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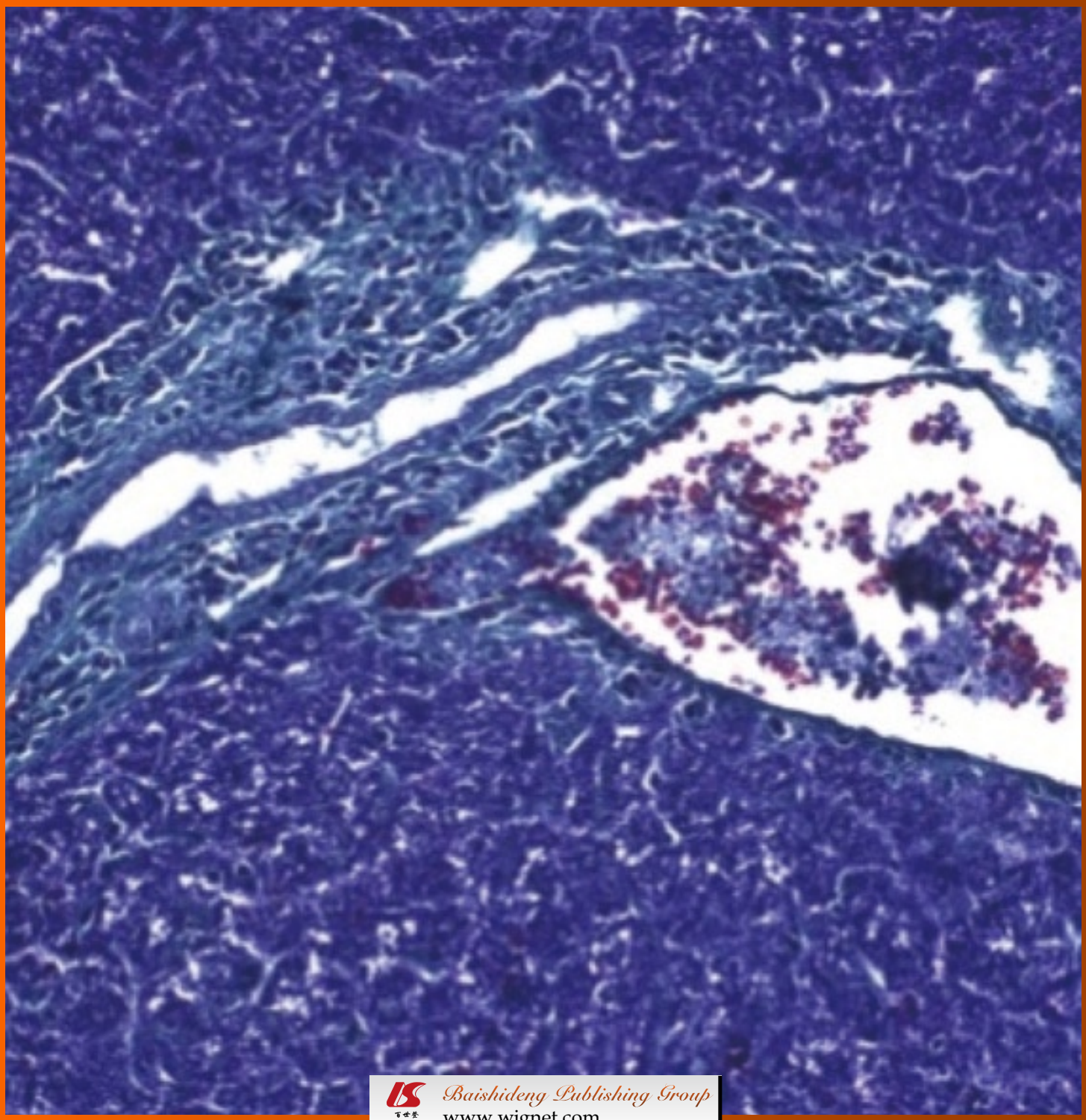

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EDITORIAL

Pathological classification of intrahepatic cholangiocarcinoma based on a new concept

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Abstract

Intrahepatic cholangiocarcinoma (ICC) arises from the lining epithelium and peribiliary glands of the intrahepatic biliary tree and shows variable cholangiocytic differentiation. To date, ICC was largely classified into adenocarcinoma and rare variants. Herein, we propose to subclassify the former, based on recent progress in the study of ICC including the gross classification and hepatic progenitor/stem cells and on the pathological similarities between biliary and pancreatic neoplasms. That is, ICC is classifiable into the conventional (bile duct) type, the bile ductular type, the intraductal neoplasm type and rare variants. The conventional type is further divided into the small duct type (peripheral type) and large bile duct type (perihilar type). The former is a tubular or micropapillary adenocarcinoma while the latter involves the intrahepatic large bile duct. Bile ductular type resembles proliferated bile ductules and shows a replacing growth of the hepatic parenchyma. Hepatic progenitor cell or stem cell phenotypes such as neural cell adhesion molecule expression are frequently expressed in the bile ductular type. Intraductal type includes papillary and tubular neoplasms of the bile duct (IPNBs and ITNBs) and a superficial spreading type. IPNB and ITNB show a spectrum from a preneoplastic borderline lesion to carcinoma and may have pancreatic counterparts. At invasive sites, IPNB is associated with the conventional bile duct ICC and mucinous carcinoma. Biliary mucinous cystic neoplasm with ovarian-like stroma in its wall is different from IPNB, particularly IPNB showing cystic dilatation of the affected ducts. Rare variants of ICC include squamous/adenosquamous cell carcinoma, mucinous/signet ring cell carcinoma, clear cell type, undifferentiated type, neuroendocrine carcinoma and so on. This classification of ICC may open up a new field of research of ICC and contribute to the clinical approach to ICC.

Key words: Intrahepatic cholangiocarcinoma; Adenocarcinoma; Bile duct; Bile ductule; Intraductal neoplasm

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INTRODUCTION

Intrahepatic cholangiocarcinoma (ICC), a primary malig-

nant neoplasm of the liver secondary to hepatocellular carcinoma, arises from intrahepatic biliary epithelia (lining epithelia and peribiliary glands) and shows a variable cholangiocytic differentiation[1,2]. Recently, the incidence of ICC has been increasing worldwide[3,4]. ICC is heterogenous in clinical features, genotypes and biological behaviors depending on anatomical location and histological differentiation. Generally, ICCs are detected and diagnosed clinically at an inoperative stage and a majority of them show a poor prognosis, even after surgical resection[3,4]. However, some patients show a rather favorable post-operative course[5,6].

ICC is usually classified into peripheral and hilar types grossly and histologically into adenocarcinoma and rare variants[7-10]. The former was simply graded into well, moderately and poorly differentiated adenocarcinomas. Recently, a new gross classification of ICC has been proposed by the Japanese Study Group of Liver Cancer[11]. While ICCs are usually adenocarcinomas, they are heterogeneous in their gross and histological features. Recently, much progress has been made in the study of precancerous and early malignant lesions of ICC in addition to molecular and genetic characteristics[12,13]. Furthermore, ICC with hepatic progenitor cell phenotypes has been proposed[2]. Interestingly, pathological similarities of ICC to pancreatic carcinoma have been proposed and biliary and pancreatic neoplasms are now being studied under the same concept and terminology[14,15].

In this review, we propose a new pathological classification of ICC based on recent progress in the study of ICC and on the pathological similarities between biliary and pancreatic neoplasms. First, the anatomy of the intrahepatic biliary tree will be briefly described.

ANATOMY OF THE BILIARY TRACT

The biliary tree is dividable into extrahepatic and intrahepatic bile ducts. The gallbladder drains into the extrahepatic bile duct *via* the cystic duct. The right and left hepatic ducts and their first to third branches are collectively called "hilar and perihilar bile ducts". The intrahepatic bile ducts, proximal to the right or left hepatic duct, are classified as intrahepatic large and small bile ducts[16]. The former are visible grossly and consist of the first to third branches of right or left hepatic bile ducts. Peribiliary glands are physiologically distributed around the large bile ducts and drain into the duct lumen *via* their own conduits. The latter are recognizable microscopically and consist of septal and interlobular bile ducts. The interlobular bile ducts are connected to bile ductules. The septal bile duct is surrounded by a fibrous wall and is over 100 μm in size while the external diameter of the interlobular bile duct is less than 100 μm. These two bile ducts are accompanied by hepatic arterial branches of similar size while bile ductules or canals of Hering are located at the periphery of portal tracts and facing hepatocytes[17].

Table 1 High risk factors and underlining causes of intrahepatic cholangiocarcinoma

Chronic inflammatory biliary diseases
Primary sclerosing cholangitis/Ulcerative colitis
Hepatolithiasis
Liver flukes and other biliary parasite infections
Others
Biliary malformation and developmental disorders
Caroli's disease
Congenital hepatic fibrosis
Biliary-pancreatic maljunction
Simple, solitary or multiple, hepatic cyst
Polycystic liver
Others
Chronic advanced, non-biliary, liver diseases
Chronic hepatitis/cirrhosis related to HCV and HBV infection
Non-alcoholic fatty liver disease
Others
Thorotrast deposition
EB virus infection
Others

HCV: hepatitis C virus; HBV: hepatitis B virus; EB: Epstein-Barr.

BACKGROUND HEPATOBILIARY LESIONS OF ICC

While ICCs usually develop in an apparently normal liver, some are associated with preceding biliary or hepatic diseases and various etiologies (Table 1). ICC may show characteristic features according to the background biliary or hepatic lesions[3,4,18-20]. Chronic inflammation of the bile ducts with sustained stress on biliary epithelial cells is reportedly at least partly responsible for the cholangiocarcinogenesis in which ICC tends to proliferate and spread along the affected intrahepatic bile ducts[3,13,21]. Primary sclerosing cholangitis (PSC) with or without inflammatory bowel disease, usually ulcerative colitis, is a common risk factor for ICC in Western countries. Clinically undetected ICCs or precursor lesions are occasionally encountered in explant livers of these biliary diseases at liver transplantation. Hepatolithiasis, not rare in the Far East, is the primary independent risk factor for ICC and about 7% of patients with hepatolithiasis eventually develop ICC[6]. Stones of most of these cases belong to calcium bilirubinate stones although cholesterol stones also occur. The hepatic lobe or segments containing stones affected by ICC are atrophic in some cases. The stone-containing bile ducts show hyperplasia of the lining epithelium and, not infrequently, premalignant lesions. Liver flukes, especially *O. viverrini* and *C. sinensis*, are risk factors for ICC[13,22]. The presence of parasites in the biliary tree leads to a chronic inflammatory response and cellular proliferation of the bile duct epithelium (adenomatous hyperplasia) with an increased risk of ICC[22,23]. As for biliary malformations and other lesions, ICC may arise with congenital segmental or multiple dilatation of the intrahepatic bile ducts (Caroli's disease) and other biliary malformations such as choledochal cyst, solitary unilocular

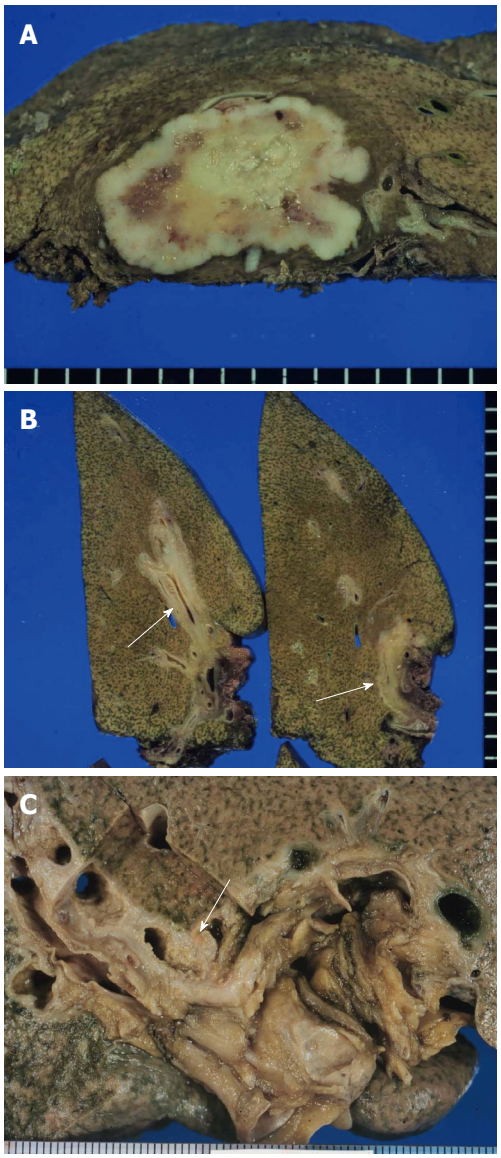

Figure 1 Gross features of intrahepatic cholangiocarcinomas. A: Mass forming type. The carcinoma forms a mass showing compressive growth; B: Periductal infiltrating type. The carcinoma spreads along the biliary tree (arrow); C: Intraductal growth type. The carcinoma shows papillary growth in the dilated intrahepatic bile duct lumen (arrow).

or multiple liver cysts and congenital hepatic fibrosis[1,22,24]. Non-biliary cirrhosis, particularly hepatitis virus-related cirrhosis, is recognized as part of the background of ICC. Hepatitis C virus (HCV) infections may also play a role in the development of ICC. In Japan, patients with cirrhosis due to HCV have about a 1000-fold higher risk of developing ICC than the general population[18]. The development of ICC seems also to be related to hepatitis B virus (HBV) infections in areas where both HBV and ICC are endemic. Such ICCs are usually of a smaller, mass-forming type when clinically detectable. Hepatic progenitor cells (HPCs) or stem cells may be involved in cholangicarcinogenesis in liver cirrhosis.

