we should decrease the dose of lipiodol during the procedure and pay great attentions to the respiratory and neurological symptoms post-operatively, which may be caused by ectopic embolism. The manifestations caused by lipiodol are different from those caused by thrombus, because lipiodol diffuses to peripheral blood vessels while thrombus usually obstructs the main branch of arteries.

A thorough patient assessment should be performed by nurses before the procedure. Some risk factors including a large size of HCC, hepatic vein invasion of HCC, live cirrhosis, congenital cardiovascular disease, and chronic pulmonary disease should be noticed before the procedure. If the patients have risk factors for pulmonary and cerebral complications of TACE, nurses should give a reassessment, extra education, psychological support.

In addition to post-embolization syndrome (PES), with its symptoms manifested as fever, pain, nausea, and vomiting, there are also some severe or rare complications of TACE, including acute hepatic failure,

intrahepatic biloma, pseudoaneurysm formation, ectopic infarction, *etc*. Nurses should keep in mind that a small number of patients after TACE will suffer from some severe or rare complications. Our patient developed a dry cough and dyspnea in the first day after TACE, followed by severe headache, nausea, vomiting, weak extremities and lost consciousness transiently in the third day. Nurses immediately provided nursing interventions such as semi-reclining position, inhaling oxygen, administration of steroids and dehydrating agents, and intensive monitoring of the patient's vital signs. She had a good recovery and was discharged. If some symptoms such as cough, chest pain, chest distress, headache, nausea, and vomiting occur in patients after TACE, nurses should make physical examination and notify the physician to exclude pulmonary and cerebral complications.

When the diagnosis of pulmonary embolism is confirmed, some nursing actions must be taken immediately. Nurses should keep the patient's airway open, and have oxygen inhaled to maintain the patient's basic respiratory function. Vital signs, blood oxygen saturation, mental status, and some laboratory values must be monitored and documented. If the patient has signs of respiratory failure, intensive care and mechanical ventilation must be provided promptly. Tiny lipiodol particles usually diffuse and stay in the peripheral bronchus and alveoli, which can damage gas exchange and cause special inflammations. Steroids, bronchodiators and prophylactic antibiotics should also be given.

The aim of nursing care during an acute phase of cerebral embolism after TACE is to minimize cerebral damage. Nurses should frequently observe the level of consciousness, pupil size and reaction to light, patient's response to commands, movement and strength, patient's vital signs, *etc*. To reduce the intracranial pressure, some nursing routines must be given such as keeping the head of bed above 30º, restriction of fluids, oxygen inhaling and administration of steroids and dehydrating agents. Sedatives and tranquilizers, which can depress the respiratory center and obscure neurological observations, should not be given except for some specific situations such as epileptic seizure attack.

In summary, even though pulmonary embolism and cerebral embolism are rare complications of TACE, we should be aware of these rare complications in patients with high risk factors and reduce the dose of iodized oil or stop the procedure.

REFERENCES

1 **Xia J**, Ren Z, Ye S, Sharma D, Lin Z, Gan Y, Chen Y, Ge N, Ma Z, Wu Z, Fan J, Qin L, Zhou X, Tang Z, Yang B. Study of severe and rare complications of transarterial chemoembolization (TACE) for liver cancer. *Eur J Radiol* 2006; **59**: 407-412

2 **Yoo KM**, Yoo BG, Kim KS, Lee SU, Han BH. Cerebral lipiodol embolism during transcatheter arterial chemoembolization. *Neurology* 2004; **63**: 181-183

3 **Wu RH**, Tzeng WS, Chang CM. Iodized oil embolization to brain following transcatheter arterial embolization of liver. *J Gastroenterol Hepatol* 2005; **20**: 1465-1467

4 **Takao H**, Makita K, Doi I, Watanabe T. Cerebral lipiodol embolism after transcatheter arterial chemoembolization of hepatocellular carcinoma. *J Comput Assist Tomogr* 2005; **29**: 680-682

5 **Matsumoto K**, Nojiri J, Takase Y, Egashira Y, Azama S, Kato A, Kitahara K, Miyazaki K, Kudo S. Cerebral lipiodol embolism: a complication of transcatheter arterial chemoembolization for hepatocellular carcinoma. *Cardiovasc Intervent Radiol* 2007; **30**: 512-514

6 **Lange PA**, Stoller JK. The hepatopulmonary syndrome. *Ann Intern Med* 1995; **122**: 521-529