GROSS FEATURES OF ICC

ICC is grossly classifiable into mass-forming (MF), periductal infiltrating (PI) and intraductal growth (IG) types[1,14]. The MF type presents as a nodular lesion or mass in the hepatic parenchyma and the carcinoma is gray to gray-white, firm and solid (Figure 1A). The PI type shows spreading of the carcinoma along the portal tracts with stricture of the affected bile ducts and dilatation of the peripheral bile ducts (Figure 1B). The IG type presents as a polypoid or papillary tumor within the variably dilated bile duct lumen (Figure 1C) and represents the malignant progression of an intraductal papillary neoplasm of the bile duct (IPNB) (see below). ICC arising in the intrahepatic small bile ducts or bile ductules is usually of the MF type while ICC arising in the intrahepatic large bile ducts (perihilar ICC) can be of the PI, MF or IG type. ICC cases involving the hepatic hilum show cholestasis, biliary fibrosis and cholangitis of the intrahepatic bile ducts. MF type ICCs can be quite large. Central necrosis or scarring is common and mucin may be visible on cut surfaces. These three gross types can overlap in a variable combination. At more advanced stages, ICCs consist of variably sized nodules, usually coalescent.

Table 2 Classification of intrahepatic cholangiocarcinoma

New classification of ICC	Traditional classification of ICC
Conventional type (bile duct type type)	**Adenocarcinoma**
Small bile duct type (peripheral type)	Well differentiated
Well differentiated	Moderately differentiated
Moderately differentiated	Poorly differentiated
Poorly differentiated	
Large bile duct type (perihilar type)	
Well differentiated	
Moderately differentiated	
Poorly differentiated	
Bile ductular type	
Intraductal type	
Papillary type	
Tubular type	
Superficial spreading type	
Rare variants	**Rare variants**
Squamous/adenosquamous cell type	Squamous/adenosquamous cell type
Mucinous/signet ring cell	Mucinous/signet ring cell
Clear cell type	Clear cell type
Undifferentiated type	Undifferentiated type
Lymphoepithelial type	Lymphoepithelial type
Others	Others

ICC: intrahepatic cholangiocarcinoma.

CLASSIFICATION OF ICCs BASED ON GROSS AND HISTOLOGICAL FEATURES

ICCs are pathologically classifiable into conventional ICCs (bile duct ICCs), bile ductular ICCs, intraductal neoplasms and rare variants (Table 2).

Conventional ICCs (bile duct ICCs)

This type is an invasive adenocarcinoma with features of variable sized tubular structures, acini formation and papillary configurations (Figure 2A, B). It is generally a well to

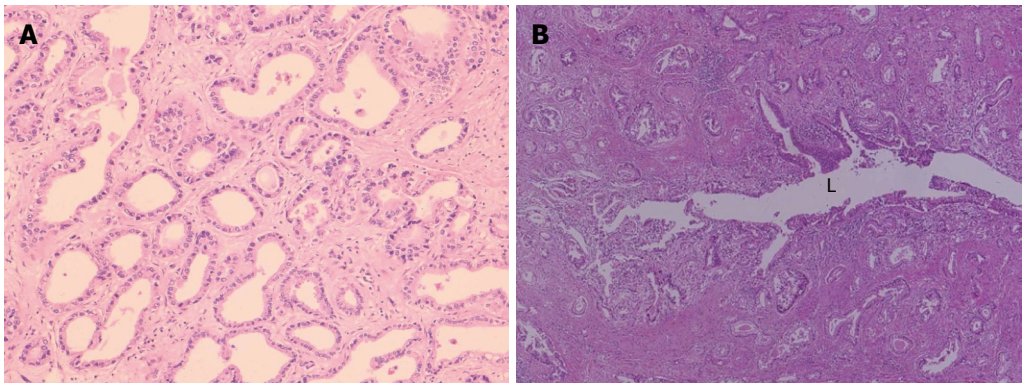

Figure 2 Conventional type (bile duct type) of intrahepatic cholangiocarcinoma. A: Small bile duct type. A well-differentiated tubular adenocarcinoma with a desmoplastic reaction is found; B: Large bile duct type. The carcinoma spreads along the bile duct lumen and infiltrates the bile duct wall. L: bile duct lumen.

moderately differentiated adenocarcinoma composed of columnar to cuboidal epithelial cells with clear or slightly granular, eosinophilic cytoplasm, resembling cholangiocytes (biliary epithelial cells). In addition, poorly differentiated adenocarcinoma is admixed, infrequently and shows solid, cord-like or cribriform growth with variable cellular and nuclear pleomorphism. Coagulative necrosis is not infrequent in the central parts. Mucin production is found in secretions of the lumen, along the luminal sides and in the cytoplasm of carcinoma cells. A prominent desmoplastic reaction is usually found and inflammatory reactions are variable. The adenocarcinoma frequently shows portal venous and lymphatic infiltration. Against the hepatic parenchyma, carcinoma may show a compressive growth although no evident fibrous capsule is formed. There are also frequent bud-like growths of the carcinoma into the surrounding liver and there is direct contact or very local admixture between hepatocytes and carcinoma cells at the interfaces. The intervening hepatic parenchyma containing the portal tracts is forcibly incorporated secondarily between or into the carcinoma tissues. The carcinoma also shows invasion between hepatocytes, appearing to infiltrate the sinusoid. Replacing infiltration of carcinoma is also found variably (see below). Grossly, this ICC is of a MF or MF + PI type or shows multinodular growth in advanced cases.

This ICC is largely dividable into two types according to the level or size of the affected bile ducts: small bile duct and large bile duct types.

Small bile duct type (peripheral type): Grossly, this ICC is of the MF type. Histologically, a variable sized tubular or acinar adenocarcinoma with variable desmoplastic and inflammatory reactions shows a nodular growth and invades the parenchyma with a replacing or compressive pattern (Figure 2A). A small solid cord-like or cribriform pattern is also found in variable combinations. The carcinomatous acini or tubules are usually larger than those of non-neoplastic small bile ducts such as interlobular bile ducts and septal bile ducts.

Large bile duct (perihilar type): Grossly, this ICC belongs to the PI type and PI with MF type. Histologically, the cancerous large bile duct shows luminal spread of carcinoma with papillary, micropapillary and flat configurations along the affected lumen and also variable invasion of carcinoma cells with a tubular, acinar or micropapillary configuration into the duct wall and surrounding parenchyma (Figure 2B). Peribiliary glands and their conduits are also frequently involved. The luminal surface of the cancerous bile duct is not infrequently ulcerated. In the invasive area, particularly in the parenchyma, their histologies are variable and some resemble small bile duct ICCs. This type of ICC might have arisen from the intrahepatic large bile duct (perihilar intrahepatic bile ducts) including the peribiliary glands although some cases might have arisen from the small bile duct ICC with the secondary involvement of intrahepatic large bile ducts (cancerization).

Bile ductular type

Grossly, this carcinoma belongs to the MF type. The adenocarcinoma cells show well-differentiated, small tubular or acinar patterns or cord-like structures with a slit-like lumen and arborization[2,25]. They resemble bile ductules or proliferating reactive bile ductules (Figure 3A, B). A small-cord like pattern with spindle cell features is occasionally predominant. The size of carcinoma cells is usually small in comparison to conventional ICC. Deposition of collagen fiber around or along the carcinoma cells is significant and carcinoma cells are squeezed here and there. Ghost-like features are found, a fibrous or hyalinous configuration reflecting pre-existing hepatic lobules or regenerative nodules is recognizable and fibrotic portal tracts are distributed regularly within the tumor. Portal vein tumor emboli are absent or else rather focal. This type is characterized by widespread replacing growth which is characterized by (1) direct contact between hepatocytes and carcinoma cells; (2) no or minimal compression of the surrounding hepatic parenchyma by the carcinoma cells; and (3) the apparent replacement of hepatocytes by carcinoma cells[2,25].

Neural cell adhesion molecule (NCAM), a marker of HPCs[2], is characteristically detected mainly on the cell membranes and to a lesser degree in the cytoplasm[25]. Normal and mature bile ducts of various sizes in non-tumorous liver are negative for NCAM while reactive

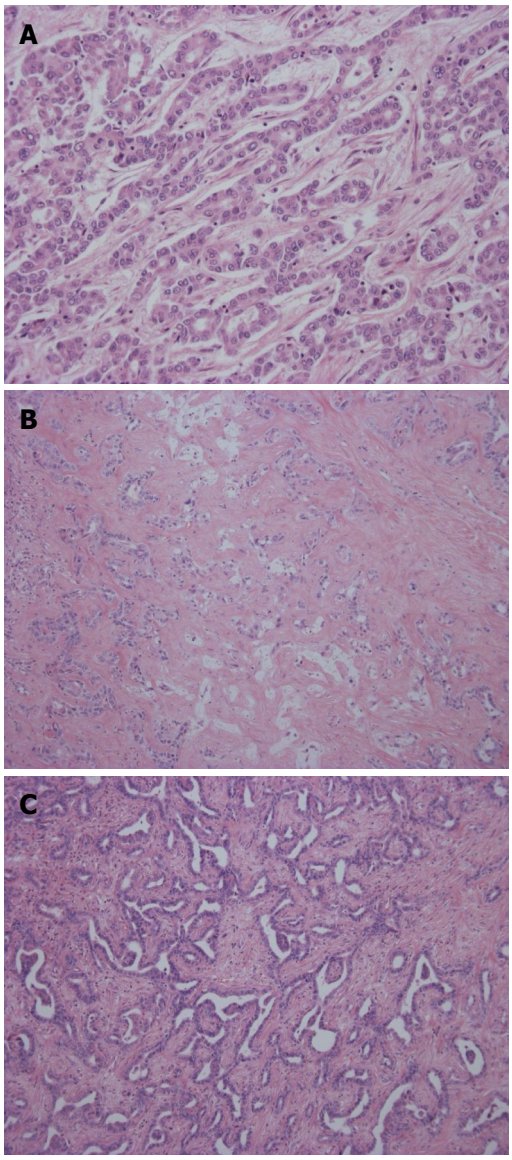

Figure 3 Bile ductular type of intrahepatic cholangiocarcinoma. A: A small ductular carcinoma grows in fibrous stroma; B: The central part of the tumor shows a dropping-out of carcinoma cells with empty spaces; C: Ductal plate malformation type.

proliferated bile ductules in diseased livers are positive for NCAM.