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CASE REPORT

Port site and distant metastases of gallbladder cancer after laparoscopic cholecystectomy diagnosed by positron emission tomography

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Abstract

We report port site and distant metastases of unsuspected gallbladder cancer after laparoscopic cholecystectomy diagnosed by positron emission tomography (PET) in two patients. Patient 1, a 72-year-old woman was diagnosed as cholelithiasis and cholecystitis and received laparoscopic cholecystectomy. Unsuspected gallbladder cancer was discovered with histological result of well-differentiated squamous cell carcinoma of the gallbladder infiltrating the entire wall. A PET scan using F-18-fluorodeoxyglucose (FDG-PET) before radical resection revealed residual tumor in the gallbladder fossa and recurrence at port site and metastases in bilateral hilar lymph nodes. Patient 2, a 69-year-old woman underwent laparoscopic cholecystectomy more than one year ago with pathologically confirmed unsuspected adenosquamous carcinoma of stage pT1b. At 7-mo follow-up after surgery, the patient presented with nodules in the periumbilical incision. Excisional biopsy of the nodule revealed adenosquamous carcinoma. The patient was examined by FDG-PET, demonstrating increased FDG uptake in the right lobe of the liver and mediastinal lymph nodes consistent with metastatic disease. This report is followed by a discussion about the utility of FDG-PET in the gallbladder cancer.

Key words: Gallbladder cancer; Positron emission tomography; Fluorodeoxyglucose

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INTRODUCTION

The vast majority of cholecystectomies are currently performed laparoscopically, and unsuspected gallbladder cancer can be discovered incidentally following 1% of routine cholecystectomies[1]. There is a suspicion that recurrence of the tumor in the abdominal incision is more common after laparoscopic operations. Several possible factors probably involved in the development of such metastases have been proposed[2,3]. Resection of the recurrent malignancy developed in the port sites is warranted, and may lead to survival benefit only when the port site metastases is the only manifestation of recurrent disease. The preoperative accurate evaluation of recurrent gallbladder cancer is essential for reasonable treatment. We report two cases of port site and distant metastases of unsuspected gallbladder cancer after laparoscopic cholecystectomy diagnosed by FDG-PET.

CASE REPORT

Patient 1

A 72-year-old woman presented with right upper quadrant pain and fever. She had a history of cholelithiasis documented by ultrasound, and intermittent attacks of biliary colic over 2 years. She was diagnosed with cholelithiasis and cholecystitis. She received laparoscopic

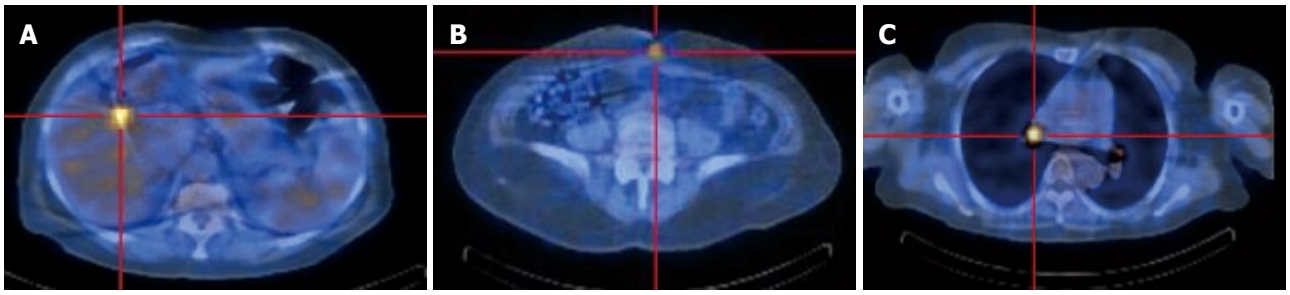

Figure 1 Increased uptake of the radiopharmaceutical in patient 1 (FDG-PET). A:Gallbladder fossa; B: Periumbilical area; C: Bilateral hilar lymph nodes.

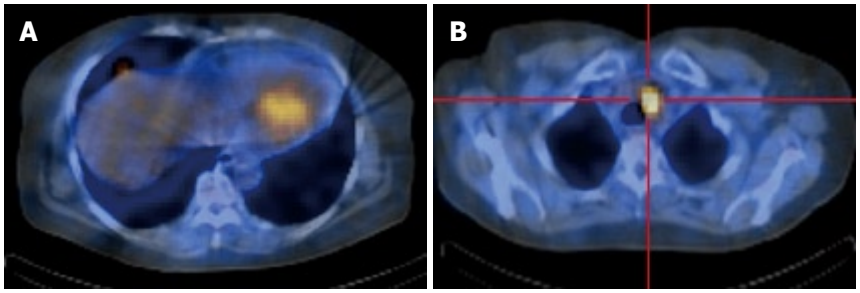

Figure 2 Increased uptake of the radiopharmaceutical in patient 2 (FDG-PET). A:Right lobe of the liver; B: Mediastinal lymph nodes.

cholecystectomy and the gallbladder was noted to be edematous and thick-walled, with multiple stones. Histological evaluation revealed an unsuspected well differentiated squamous cell carcinoma of the gallbladder infiltrating the entire wall. More than one month after surgery, she visited our hospital for further radical surgery. Palpable nodules in the periumbilical incision were found by physical examination during admission. A FDG-PET scan was performed, demonstrating increased uptake of the radiopharmaceutical in the gallbladder fossa and periumbilical area as well as bilateral hilar lymph nodes (Figure 1). The lesions were interpreted as residual tumor in the gallbladder fossa and recurrence at port site and metastases in bilateral hilar lymph nodes. Tru-cut biopsy confirmed metastatic squamous cell carcinoma similar to the previous histology. The patient refused further treatment and was discharged.

Patient 2

A 69-year-old woman with a history of intermittent right upper quadrant pain over 11 years underwent laparoscopic cholecystectomy more than 1 year ago. The histological examination revealed an unsuspected adenosquamous carcinoma of stage pT1b (tumor invades into muscularis). At 7-mo follow-up after surgery, the patient presented with nodules in the periumbilical incision interpreted as inflammation or postoperative change. The nodule enlarged progressively, and she visited our hospital 19 mo after surgery. Abdominal CT scan revealed a small nodule in the right lobe of the liver, which was difficult to interpret. Excisional biopsy of the nodule in the periumbilical incision was performed and histological examination revealed adenosquamous carcinoma. The patient was examined by FDG-PET, demonstrating increased FDG uptake in the right lobe of the liver and mediastinal lymph nodes consistent with metastatic disease (Figure 2). Chest computed tomography demonstrated enlarged mediastinal lymph nodes consistent with metastases. The patient has subsequently been treated with Gemzar and oxaliplatin with regression of tumor. She died of a non-cancer related cause 4 mo after the second operation.

DISSCUSSION

PET is a noninvasive scanning method to assess metabolism *in vivo* by means of positron-emitting radiolabeled tracers. This is in contrast with conventional imaging modalities, including ultrasonography (US), computed tomography (CT) and magnetic resonance imaging (MRI) which evaluate structural or anatomical changes[3]. The tracer to measure cellular metabolism commonly used in PET is FDG. FDG is a glucose analogue that is phosphorylated in the cells, but not further metabolized. Most malignant tumors show increased uptake of FDG because malignant transformation and growth of tumor cells is associated with overexpression of glucose transporters and increased hexokinase activity[4].

FDG-PET imaging has been increasingly used to identify and stage various tumors. The majority of these studies show FDG-PET to be superior to traditional imaging in the differential diagnosis of malignancy. This has been particularly notable in the evaluation of recurrent or metastatic disease[5-7]. A few studies have evaluated the use of FDG-PET in the assessment of biliary system tumors or gallbladder carcinoma[4,8-10]. In the study of Anderson *et al*, nine of 14 gallbladder cancer patients had residual carcinoma at the time of PET[9]. FDG-PET was useful in our cases to delineate recurrent gallbladder cancer, and its extent and had an important clinical impact on the selection of proper treatment. In both cases, FDG-PET detected residual tumor or port site and distant metastatic diseases and

changed the surgical plan of radical resection with intent for cure. This report emphasizes that FDG-PET may play an important role in the posttherapy follow-up of gallbladder cancer. Taking the accumulation of FDG in the malignant gallbladder cancer cells into consideration, FDG-PET can be considered as a complementary preoperative staging method. In cases in which it is easy to understaging gallbladder cancer before surgery such as peritoneal seeding, small hepatic metastases, and small regional lymph nodes involvement, FDG-PET may be able to provide important diagnostic information to obtain a correct presurgical staging, and sometimes lead to the change of treatment.