Ductal plate malformation variant: This type mimics ductal plate malformation (DPM) which is found in Caroli' s disease or congenital hepatic fibrosis. That is, the carcinoma shows an irregular configuration and lumen lined by one columnar or cuboidal layer of carcinoma cells (Figure 3C). A central dot or bridge is found microscopically. A fibrous stroma is sometimes found. They look benign although they show infiltrative growth and occasionally venous invasion. These features are not infrequently admixed with the bile ductular type while some cases are exclusively composed of such DPM features.

Intraductal neoplasm of the intrahepatic bile duct

This type is usually seen in the intrahepatic large bile ducts and similar lesions are seen in the hilar and extrahepatic bile ducts.

Intraductal papillary neoplasm of bile duct: This tumor shows a spectrum from preneoplastic lesion to non-invasive and invasive carcinoma, and intraductal papillary neoplasm of bile duct (IPNB) of carcinoma corresponds to the IG type of ICC grossly. In the dilated bile duct it shows papillary growth which is grossly visible[5,9]. Histologically, it is a well-differentiated papillary adenocarcinoma which is invasive or non-invasive while some IPNB can be recognized as a preneoplastic borderline lesion (Figure 4A). Intraductal, intraepithelial spread involving the large bile ducts and even small bile ducts is found variably and constantly. IPNB shows four phenotypes: pancreatobiliary type, oncocytic type, gastric type and intestinal type[14,26]. Clinicopathologically, IPNB is classified into a papillomatosis or papilloma type, intra ductal growing type, mucin-producing type and cystic type[14,26]. The bile duct affected by IPNB shows variable dilatation, not infrequently cystic dilatation. Such cystic type should be differentiated from a hepatobiliary mucinous cystic neoplasm (MCN) in which an ovarian-like stroma is detectable in the wall of the cystic tumor[14,26] and the mucin-producing type shows massive mucin secretion. At the invasion site of IPNB, mucinous carcinoma and more frequently conventional tubular adenocarcinoma are found[5,6].

Intraductal tubular neoplasm of bile duct: This type is occasionally encountered in the intrahepatic large bile duct[27]. The neoplasm is mainly composed of a tubular component and focally papillary. Mucin secretion is usually absent and appears as a cast in the slightly dilated bile duct (Figure 4B, C). Intraductal tubular neoplasm of bile duct (ITNB) also shows a spectrum from preneoplastic lesion to carcinoma, even within the same tumor.

Superficial spreading type: This is a rare type of ICC showing extensive spread along the luminal surface of the intrahepatic bile duct. While the affected bile ducts show a variable luminal dilatation, the grossly visible nodular or tumor lesions are usually not identifiable or conspicuous in the lumen of the affected biliary tree (Figure 4D). Rarely, they show invasion into the surrounding tissue.

Variants

The following variants are only occasionally encountered.

Squamous and adenosquamous cell carcinoma: Both squamous and adenocarcinoma components are mixed, isolated or adjoining in adenosquamous cell carcinoma. Squamous carcinoma is usually well-differentiated and keratinizing. Chronic cholangitis such as hepatolithiasis or liver fluke infection is a common background to these variants and this variant is also reported to occur in polycystic liver.

Mucinous carcinoma/signet ring cell carcinoma: In

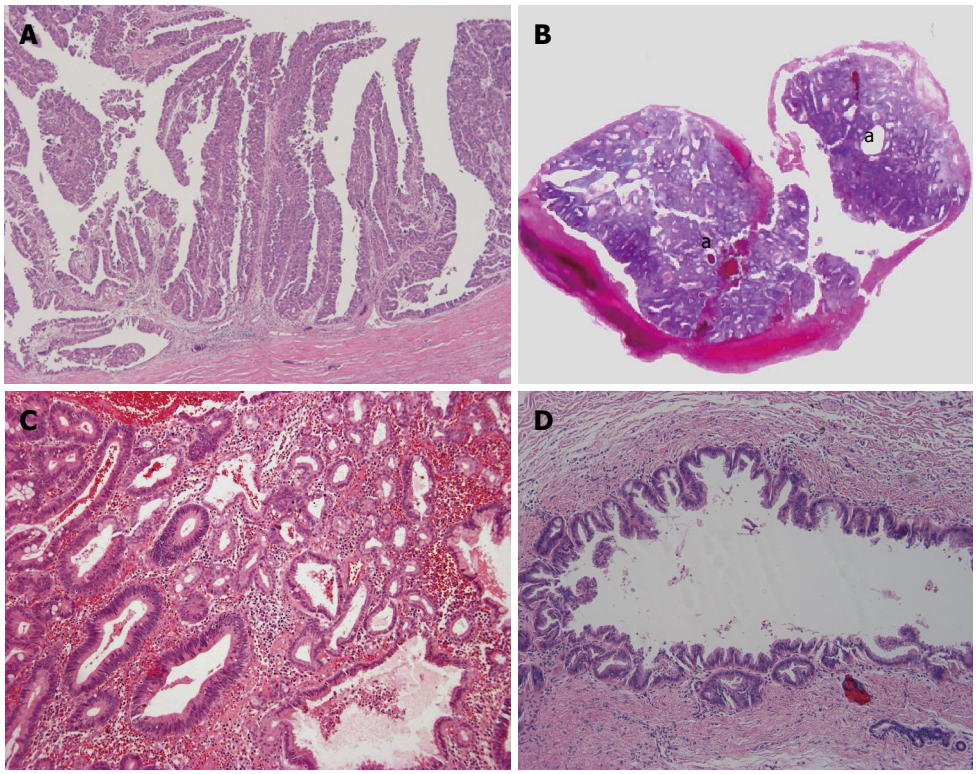

Figure 4 Intraductal type of intrahepatic cholangiocarcinoma. A: Intraductal papillary neoplasm of bile duct. Neoplastic biliary epithelia show papillary growth in the dilated lumen. There is no invasion into the duct wall; B: Intraductal tubular neoplasm of bile duct. The neoplasm (a) appears as a cast in the dilated lumen; C: Intraductal tubular neoplasm of bile duct. The tubular pattern is predominant. Higher magnification of Figure 4B; D: Superficial spreading type. Carcinoma cells show intraductal, intraepithelial growth with a micropapillary configuration and intraglandular involvement. There is no evident invasion into the duct wall.

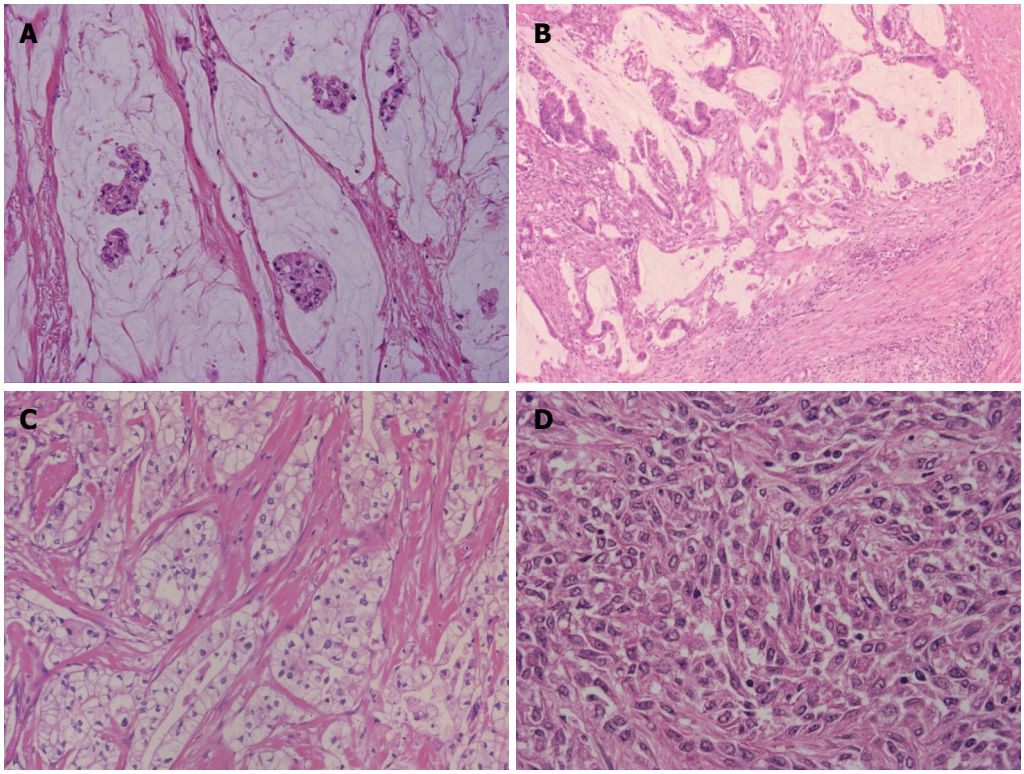

Figure 5 Variants of intrahepatic cholangiocarcinoma. A: Mucinous type and carcinoma cells are floating in a mucinous lake; B: Mucinous type and in the invasive part of the intraductal papillary neoplasm of bile duct, mucinous changes are found; C: Clear cell type and clear cell carcinoma shows a tubular pattern with a desmoplastic reaction; D: Sarcomatous type and spindle cell sarcoma grows medullary.

mucinous carcinoma, carcinoma cells are floating within a mucinous lake. This type is usually found at the invasive parts of IPNB (Figure 5A, B). Signet ring cell carcinoma is occasionally encountered in mucinous carcinoma or conventional ICC but pure signet ring cell carcinoma is extremely rare.

Clear cell carcinoma: This type is occasionally experienced. Tubules or acini composed of columnar epithelial cells with abundant clear cytoplasm and eccentric small nuclei are predominant (Figure 5C). Other histologies of adenocarcinoma such as micropapillary or tubular configuration are focally encountered in this type.

Undifferentiated carcinoma: This carcinoma is heterogeneous in its histology and has several subcategories.

Coagulative necrosis is frequently seen. Lymphatic or portal venous emboli are frequent. (1) Sarcomatous type: Spindle cell sarcoma type is common and spindle shaped sarcomatous cells grows medullary (Figure 5D) and these cells express epithelial phenotypes variably. Adenocarcinoma or cord-like growth is usually admixed focally. When a more mature sarcomatous component such as osteosarcoma or angiosarcoma is found in addition to the adenocarcinoma, carcinosarcoma of the liver or biliary tract is the preferred term; (2) Anaplastic type: Pleomorphic carcinomas with loose cell adhesion are one example and anisocytotic carcinoma cells grow with cord or nest-like patterns. Cohesive carcinoma cell nests without glandular differentiation occur here and there. Giant cells are mingled with the carcinoma. Growth may be medullary and no fibrous stroma is found.

Lymphoepithelioma-like carcinoma: Carcinomas with features of undifferentiated tumors and intense lymphoid stroma are classified as lymphoepithelioma-like carcinomas (LELCs). This type is reported in the liver. Like nasopharyngeal carcinoma, most LELC are strongly linked to Epstein-Barr virus (EBV)[28]. The role of EBV implicated in the cholangiocarcinogenesis is not fully delineated but a strong lymphoplasmacytic response to these neoplasms characterizes most of these lesions. The prognosis of LELC-type ICC seems to be better than that for conventional ICC.

Neuroendocrine type: Most cases reported so far are adenocarcinomas accompanying a neuroendocrine component positive for chromogranin A and/or synaptophysin. The neuroendocrine component is usually a well-differentiated neuroendocrine carcinoma (low grade malignancy) or poorly differentiated neuroendocrine carcinoma (high grade malignancy). Histologically, the adenocarcinoma is usually located at the surface of the tumor and the majority of the stromal invasion involves the neuroendocrine component.

DISCUSSION

ICC has been usually classified grossly into peripheral and hilar types and histologically into well, moderately and poorly differentiated adenocarcinomas and rare variants[7-10]. Herein, we propose a new histopathological classification of ICC based on the recent studies of ICC including the gross classification of ICC and on the pathological similarities between pancreatic and biliary neoplasms[1,5,14]. That is, ICC is largely classified histologically into four categories: a conventional (bile duct) type, a bile ductular type, an intraductal neoplasm type and rare variants.

Conventional ICC is further classified into small and large bile duct types. The former is characterized by a mass forming tumor grossly with or without involvement of the small bile ducts and the latter by evident cancerous large bile duct(s) showing periductal infiltration grossly. However, the differentiation of these two types is occasionally arbitrary, particularly in advanced cases, because the small bile duct type may secondarily involve the large bile duct type (cancerization) appearing as the large bile duct type of ICC and the large bile duct often shows a mass around the cancerous large bile duct at progressive stages.

Bile ductular ICC is proposed based on morphological similarities to proliferating and reactive bile ductules which frequently present with features of HPCs[2,25]. There is extensive replacement of hepatocytes of hepatic lobules or regenerative nodules by infiltrating carcinoma cells. Recent studies of biliary pathology show that HPCs, which exist in bile ductules and/or canals of Hering, can differentiate into hepatocytes and cholangiocytes and are activated in most chronic liver diseases. Interestingly, HPCs are potential targets for carcinogenesis and, eventually, primary liver tumors with HPC features may develop and such neoplasms may correspond to this type of ICC.

Intraductal neoplasm of the bile duct is a new category of intrahepatic biliary neoplasm and is classified into three types: IPNB, ITNB and intraductal superficial spreading type. IPNB is now being accepted as a counterpart of intraductal papillary mucinous neoplasm (IPMN) of the pancreas[5] and IPNB and IPMN share many features such as four types of phenotypes. At present, a majority of IPNB is regarded as the IPMN of main pancreatic duct type. Biliary mucinous cystic neoplasm (biliary MCN) is characterized by an ovarian-like stroma in the wall of the cystic neoplasm[14,26]. This type of neoplasm usually lacks luminal communication with the bile duct lumen. These points are used for the differentiation of this tumor from cystic IPNB. ITNB is only rarely reported and is characterized by a cast-like growth in the dilated duct lumen. ITNB may also correspond to the pancreatic counterpart, intraductal tubular neoplasm of the pancreas (adenoma or carcinoma). Several cases of intraductal, superfical spreading ICC are being reported and a detailed analysis of this type is mandatory.

The biliary tree and pancreas are closely located anatomically and share several physiological functions. Both derive from the foregut at almost the same time and recent studies using animals revealed that they show plasticity to each other during development[14]. Experimental studies using animals suggest that the biliary tract shows some potential for pancreatic differentiation. There are peribiliary glands around the biliary tract in humans and these glands drain into the bile duct lumen. Interestingly, small amounts of pancreatic exocrine acini are intermingled with these glands, raising the possibility that these glands may be abortive pancreatic exocrine acini which are prevented from differentiation into fully exocrine pancreatic acini in humans.

In this context, the biliary pathology is being considered given the similarities between the biliary tract and pancreas[14]. IgG4-related sclerosing cholangitis and autoimmune pancreatitis is one example[29,30]. Mucinous cystic neoplasm is also reported to develop in the pancreas and along the hepatobiliary system. In addition, advanced cho-

langiocarcinoma and preneoplastic or early intraepithelial neoplasms of the biliary tract show similar morphological or genetical changes to their pancreatic counterparts[6,14]. That is, invasive duct carcinoma of the pancreas and conventional ICC of large bile duct type share many biological and clinical features. In addition, similar intraepithelial neoplasms are also reported in the biliary tract and pancreas: biliary intraepithelial neoplasm (BilIN) and pancreatic intraepithelial neoplasm (PanIN)[15,21,31]. BilIN and PanIN are followed by conventional invasive duct adenocarcinoma. In this context, conventional ICC of large bile duct type can be regarded as a "bilio-pancreatic duct adenocarcinoma" along with extrahepatic and hilar cholangiocarcinomas as well as invasive ductal adenocarcinomas of the pancreas.

CONCLUSION

Based on recent progress in ICC pathology, including the gross classification of ICC and the similarities between biliary and pancreatic neoplasms, ICC was pathologically classified into a conventional (bile duct) type, a bile ductular type, an intraductal type and rare variants. The conventional type was further divided into small bile duct type (peripheral type) and large bile duct type (perihilar type). This new classification of ICC proposed here needs extensive discussions at an international consensus meeting and clinical and practical applications, especially a correlational study with TNM staging and prognostic study. This classification may also lead to a novel approach for research of ICC.

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REVIEW

Spontaneous rupture of hepatic hemangiomas: A review of the literature

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Abstract

Hepatic hemangiomas are congenital vascular malformations, considered the most common benign mesenchymal hepatic tumors, composed of masses of blood vessels that are atypical or irregular in arrangement and size. Hepatic hemangiomas can be divided into two major groups: capillary hemangiomas and cavernous hemangiomas These tumors most frequently affect females (80%) and adults in their fourth and fifth decades of life. Most cases are asymptomatic although a few patients may present with a wide variety of clinical symptoms, with spontaneous or traumatic rupture being the most severe complication. In cases of spontaneous rupture, clinical manifestations consist of sudden abdominal pain, and anemia secondary to a haemoperitoneum. Disseminated intravascular coagulopathy can also occur. Haemodynamic instability and signs of hypovolemic shock appear in about one third of cases. As the size of the hemangioma increases, so does the chance of rupture. Imaging studies used in the diagnosis of hepatic hemangiomas include ultrasonography, dynamic contrast-enchanced computed tomography scanning, magnetic resonance imaging, hepatic arteriography, digital subtraction angiography, and nuclear medicine studies. In most cases hepatic hemangiomas are asymptomatic and should be followed up by means of periodic radiological examination. Sur gery should be restricted to specific situations. Absolute indications for surgery are spontaneous or trau matic rupture with hemoperitoneum, intratumoral blee ding and consumptive coagulopathy (Kassabach-Merrit syndrome). In a patient presenting with acute abdo minal pain due to unknown abdominal disease, sponta neous rupture of a hepatic tumor such as a hemangio ma should be considered as a rare differential diagnosis.

Key words: Hepatic hemangioma; Giant hepatic hemangioma; Liver tumor; Spontaneous rupture; Surgery

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INTRODUCTION

Hepatic hemangiomas are congenital vascular malformations, considered the most common benign mesenchymal hepatic tumors, composed of masses of blood vessels that are atypical or irregular in arrangement and size[1]. Malignant transformation is extremely rare. They are often diagnosed as incidental findings on imaging studies of the abdomen during exploratory surgeries[2]. It is estimated that about 20% of the general population present hepatic hemangiomas, and the prevalence in autopsy studies ranges between 0.4%-7.4%[3-5]. These tumors most frequently affect females (80%) and adults in their fourth and fifth decades of life[1,6,7].

The hepatic hemangiomas are often solitary although multiple lesions may be present in both hepatic lobes in up to 40% of the patients. Their size varies from a few millimeters to over 20 cm. Those lesions larger than 5 cm are reported as giant hemangiomas. Most cases are asymptomatic (especially when smaller than 4 cm), but a few patients may present a wide variety of clinical symptoms with spontaneous or traumatic rupture being the most severe complication. This has a catastrophic outcome if not promptly managed[3], and is the reason why correct diagnosis and management are extremely important[5]. A study by Jain *et al* indicated that the operative mortality rate of ruptured lesions is around 36.5%[1].

The first case of spontaneous rupture of a hepatic hemangioma was described by Van Haefen in an autopsy in 1898[8]. In 1961, Swell and Weiss reviewed 12 cases of spontaneous rupture of hemangiomas from literature and reported the mortality rate to be as high as 75%[3].

Bleeding of spontaneous rupture is a severe complication in liver diseases, as its clinical signs are not usually specific. The risk of rupture is generally considered to be one reason for performing surgical resection of the hemangioma[9].

CLASSIFICATION

Hepatic hemangiomas are classified as primary benign vascular tumors of the liver, and can be divided into two major groups: (1) capillary hemangiomas, generally peripheral, small, and sometimes multiple; and (2) cavernous hemangiomas, which are rarer and larger, also known as giant hemangiomas when larger than 4-5 cm. Occasionally these can reach up to 20-30 cm[7], as seen in Figures 1 and 2.

EPIDEMIOLOGY

According to the case report and literature review by Vokaer *et al*[8], since 1898 when Van Haefen described the first case of spontaneous rupture of a liver hemangioma, only 33 cases of ruptured hemangioma in adults have been reported in the literature. Spontaneous rupture of this tumor is an uncommon complication, representing 1%-4% in Jain's case series (spontaneous rupture with hemoperitoneum)[3] and 2.9% in the study of Chen *et al*[5] (a study of 70 cases admitted from 1992-2001 with spontaneous liver rupture including primary liver cancer, cirrhosis, liver adenoma, secondary liver cancer and liver hemangioma as causes of rupture). Treatment methods for spontaneous liver rupture in these 70 patients were suture in 17 (24.3%), packing in 7 (10%), ligation of hepatic artery in 23 (32.9%), hepatic artery chemoembolization in 2 (2.9%) and hepatic partial resection in 40 (57.1%)[5]. Jain *et al* described a mortality rate ranging from 60% to 75% for spontaneous rupture with an operative mortality rate from this complication of 36.4%[3].

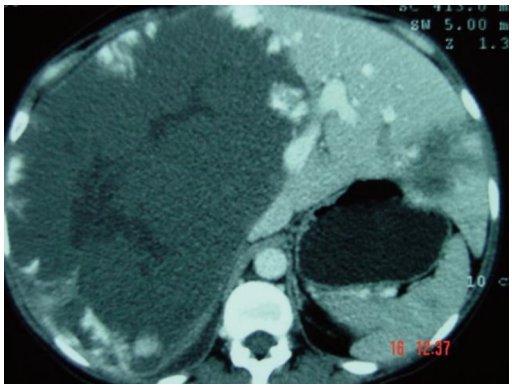

Figure 1 Computed tomography scan of a huge liver cavernous hemangioma compromising the right liver lobe.

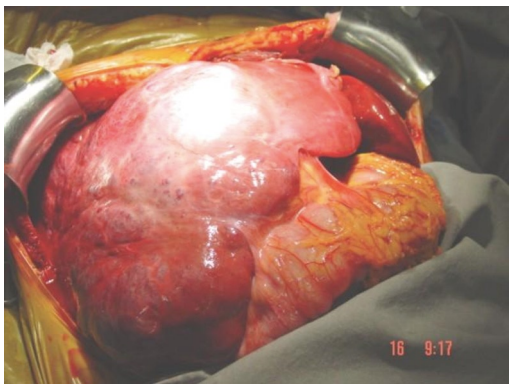

Figure 2 Intraoperative finding of a huge liver cavernous hemangioma compromising the right liver lobe.

Yamamoto *et al* researched 28 cases of spontaneous rupture of hepatic hemangiomas (19 adults and 9 children), and reported that surgical treatment was carried out on 20 patients, of whom only 5 survived. The ruptured tumors ranged in size from 3 to 25 cm, many of them located on the surface of the liver[10].

Corigliano *et al* reviewed 27 of 32 cases reported in the literature up to 2003, and indicated that 16 (84.2%) of 19 tumors were giant hemangiomas (range 6-25). Twenty-two (95.7%) underwent surgery (13 resections, 5 sutures, 4 tamponade). Three (23%) of 13 resected patients had died. Among the sutured patients, 2 died (40%) as well as 3 (75%) of the 4 patients who underwent tamponade. The mortality rate of all surgery patients researched by Corigliano *et al*[11] was 36.4% (8/22), as noted by Jain *et al* in their literature review.

ETIOLOGY

Some authors believe that hepatic hemangiomas are congenital hamartomatous lesions of the liver that grow silently over the years[12]. No genetic or familial mode of inheritance has been clearly described, although Moser *et al* reported on a large family of Italian origin in which 3 female patients in 3 successive generations had large symptomatic hepatic hemangiomas[13].