Gallbladder cancer is a relatively rare disease that has no specific symptoms or signs, and the clinical presentations of gallbladder cancer, and gallstone disease are commonly difficult to distinguish. The only effective treatment for carcinoma of the gallbladder is operative resection, and an open technique is preferred. Unfortunately, as is often the case, the lack of presurgical differential diagnosis hampers the planning of surgery. Recently, a few published articles have studied the utility of FDG-PET in gallbladder cancer focusing on not only posttherapy follow-up and preoperative staging, but also the establishment of the benign or malignant natures of gallbladder lesions. Koh *et al*[10] reported that FDG- PET provided reliable differential diagnoses, identifying gallbladder carcinoma with 75% sensitivity, 87.5% specificity, and 81.3% accuracy. Anderson *et al*[9] report that the sensitivity of this modality was 78% in their series of 14 gallbladder cancer cases. In the study of Antonio *et al*, which comprises a series of 16 patients, FDG-PET showed a sensitivity of 0.80, a specificity of 0.82 in diagnosing gallbladder cancer[4]. These studies revealed that FDG-PET can provide important information for establishing the nature of gallbladder lesions especially when in conjunction with conventional modalities.

Because FDG is taken up not only by malignant tumor cells, but also by activated inflammatory cells, benign inflammatory or infectious lesions typically without obvious increase of FDG uptake under some circumstances can produce false positive results[11,12]. The most common reason for false positive FDG-PETs is an inflammatory lesion. Xanthogranulomatous cholecystitis and polypoid lesion with adenomyomatosis are also the common reasons caused false positive result[4,10,11]. PET imaging must be interpreted with caution in patients with known severe inflammatory or granulomatous disease. Nishiyama *et al*[12] illustrated the relationship between the severity of inflammation and the specificity of PET, and proposed that patients with signs of acute inflammation should be excluded from examination. If the PET scans are performed under conditions with no or low-grade inflammation, an accurate diagnosis of acute or chronic cholecystitis as a benign lesion may be possible.

Although PET was sensitive for the detection of gallbladder cancer, some false negative findings also occurred. The limited sensitivity of FDG-PET for small lesions may have several causes[10,12]. Some factors illustrate the intrinsic limitations of PET resolution for small lesions: activity in small lesions may be underestimated because of the partial-volume effect, movement artifacts caused by nongated breath holding, or physiologic liver FDG uptake. PET scanning performed under suboptimal conditions can also decrease the sensitivity: patient fasting may be too short, and lead to an unnecessarily high liver FDG uptake; the duration of FDG administration and data acquisition may be too short. In diabetic patients, the rate of FDG accumulation in the tumor is decreased, impaired the sensitivity of FDG-PET[10]. In patients with mucinous adenocarcinoma of the gallbladder, a false-negative result has also been reported, probably secondary to poor cellular density[4]. To increase the sensitivity for small lesions, the underestimation due to the partial-volume effect may be reduced by improving the spatial resolution of PET; movement artifacts may be reduced by breath gating of the measurement, and by avoiding reintroduction of the patient to the scanner; PET scanning can be well performed under optimal conditions. Nishiyama *et al* adopted dual-time-point FDG-PET to evaluate the nature of gallbladder lesions, and demonstrated that delayed FDG-PET was more helpful than early FDG-PET in the evaluation of malignancy, because of the increased uptake by lesions, and the increased lesion-to-background contrast[12]. Recent hybrid PET-CT systems provide structural and functional information simultaneously, and may offer early and accurate staging with an improved specificity[13,14].

In conclusion, despite the relatively small number of gallbladder cancer patients, received FDG-PET scan, this imaging may play an important role in the differential diagnosis, staging, restaging, and posttherapy follow-up of gallbladder cancer.

REFERENCES

1 **Akyurek N**, Irkorucu O, Salman B, Erdem O, Sare M, Tatlicioglu E. Unexpected gallbladder cancer during laparoscopic cholecystectomy. *J Hepatobiliary Pancreat Surg* 2004; **11**: 357-361

2 **Lundberg O**. Port site metastases after laparoscopic cholecystectomy. *Eur J Surg Suppl* 2000; 27-30

3 **Lomis KD**, Vitola JV, Delbeke D, Snodgrass SL, Chapman WC, Wright JK, Pinson CW. Recurrent gallbladder carcinoma at laparoscopy port sites diagnosed by positron emission tomography: implications for primary and radical second operations. *Am Surg* 1997; **63**: 341-345