Several pharmacologic agents have been postulated to cause tumor growth. Steroid therapy, estrogen therapy and pregnancy can increase the size of an already existing hemangioma. Experimental studies have revealed that estrogens augment endothelial cell proliferation, migration and organization into capillary-like structures. *In vitro*, they promote the proliferation of the endothelial cells of the hemangioma and also work synergistically with vascular endothelial growth factor[14]. In another study, Xiao described that hemangiomas have estrogen receptors, an indication that these tumors may be a target tissue for estrogens[15].

Spitzer *et al* studied prospectively 94 women with hepatic hemangiomas over a period of 7.3 years and noticed an increase in the size of the lesions in women who received hormonal therapy -23% *vs* 10% in control subjects[16]. Although several possibilities have been described, the exact etiology still remains unknown.

CLINICAL MANIFESTATIONS

Hepatic hemangiomas are mainly asymptomatic, although they can induce intermittent right upper quadrant pain related to focal necrosis or pain from capsular distension as the tumor grows. Thrombosis, infarction, hemorrhage into the lesion and compression of adjacent structures are other possible causes of pain. Giant hemangiomas can also cause biliary colic, obstructive jaundice, and gastric outlet obstruction[4].

Spontaneous hepatic hemorrhage is an uncommon condition, and in the absence of anticoagulant therapy or trauma, it frequently occurs as a consequence of hepatocellular carcinoma, hepatic adenoma or, in a few cases, of spontaneous rupture of a cavernous hemangioma[17].

In cases where spontaneous rupture occurs, clinical manifestations consist of sudden abdominal pain, and anemia secondary to a hemoperitoneum. Disseminated intravascular coagulopathy can also occur[6,7]. Hemodynamic instability and signs of hypovolemic shock appear in about one third of cases[8]. The global mortality of rupture is approximately 35%, and it seems to be related to the size of the lesions. As the size of the hemangioma increases, so does the chance of rupture[8,9], especially if the tumor is located on the surface of the liver and shows extrahepatic growth. If the patient receives steroid therapy for a coexisting disorder, the chance of rupture is even higher[9].

The clinical presentation of liver hemangioma in pregnancy does not differ from the same mass in a non-pregnant woman. Laboratory studies usually show some an elevation of transaminases, bilirubin and alkaline phosphatase even in asymptomatic cases. The rupture of a small hemangioma may lead to serious intra-abdominal hemorrhage. In fact, liver hemangiomas during pregnancy are potentially serious lesions, especially as their rupture during labor can precipitate an hemorrhagic shock[6].

DIAGNOSTIC TOOLS

Imaging studies used in the diagnosis of hepatic hemangiomas include ultrasonography, dynamic contrast-enhanced computed tomography (CT) scanning, magnetic resonance imaging (MRI), hepatic arteriography, digital subtraction angiography, and nuclear medicine studies[1].

Ultrasonography is commonly used as an initial diagnostic tool as it is widely available and inexpensive. Hemangiomas are seen as sharp edged hyperechoic lesions with clear borders (when small, they are strongly hyperechoic) although in cases with hemorrhage, fibrosis and necrosis, their appearance may be different[1,16,18]. The addition of color Doppler provides qualitative and quantitative data and increases the sensitivity and specificity of the test. In general, the finding on ultrasonography of a suspected hemangioma should be diagnostically integrated with CT scan or MRI to ensure a correct diagnosis.

Dynamic contrast-enhanced CT scanning, especially triple phase CT with delayed imaging, is preferred (Figure 1). In this exam, hemangiomas are typically hypodense on precontrast imaging; in the arterial phase there may be enhancement of the peripheral portions of the lesion while the center of the lesion remains hypodense[1,17]. In cases which may can be diagnosed conclusively by US and CT, MRI may provide more specific diagnostic features (Figures 3A and B), with a sensitivity upwards of 90%[1]. The lesions have markedly high signal intensity on T2 weighted images and a specific dynamic contrast enhancement pattern (in a fashion similar to that seen on CT)[17]. T1-weighted images have low signal density[19,20].

Giant cavernous hemangiomas may exhibit internal fluid levels on MRI and CT scan, because of the slow blood flow through the tumor that allows separation of the blood cells.

When hemangiomas are ruptured, radiological findings reveal hemoperitoneum and heterogeneous hepatic mass. Intraperitoneal clots may also be identified near to the site of the bleeding[17].

Nuclear medicine studies include single-photon emission computerized tomography (SPECT) using Tc-99m pertechnetate-labeled RBCs. SPECT is more specific than MRI, but less sensitive. Some authors consider SPECT to be the standard diagnostic tool. However, the test may miss some lesions and it is unfortunately not available at all medical centers[21,22].

Arteriography is an invasive modality that can be useful to diagnose some hepatic hemangiomas that are characterized by early opacification or irregular areas or lakes with persistence of contrast long after arterial emptying. The hemangioma may appear as a C-shaped lesion with an avascular center[1].

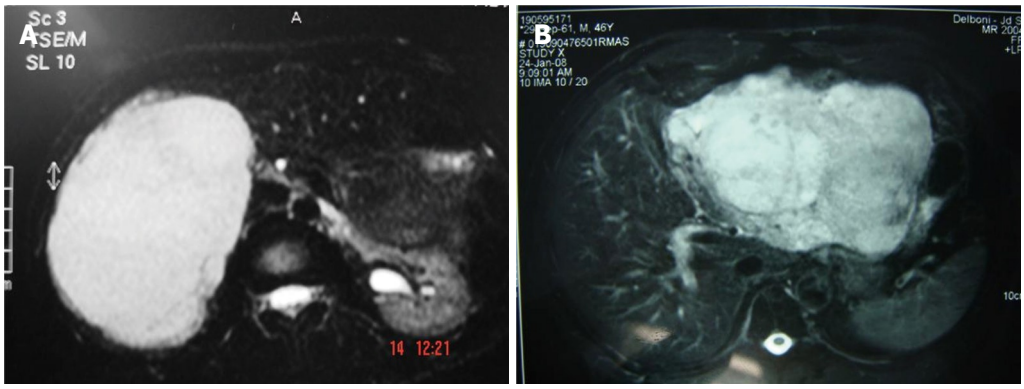

Figure 3 Magnetic resonance imaging of hemangioma. A: Right lobe; B: Left lobe.

In a retrospective study of 27 patients with 35 hemangiomas De Franco *et al* compared the diagnostic capabilities of ultrasonography, Doppler color ultrasonography, dynamic CT scanning and MRI. The results were as follows: ultrasonography -46% sensitivity; combined color Doppler ultrasonography and B-mode -60% sensitivity; T2-weighted MRI -96% sensitivity; gadolinium enchanced MRI with dynamic CT scanning -100% sensitivity[23].

Diagnostic accuracy diminishes in all imaging modalities when lesions are smaller than 2 cm in diameter. In these cases, MRI and 99mTc-RBC SPECT are the most accurate radiological methods for establishing a diagnosis.

In some cases the hemangioma may be a differential diagnosis of a hepatic mass. Liver biopsy is contraindicated in most cases because of an increased risk of hem orrhage. In cases where a small liver lesion must be differentiated from hepatocellular carcinoma, either percutaneous or laparoscopic liver biopsy may be reasonable. However, over the years, hepatologists and surgeons have been increasingly resistant to biopsy in the vast majority of cases. Biopsy should be used only when radiologic studies and alpha fetoprotein testing are inconclusive.

TREATMENT

The treatment of hepatic hemangioma should be decided based on the size and location of the tumor[9]. Small hemangiomas (< 4 cm) can be managed by observation and as, in most cases, hepatic hemangiomas are asymptomatic they should be followed up by means of periodic radiological examination. Surgery should be restricted to specific situations.

Absolute indications for surgery are spontaneous or traumatic rupture with hemoperitoneum, intratumoral bleeding and consumptive coagulopathy (Kassabach-Merrit syndrome). Rupture of a hemangioma with hemoperitoneum is a dreadful situation and often fatal if not promptly managed. Persistent abdominal pain, obstructive jaundice, portal hypertension, superficial location of tumors larger than five cm with a risk of trauma, pain and uncertain diagnosis are all relative surgical indications[3,4,7]. The proposed surgical procedures for the treatment of liver hemangioma are as follows: (1) anatomic, nonanatomic resection, enucleation (the procedure of choice to treat giant hemangiomas, especially in superficial lesions but should be discouraged for intrahepatic lesions because of large scale bleeding. This procedure has a major advantage when compared to hepatectomy, the greater preservation of the parenchyma), ligation of the hepatic artery (in cases where it is not possible to remove the tumor, but its benefit is suspicious); (2) selective portal vein embolization (reduces the size of the lesion when the tumor is too big to be removed,); and (3) liver transplantation.

Spontaneous rupture of a liver hemangioma is challenging because it is considered a life threatening situation. Conservative treatment runs the risk of hypovolemic shock, and aggressive surgical treatment is associated with a high mortality risk. Surgical haemostatic methods such as packing, hepatic artery ligation and hepatic suture may be helpful to contain the bleeding in cases of ruptured hemangioma[8].

Surgical resection and enucleation are considered the treatments of choice. The size and location of a lesion are decisive when the surgeon has to determine whether to perform either a formal segmental resection or an enucleation. Both procedures are typically performed by an open approach although laparoscopy can be both safe and well tolerated in some cases. Lesions of massive or diffuse nature, proximity to vascular structures and pre-existing comorbidities are limiting factors to surgical resection. In the absence of tumor promoting factors such as estrogen therapy, hemangiomas rarely recur after resection[1].

Recent studies have emphasized the use of transcatheter hepatic arterial embolization (TAE) in the effective treatment of larger symptomatic hemangiomas, for those at risk of bleeding and before exploratory laparotomy to treat patients with a hemorrhagic hemangioma. It can significantly improve outcome in such patients[16]. TAE as an alternative to surgery is still controversial because of the risk of ischemia, infection, abscess or intra abdominal bleeding[8].

The successful use of TAE before surgery of a ruptured hemangioma was first reported by Yamamoto *et al* in 1991[3]. A significant improvement in coagulative factors and a decrease in intraoperative blood loss was observed by Suzuki *et al* in patients with consumptive coagulopathy related to intravascular coagulation who were treated with preoperative TAE[3].

Radiofrequency ablation (open or laparoscopic) has been successfully used to improve abdominal pain in sym-

ptomatic hemangiomas. Other procedures such as radiotherapy should be reserved for poor candidates for surgery. It can produce regression of the hemangioma with minimal morbidity.

Orthotopic liver transplantation is occasionally offered to specific patients, including those with symptomatic and large or diffuse lesions.

CONCLUSION

Hemangiomas are common benign tumors of the liver, generally detected accidentally during a radiological screening performed for other reasons. In symptomatic cases, surgical treatment should be preferred[22]. Emergent hepatic resection has been the treatment of choice, but has high operative mortality. Preoperative transcatheter arterial embolization (TAE) can significantly improve outcomes in such cases[3].

Spontaneous rupture in hemangiomas is not usual, but can be dramatic and very dangerous. Patients can die of massive hemorrhage in a short space of time and, in this situation, the patient is usually too weak to endure an operation[5] Ligation of hepatic artery or packing should be performed to control bleeding as soon as possible while hemodynamic stabilization is accomplished. If the hemorrhage is stopped and the patient's condition is stable, a secondary operation to cure the hemangioma should be performed[4,8]. In a patient presenting with acute abdominal pain due to unknown abdominal disease, spontaneous rupture of a hepatic tumor such as hemangioma should be considered as a rare differential diagnosis[3].

According to the current literature, we conclude that the rate of spontaneous rupture of hepatic hemangiomas ranges from 1% to 4%, occurring mostly in giant hemangiomas (6-25 cm), with a mortality rate that can reach up to 75%. The operative mortality rate of this complication is around 36.4%[3,5,9,22,23]. So far, only 33 cases of spontaneous rupture of hepatic hemangiomas have been reported and published.

The right hepatic lobe, especially its posterior segment, is the most common site of appearance of these lesions. They are often subcapsular, well circumscribed and unencapsulated. Structurally, hemangiomas are composed of venous lakes, coated with endothelial tissue plus clots and calcification, separated by a connective tissue septa, where the blood circulates slowly. The growth of these tumors occurs by vascular ectasia, and never by hyperplasia or hypertrophy.

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ORIGINAL ARTICLE

Does granulocyte-colony stimulating factor administration induce damage or repair response in schistosomiasis?

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Abstract

AIM: To introduce Granulocyte-colony stimulating factor (G-CSF) as a new therapeutic modality for schistosomiasis through stem cell mobilization, immunomodulation or fibrosis remodeling.

METHODS: In this study, a 5 d course of human recombinant G-CSF (100 μg/kg sc) was applied to Schistosoma mansoni-infected mice at different stages of disease (5 d before infection as well as 3, 5 and 7 wk post-infection). The animals were sacrificed at 10 d as well as 4, 6 and 8 wk post infection. Mice were examined for: (1) Total leukocyte count which is an accepted surrogate marker for the stem cell mobilization into the circulation; (2) Egg count in intestine and liver tissue to assess the parasitic load; and (3) Histopathological changes in Hx/E and Masson trichrome stained sections as well as collagen content in Sirius red-stained liver sections to determine the severity of liver fibrosis.

RESULTS: Mice developed leukocytosis. The egg load and the number of granulomas were not affected by the G-CSF treatment but there was an obvious change in the composition of granulomas towards an increased cellularity. Moreover, fibrosis was significantly decreased in treated groups compared to untreated animals (collagen content either preinfection or at 3 and 5 wk post infection: 5.8 ± 0.5, 4.7 ± 0.5, 4.0 ± 0.7 *vs* 8.2 ± 0.9; $P \leqslant 0.01$).

CONCLUSION: Although G-CSF did not cause direct elimination of the parasite, it enhanced granulomatous reaction and reduced the fibrosis. Further investigation of the underlying mechanisms of these two actions is warranted.

Key words: Schistosomiasis; Granulocyte-colony stimulating factor; Periovular granuloma; Fibrosis; Immunomodulation; Stem cell mobilization

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INTRODUCTION

Schistosomiasis is a parasitic disease that mainly affects the rural population in developing countries. An estimated 200 million people suffer from this disease, 85% of whom live in sub-Saharan Africa, and 650 million people are at risk of infection[1].

Schistosoma mansoni (*S. mansoni*) is a leading cause of chronic liver disease. Morbidity from this infection is primarily the result of a granulomatous reaction to eggs deposited in the liver leading to the induction of fibrosis and portal hypertension[2]. The immuno-pathology of the granulomatous response has been studied extensively in murine models as there is a close similarity to that in human schistosomiasis[3]. Granulocyte-colony stimulating factor (G-CSF) is a cytokine that shows a variety of biological functions. Its action ranges from mobilization of bone marrow-derived stem cells to immuno-modulation of a variety of host responses and even anti-infectious properties. It is in clinical use for the mobilization of bone marrow-derived stem cells before peripheral stem cells are harvested and stem cell transplantation is performed[4]. A new strategy employing bone marrow-derived stem cells has recently been attempted for organ repair. Stem cell mobilization with G-CSF had a dramatic effect on the enhancement of stem cell "transdifferentiation" into cardiomyocytes in a rat model of myocardial infarction[5]. Petersen *et al*[6] first reported "transdifferentiation" of hemato poietic stem cells into liver cells. The frequency of transdifferentiation and thereby its biological relevance has been a controversial discussion[7]. Granulocyte-colony stimulating factor can also modulate the immune response by affecting T cells and their cytokine secretion. Some authors[8-10] have described polarization of T cell response towards T helper 2 cell type by G-CSF. Another study[11] documented inhibition of both Th1 and Th2 with reduced INF-γ and IL-4 production by G-CSF. Moreover, it was found that G-CSF is in clinical use to battle cytomegalovirus (CMV) infection in immuno-suppressed patients. Noursadeghi *et al*[12] reported the effect of G-CSF on the non-specific acute phase response associated with increased resistance to bacterial infection. Schneider *et al*[13] evaluated the effect of G-CSF in 60 patients undergoing major surgery and observed an increase in anti-infectious mediators, reduction in the incidence and severity of postoperative infections plus attenuation of the postoperative acute phase response. It was demonstrated that G-CSF upregulates lipopolysaccharide binding protein (LBP) in the liver, thus potentially contributing to increased resistance against lipopolysaccharide (LPS)[14]. This result hints at the potential influence of G-CSF on the innate immune system.

The aim of this study is to employ an interesting drug candidate, G-CSF, as a new therapeutic approach that may potentially influence the damage response and consequently the course of liver disease caused by *S. mansoni* infestation.

MATERIALS AND METHODS

Materials

Experimental animals and experimental design: CD1 mice, aged 6-7 wk and weighing 15-18 g, were used in this study. Mice were bred at the Schistosome Biological Supply Program (SBSP) (Theodor Bilharz Research Institute, Giza, Egypt). They were fed on a standard pelleted diet (containing 24% protein, 4% fat and about 4%-5% fiber and water ad libitum) according to a recipe prepared by Lowell University, USA. The study met the national guidelines of experimental animal research.

CD1 mice (6-8/subgroup) were infected with cercariae of *S. mansoni* and treated with G-CSF. In the first group, G-CSF treatment (100 μg/kg) started 5 d prior to infection and continued every other day for 10 d post infection (PI). In other groups, G-CSF treatment started 3, 5, and 7 wk post infection (wpi). Animals were sacrificed on day 10 and week 4, 6 and 8 post infections (Figure 1).

Parasites: *S. mansoni* cercariae were obtained from infected Biomphalaria alexandrina snails which were reared and maintained at the SBSP. The original strain of *S. mansoni* was obtained from Lowell University, Lowell, Massachusetts, USA. by passage throughout bred mice and B. alexandrina snails.

Estimating cercarial densities: The cercarial suspension was mixed with a magnetic stirring bar and ten times of 0.1 mL aliquots were removed from the center of the suspension by a syringe and placed into a petri dish. Before counting under a dissecting microscope, one drop of Lugol's iodine solution was added. The counts were averaged and the cercarial density was determined[15].

Infection: About 70-80 *S. mansoni* cercariae suspended in 0.2 mL solution was injected subcutaneously into the back of each mouse using a 22 gauge needle syringe[16].

Methods

Mice were examined for general condition, body weight, liver weight at time of sacrifice and liver/body weight ratio.

Confirmation of G-CSF effect was based on: (1) Relative amount of mobilized leukocytes. Total leukocytic count was performed using an automated cell analyzer (Sysmex Corporation, KX_21, Japan) and differential counts were made from the slide-mounted blood films stained with Leishman stain; (2) Egg count in intestine

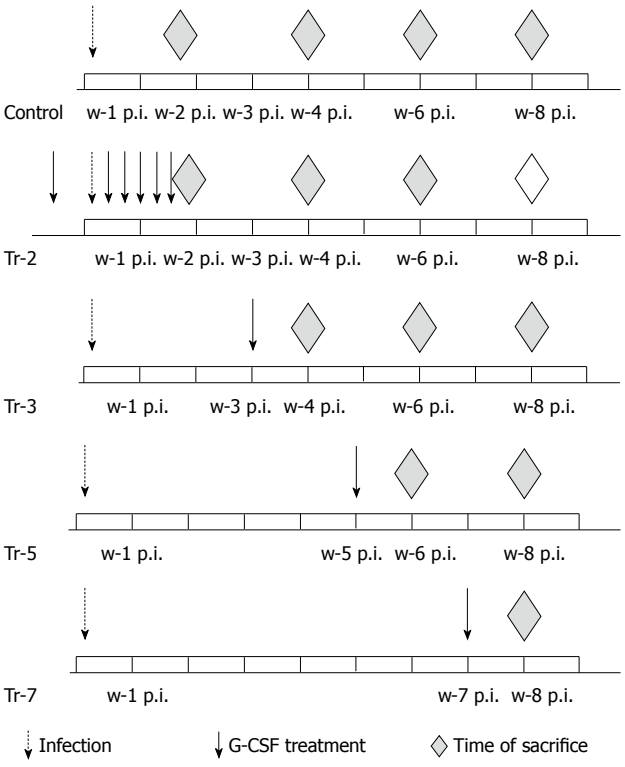

Figure 1 Experimental design. w: week; p.i.: post infection.

and liver[17]. The number of ova/gm intestinal or hepatic tissue was counted after digestion overnight in 5% KOH; and (3) Assessment of disease severity by detailed liver histology including assessment of liver fibrosis.

Histopathology: Following sacrifice of animals by cervical dislocation, the liver was removed, rinsed with phosphate buffered saline and weighed. Liver specimens from the right lobe were processed and stained with hematoxylin and eosin and Masson trichrome stains. Sections were examined for hepatic parenchyma, periovular granulomas as well as inflammatory cell exudation and fibrous tissue deposition in portal tracts together with bile ductular and blood vascular changes. The diameter of granulomas was measured (5 granulomas/section) using the ocular micrometer. This was done only for granulomas containing ova in their centers and not confluent ones. The mean diameter for each group was calculated. Evaluation of liver fibrosis was done by morphometry using 10 μm thick, Sirius Red-stained sections. This is a strong anionic dye that stains collagen by reacting, *via* its sulphonic acid groups, with basic groups present in the collagen molecule. The elongated dye molecules are attached to the collagen fiber in such a way that their long axes are parallel[18]. Morphometric assessment of the collagen content in portal tracts and around granulomas was performed using a Zeiss microscope connected to a Kontron Image Analysis System. Quantification of hepatic collagen deposition was done under x50 magnification employing the CIRES software program and represented as mean percentage of fibrotic area ± SD in 3 sections/animal.

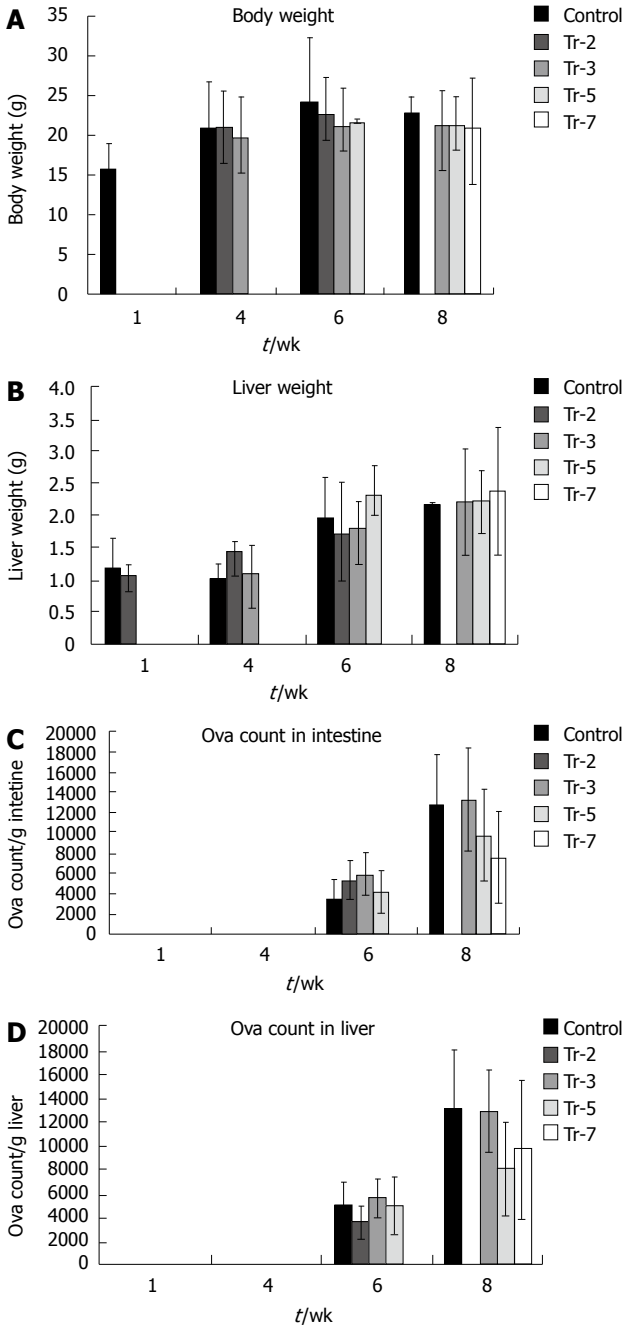

Figure 2 Effect of treatment on. A: body weight; B: liver weight; C: egg load in intestine; and D: egg load in liver.