4 **Rodriguez-Fernandez A**, Gomez-Rio M, Llamas-Elvira JM, Ortega-Lozano S, Ferron-Orihuela JA, Ramia-Angel JM, Mansilla-Rosello A, Martinez-del-Valle MD, Ramos-Font C. Positron-emission tomography with fluorine-18-fluoro-2-deoxy-D-glucose for gallbladder cancer diagnosis. *Am J Surg* 2004; **188**: 171-175

5 **Herder GJ**, Kramer H, Hoekstra OS, Smit EF, Pruim J, van Tinteren H, Comans EF, Verboom P, Uyl-de Groot CA, Welling A, Paul MA, Boers M, Postmus PE, Teule GJ, Groen HJ. Traditional versus up-front [18F] fluorodeoxyglucose-positron emission tomography staging of non-small-cell lung cancer: a Dutch cooperative randomized study. *J Clin Oncol* 2006; **24**: 1800-1806

6 **Wiering B**, Krabbe PF, Jager GJ, Oyen WJ, Ruers TJ. The impact of fluor-18-deoxyglucose-positron emission

tomography in the management of colorectal liver metastases. *Cancer* 2005; **104**: 2658-2670
7 **Sperti C**, Pasquali C, Fiore V, Bissoli S, Chierichetti F, Liessi G, Pedrazzoli S. Clinical usefulness of 18-fluorodeoxyglucose positron emission tomography in the management of patients with nonpancreatic periampullary neoplasms. *Am J Surg* 2006; **191**: 743-748
8 **Wakabayashi H**, Akamoto S, Yachida S, Okano K, Izuishi K, Nishiyama Y, Maeta H. Significance of fluorodeoxyglucose PET imaging in the diagnosis of malignancies in patients with biliary stricture. *Eur J Surg Oncol* 2005; **31**: 1175-1179
9 **Anderson CD**, Rice MH, Pinson CW, Chapman WC, Chari RS, Delbeke D. Fluorodeoxyglucose PET imaging in the evaluation of gallbladder carcinoma and cholangiocarcinoma. *J Gastrointest Surg* 2004; **8**: 90-97
10 **Koh T**, Taniguchi H, Yamaguchi A, Kunishima S, Yamagishi H. Differential diagnosis of gallbladder cancer using positron emission tomography with fluorine-18-labeled fluoro-deoxyglucose (FDG-PET). *J Surg Oncol* 2003; **84**: 74-81
11 **Fletcher JW**, Djulbegovic B, Soares HP, Siegel BA, Lowe VJ, Lyman GH, Coleman RE, Wahl R, Paschold JC, Avril N, Einhorn LH, Suh WW, Samson D, Delbeke D, Gorman M, Shields AF. Recommendations on the use of 18F-FDG PET in oncology. *J Nucl Med* 2008; **49**: 480-508
12 **Nishiyama Y**, Yamamoto Y, Fukunaga K, Kimura N, Miki A, Sasakawa Y, Wakabayashi H, Satoh K, Ohkawa M. Dual-time-point 18F-FDG PET for the evaluation of gallbladder carcinoma. *J Nucl Med* 2006; **47**: 633-638
13 **Rodriguez-Fernandez A**, Gomez-Rio M, Medina-Benitez A, Moral JV, Ramos-Font C, Ramia-Angel JM, Llamas-Elvira JM, Ferron-Orihuela JA, Lardelli-Claret P. Application of modern imaging methods in diagnosis of gallbladder cancer. *J Surg Oncol* 2006; **93**: 650-664
14 **Casneuf V**, Delrue L, Kelles A, Van Damme N, Van Huysse J, Berrevoet F, De Vos M, Duyck P, Peeters M. Is combined 18F-fluorodeoxyglucose-positron emission tomography/computed tomography superior to positron emission tomography or computed tomography alone for diagnosis, staging and restaging of pancreatic lesions? *Acta Gastroenterol Belg* 2007; **70**: 331-338

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Events Calendar 2008-2009

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January 24-25, Frankfurt, Germany
Falk Workshop: Perspectives in Liver
Transplantation

International Gastroenterological Congresses 2008
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EASL-AASLD-APASL-ALEH-IASL Conference Hepatitis B and C virus resistance to antiviral therapies
www.easl.ch/hepatitis-conference