Statistical analysis

Statistical analysis was performed using the analysis of variance method (ANOVA)[19]. Significant level was reached at $P \leqslant 0.05$.

RESULTS

All groups (treated and untreated) had a good health status throughout the experimental period and apparent manifestations of schistosomiasis did not develop.

Body weight (Figure 2A) was not affected significantly by G-CSF treatment before infection and 3 wpi in animals sacrificed 4 wpi with an average body weight of 20.95

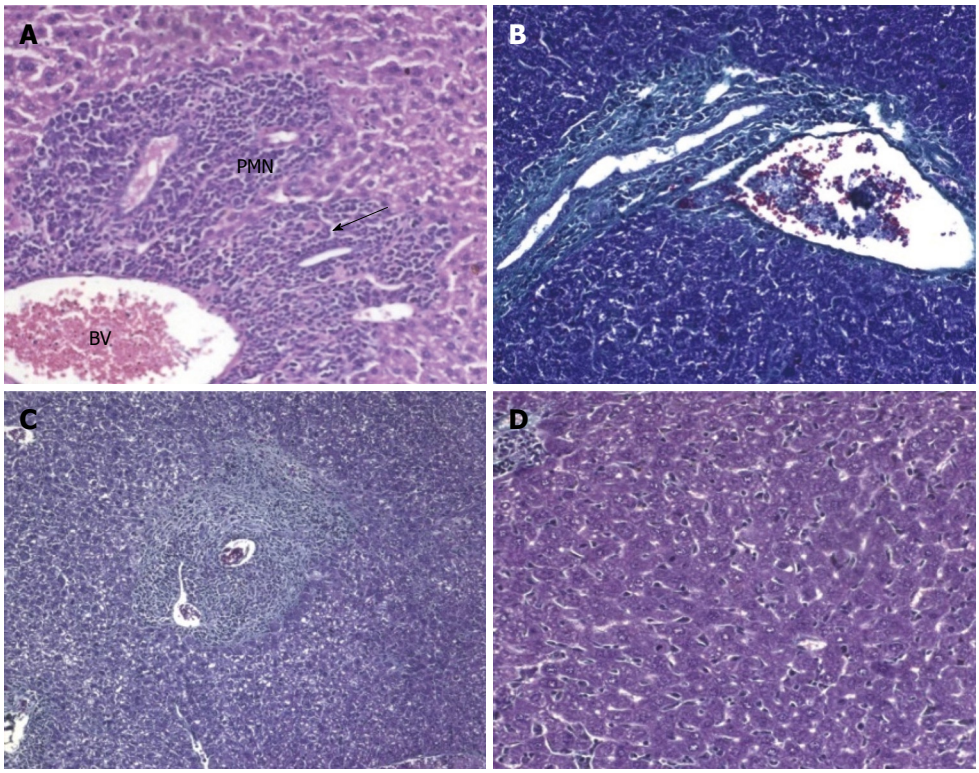

Figure 3 Preinfection treated animals sacrificed. A: 10 d post infection (H&E × 200); B: 4 wk post infection (wpi) (Masson trichrome × 200); C,D: 6 wpi (Masson trichrome and H&E × 200). PMN: polymorphonuclear.

g, 21.10 g and 19.89 g in untreated, preinfection treated and 3 wpi treated animals respectively. Body weight in mice sacrificed 6 wpi was not affected significantly by treatment, being 24.2 *vs* 22.75, 21.2, 21.75 g in controls untreated, preinfection treated and treated 3 and 5 wpi respectively. Also, at 8 wpi, the body weight of controls and those receiving treatments did not differ significantly (22.9, 20.9, 21.4 and 21.08 g in controls, treated 3, 5 and 7 wpi respectively).

The effect of treatment on liver weight by treatment was dependent on the stage of infection (Figure 2B). Liver weight was not significantly affected in mice sacrificed 10 d PI to be 1.06 g in the group treated preinfection compared to 1.17 g in untreated controls. A highly signifi cant increase occurred at 4 wpi in groups treated before infection and 3 wpi relative to controls (1.44 and 1.1 g *vs* 1.01 respectively) ($P \leqslant 0.01$). Liver weight was significantly affected at 6 wpi by G-CSF treatment 5 wpi (2.32 g *vs* 1.97 g of controls) ($P \leqslant 0.01$). However, it was not sig nificantly affected 8 wpi to be 2.23, 2.23, 2.37 g in those treated 3, 5 and 7 wpi respectively compared to 2.17 g in control.

Liver/body weight ratio

All treated groups had significantly higher liver/body weight ratio at 4 wpi (4.85% in untreated controls vs 6.98% in preinfection and 5.47% in 3 wpi treated groups). However, treatment did not affect this parameter in any of the groups sacrificed at either 6 or 8 wpi.

Stem cell mobilizing effect of G-CSF

The average total leukocytic count (TLC) in animals receiving G-CSF treatment was significantly higher compared to the untreated group. In mice sacrificed at 6 wpi, the TLC in the untreated group was $9.04 \times 10^3/\mu L$. However, it was 8.4, 14.87 and $10 \times 10^3/\mu L$ in animals receiving treatment before infection or treated 3 and 5 wpi respectively. Also, animals sacrificed at 8 wpi showed significantly higher levels ($P \leqslant 0.01$) in mice treated 3, 5 and 7 wpi (13.3, 12.06 and $18.36 \times 10^3/\mu L$ respectively) compared to the untreated group ($9.07 \times 10^3/\mu L$).

Effect of G-CSF treatment on egg loads in intestine and liver

The number of eggs retained in the small bowel and the liver of treated and untreated animals (Figure 2C, D) were not affected significantly in mice sacrificed at 6 and 8 wpi. A gradual non significant reduction of liver egg burden was observed at 8 wpi when comparing the untreated and treated groups 3, 5 and 7 wpi (13.2×10^3 eggs/g liver *vs* 13.0, 8.1 and 9.7×10^3 eggs/g liver weights respectively).

Histopathological results

At the acute stage of infection (10 d PI), animals treated before and for a few days after infection showed an increase of infiltrating polymorphonuclear cells (PMNCs) in the portal tract (Figure 3A) in comparison to the untreated group (Figure 4A). Both groups showed dilated portal blood vessels.

At 4 wpi, animals treated preinfection showed milder inflammation in their portal tracts compared to untreated mice (Figure 4B). A comparable degree of inflammation was presented in animals treated at 3 wpi (Figures 3B and 5A). Kupffer cell hyperplasia and intralobular inflammatory cell infiltration seen in the untreated group (Figure 4C) were not found in treated animals at 3 wpi.

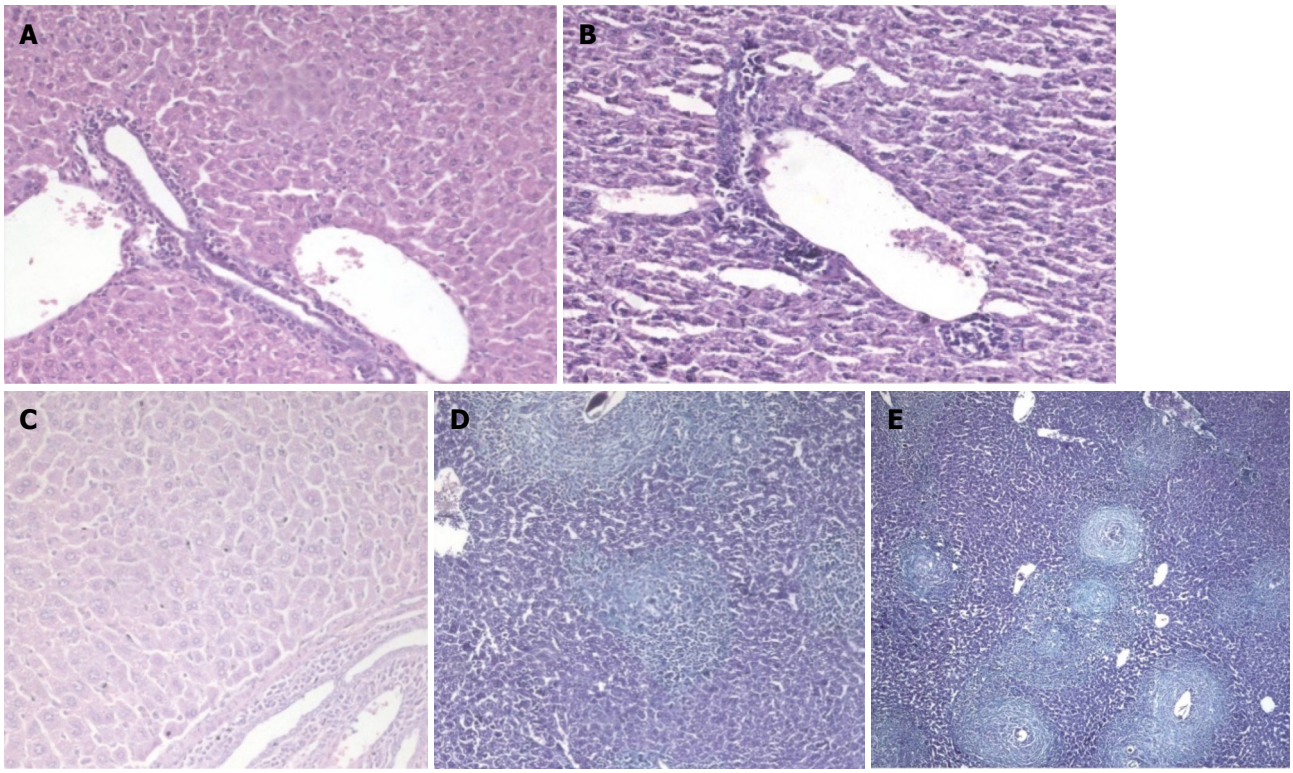

Figure 4 Liver sections in Schistosoma mansoni-infected untreated mice. A,B: 10 d post infection (H&E × 200); C: 4 wk post infection (wpi) (H&E × 200); D: 6 wpi (Masson trichrome × 100); E: 8 wpi (Masson trichrome × 100).

At 6 wpi, a considerable decrease in portal tract inflammatory infiltrate was seen in G-CSF-treated animals compared to untreated ones regardless of the time of treatment. Although the effect of treatment on granuloma diameter was not significant at this stage compared to untreated animals (Figure 4D), cellular granulomas became more predominant (Figures 3C, 5B, 6A) but their number did not change. Hyperplastic Kupffer cells and lobular inflammatory infiltrates continued to be present in animals treated with G-CSF before the infection (Figure 3D) but were absent in those treated at 3 and 5 wpi. Fibrous granulomas were encountered in animals treated at 3, 5 and 7 wpi (Figures 5C, 6B, 7) compared to the untreated group.

At 8 wpi, an intense portal tract inflammation was observed in G-CSF treated groups similar to untreated animals (Figures 6B, 4E respectively). However, animals treated at 3 wpi showed a decreased inflammatory reaction with a significant increase in granuloma diameter (Figure 5C). It was 275.8 μm in untreated animals versus 336.12, 308.3 and 325 μm in 3, 5 and 7 wpi treated mice respectively (Figure 8). Granulomas showed lobular and portal distribution.

Collagen content as determined by morphometric image analysis

Treatment did not affect collagen deposition in untreated animals at 4 wpi (2.1 ± 0) compared to animals treated preinfection 2.6 ± 0.5 and animals treated at 3 wpi 2.6 ± 0.47. On the other hand, a highly significant decrease in collagen content ($P \leqslant 0.01$) was found at 6 wpi in treated animals either preinfection or at 3 and 5 wpi (5.8 ± 0.5, 4.7 ± 0.5, 4.0 ± 0.7 *vs* untreated group 8.2 ± 0.9). Although treatment did not fully inhibit the progressing collagen deposition, the effect of treatment was also visible in animals at 8 wpi in animals treated at 3, 5 and 7 wpi respectively (8.9 ± 0.4, 10.0 ± 0.8 and 11.6 ± 0.9) compared to untreated ones (12.0 ± 1.0) (Figure 9).