February 14-17, Berlin, Germany
8th International Conference on New Trends in Immunosuppression and Immunotherapy
www.kenes.com/immuno

February 28, Lyon, France
3rd Congress of ECCO - the European Crohn's and Colitis Organisation
Inflammatory Bowel Diseases 2008
www.ecco-ibd.eu

February 29, Québec, Canada
Canadian Association of Gastroenterology
E-mail: general@cag-acg.org

March 10-13, Birmingham, UK
British Society of Gastroenterology Annual Meeting
E-mail: BSG@mailbox.ulcc.ac.uk

March 14-15, HangZhou, China
Falk Symposium 163: Chronic Inflammation of Liver and Gut

March 23-26, Seoul, Korea
Asian Pacific Association for the Study of the Liver
18th Conference of APASL: New Horizons in Hepatology
www.apaslseoul2008.org

March 29-April 1, Shanghai, China
Shanghai-Hong Kong International Liver Congress
www.livercongress.org

April 05-09, Monte-Carlo (Grimaldi Forum), Monaco
OESO 9th World Congress, The Gastro-esophageal Reflux Disease: from Reflux to Mucosal Inflammation-Management of Adeno-carcinomas
E-mail: robert.giuli@oeso.org

April 9-12, Los Angeles, USA
SAGES 2008 Annual Meeting - part of Surgical Spring Week
www.sages.org/08program/html/

April 18-22, Buenos Aires, Argentina
9th World Congress of the International Hepato-Pancreato Biliary Association
Association for the Study of the Liver
www.ca-ihpba.com.ar

April 23-27, Milan, Italy
43rd Annual Meeting of the European Association for the Study of the Liver
www.easl.ch

May 2-3, Budapest, Hungary
Falk Symposium 164: Intestinal Disorders

May 18-21, San Diego, California, USA
Digestive Disease Week 2008

May 21-22, California, USA
ASGE Annual Postgraduate Course
Endoscopic Practice 2008: At the Interface of Evidence and Expert Opinion
E-mail: education@asge.org

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www.congrex.com/ngc2008

June 5-8, Sitges (Barcelona), Spain
Semana de las Enfermedades Digestivas
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June 6-8, Prague, Czech Republic
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June 13-14, Amsterdam, Netherlands
Falk Symposium 165: XX International Bile Acid Meeting. B ile Acid Biology and Therapeutic Actions

June 13-14, Prague, Czech Republic
Central and Eastern European Conference on Colorectal "Cancer" Screening, Prevention and Management
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Imedex and ESMO
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www.czech-hepatology.cz/phm2008

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In press

3 **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci USA* 2006; In press

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Both personal authors and an organization as author

5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764]

No author given

6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303]

Volume with supplement

7 **Geraud G**, Spierings EL, Keywood C. Tolerability and safety of frovatriptan with short- and long-term use for treatment

of migraine and in comparison with sumatriptan. *Headache* 2002; **42** Suppl 2: S93-99 [PMID: 12028325]

Issue with no volume

8 **Banit DM**, Kaufer H, Hartford JM. Intraoperative frozen section analysis in revision total joint arthroplasty. *Clin Orthop Relat Res* 2002; (**401**): 230-238 [PMID: 12151900]

No volume or issue

9 Outreach: Bringing HIV-positive individuals into care. *HRSA Careaction* 2002; 1-6 [PMID: 12154804]

Books

Personal author(s)

10 **Sherlock S**, Dooley J. Diseases of the liver and billiary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

Chapter in a book (list all authors)

11 **Lam SK**. Academic investigator's perspectives of medical treatment for peptic ulcer. In: Swabb EA, Azabo S. Ulcer disease: investigation and basis for therapy. New York: Marcel Dekker, 1991: 431-450

Author(s) and editor(s)

12 **Breedlove GK**, Schorfheide AM. Adolescent pregnancy. 2nd ed. Wieczorek RR, editor. White Plains (NY): March of Dimes Education Services, 2001: 20-34

Conference proceedings

13 **Harnden P**, Joffe JK, Jones WG, editors. Germ cell tumours V. Proceedings of the 5th Germ cell tumours Conference; 2001 Sep 13-15; Leeds, UK. New York: Springer, 2002: 30-56

Conference paper

14 **Christensen S**, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer, 2002: 182-191

Electronic journal (list all authors)

15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: http//www.cdc.gov/ncidod/EID/eid.htm

Patent (list all authors)

16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

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