DISCUSSION

S. mansoni infection is detected by the first line of defense - the innate immune system which does not detect the whole parasite but reacts to molecules released by *S. mansoni* or molecular changes induced by the parasite. Accordingly, the innate immunity is able to detect the pathogen associated molecular pattern (PAMP) and a primary inflammatory response is induced, eventually resulting in an immune response.

Administration of G-CSF has been shown to protect rodents against LPS toxicity[20] and to induce an upregulation of LBP synthesis in the liver[14] that decrease LPS toxicity through TLR4[21]. Since G-CSF treatment influences the "damage detection" in this model, we evaluated its effect on *S. mansoni* infection especially since it was reported that parasites directly affect TLR3 and TLR4[22]. In the course of disease, periovular granuloma and the induction of fibrosis cause most of the clinical symptoms. The number of granulomas correlates with the severity of disease but is not affected by G-CSF. As granulomas develop in response to egg deposition in the tissue, we assessed

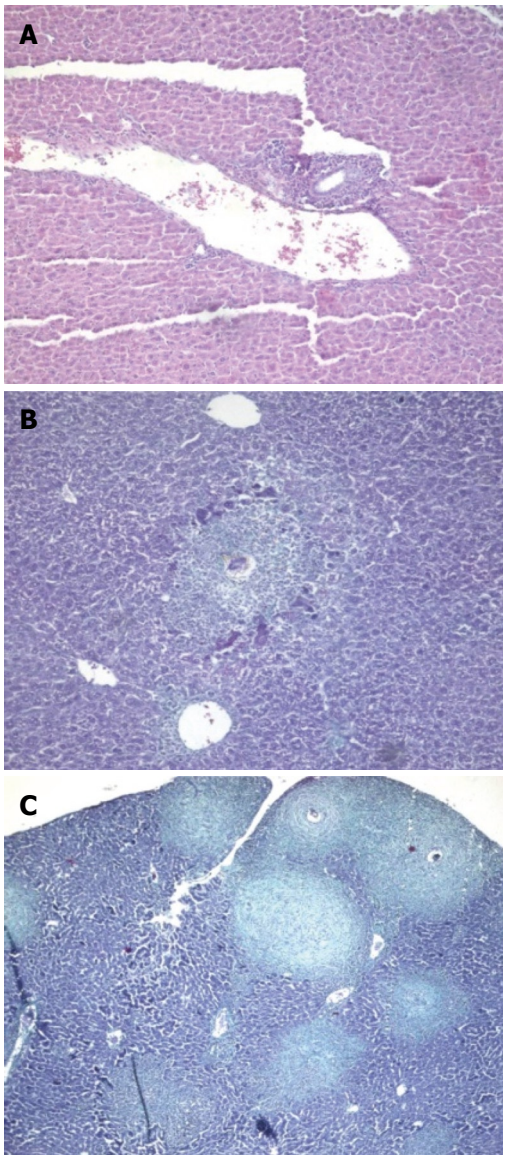

Figure 5 Animals treated 3 wk post infection and sacrificed. A: 4 wk post infection (wpi) (H&E × 100); B: 6 wpi (Masson trichrome × 100); C: 8 wpi (Masson trichrome × 100).

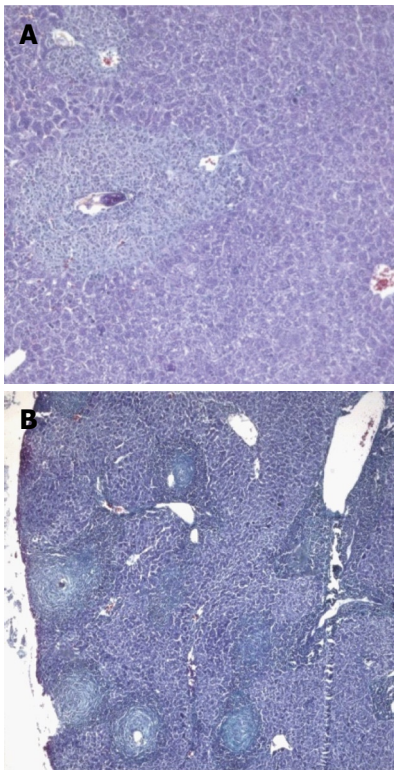

Figure 6 Mice treated 5 wk post infection and sacrificed. A: 6 wk post infection (wpi) (Masson trichrome × 200); B: 8 wpi (Masson trichrome × 100).

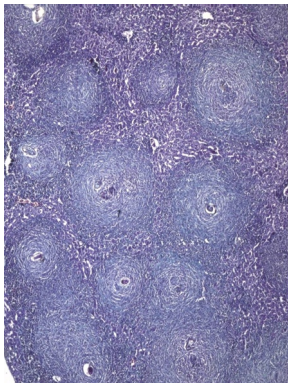

Figure 7 Mice treated at 7 wk post infection and sacrificed at 8 wk post infection showing multiple variable-sized fibrocellular and fibrous lobular granulomas distorting the hepatic lobular architecture (Masson trichrome x 200).

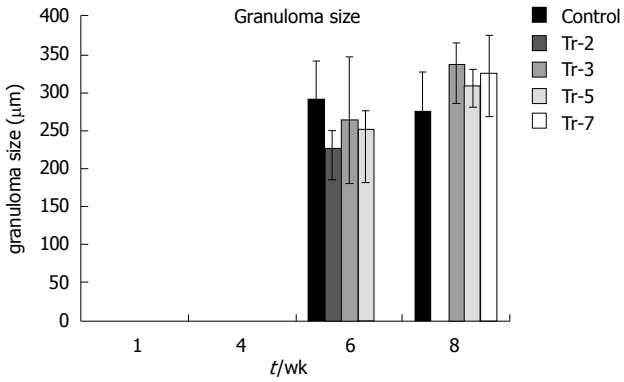

Figure 8 The effect of therapy on granuloma diameter.

the amounts of eggs retained in liver and intestine. As expected, we found increased egg load during the course of the disease with no significant reduction in treated mice. The egg load is generally employed as an important parameter to assess the efficiency of *S. mansoni* treatment[23] and consequently a lack of a significant effect of G-CSF treatment on the egg load indicates that G-CSF does not enhance the primary damage response.

The number of granulomas did not change in response to treatment; however, the composition of granuloma was different in G-CSF treated animals with a predominance of cellular ones. This could be caused by GCSF-mediated recruitment of inflammatory cells from extrahepatic sources or due to endogenous hematopoiesis. Lenzi *et al*[24] presented evidence of internal cellular production by granulomas and expression of G-CSF receptors. Exploration of the respective cytokine profile might help to elucidate the mechanisms of these flared up reactions and whether this reaction was biased to either the Th1 or Th2 type.

Lack of full recovery by elimination of the parasites leads to the secondary damage response associated with the onset of a marked immune reaction, progressive tis-

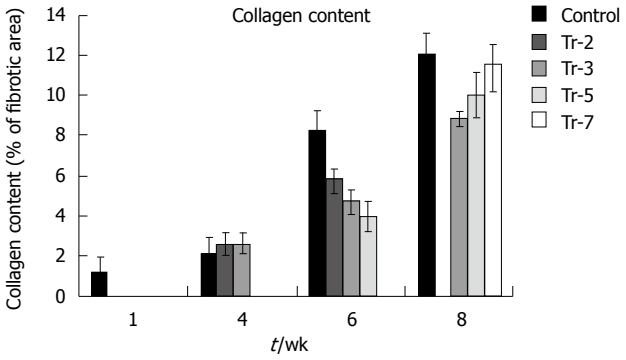

Figure 9 Effect of treatment on collagen content.

sue damage and repair with the development of a marked fibrosis. Portal fibrosis eventually results in clinical symptoms due to marked portal hypertension[2].

In this study, the extent of fibrosis, as estimated by the quantitative morphometric analysis of collagen content in Sirius red-stained liver sections, was significantly decreased in animals sacrificed at 6 and 8 wpi in all G-CSF treated groups. Fibrosis develops during the chronic phase of granulomatous inflammation in murine schistosomiasis. Fibrosis seems to represent a protective function during infection by neutralizing and sequestering egg antigens that can potentially damage host tissues[25]. An abrogation of Th2 response is then required to prevent excessive fibrosis. However this mechanism cannot explain the antifibrotic action of G-CSF administered in this experiment as the latter produces Th2 polarization rather than abrogation. It could be speculated that the inhibitory effect of G-CSF on collagen deposition may be related to enhanced fibrotic degradation rather than decreased fibrous synthesis. G-CSF was found in previous studies[26] to enhance the matrix metalloproteinase family (MMP 1 and 9). Whether this is also the mechanism involved in the current experiment is the topic of ongoing experiments.

In conclusion, the most prominent effect of treatment with G-CSF consisted of a slight effect on the inflammatory reaction but a marked reduction of collagen deposition irrespective of the time of starting treatment was initiated. Further work is needed to dissect the molecular events underlying immunomodulation and fibrous remodeling actions of G-CSF covering later stages of the disease for better evaluation of its therapeutic effect in schistosomiasis.

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COMMENTS

Background

Granulocyte-colony stimulating factor (G-CSF) has a variety of biological functions including mobilization of bone marrow-derived stem cells, immuno-modulation of a variety of host responses and anti-infectious properties.

Research frontiers

This article introduces G-CSF as a new therapeutic modality in *Schistosoma mansoni* (*S. mansoni*).

Innovations and breakthroughs

Treatment with G-CSF revealed slight affection of the inflammatory reaction and marked reduction of collagen deposition irrespective of the time of starting treatment.

Applications

Further work is needed to dissect the molecular events underlying immunomodulation and fibrous remodeling actions of G-CSF covering later stages of the disease for better evaluation of its therapeutic effect in schistosomiasis.

Peer reviews

The manuscript describes the effects of human recombinant G-CSF on liver histopathology of mice infected with *S. mansoni* and prevents progression of granuloma formation. The findings are of interest, but observational.

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CASE REPORT

Development of osteomalacia in a post-liver transplant patient receiving adefovir dipivoxil

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Abstract

We report the case of a patient treated with living donor-related liver transplantation who suffered from osteomalacia during adefovir dipivoxil (ADV)-containing antiviral therapy for lamivudine-resistant hepatitis B virus infection. The patient had generalized bone pain, with severe hypophosphatemia after 20 mo of ADV therapy. Radiographic studies demonstrated the presence of osteomalacia. The peak plasma ADV level was 38 ng/mL after administration of ADV at 10mg/day. It was also found that ADV affected the metabolism of tacrolimus, a calcineurin-inhibitor, and caused an increase in the plasma levels of tacrolimus. The disability was reversed with the withdrawal of ADV and with mineral supplementation. ADV can cause an elevation of plasma tacrolimus levels, which may be associated with renal dysfunction. High levels of ADV and tacrolimus can cause nephrotoxicity and osteomalacia. This case highlights the importance of considering a diagnosis of osteomalacia in liver transplantation recipients treated with both ADV and tacrolimus.

Key words: Hepatitis B virus; Osteomalacia; Adefovir dipivoxil; Living donor-related liver transplantation; Tacrolimus

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INTRODUCTION

In hepatitis B virus (HBV) DNA-positive patients, the rate of HBV recurrence after liver transplantation (LT) remains high without prophylaxis using hepatitis B immunoglobulins (HBIG) and lamivudine (LAM)[1-3]. Resistance to LAM is characterized by the substitution of methionine with valine or isoleucine at residue 204 within the tyrosine-methionine-aspartate-aspartate (YMDD) motif of the viral DNA polymerase[4-5]. Adefovir dipivoxil (ADV) is a potent nucleotide analogue against both the wild-type and LAM-resistant HBV[6-7], and it has been reported that ADV can prevent and treat recurrences of HBV infection following LT[8-10].

Hypophosphatemic osteomalacia results from a ge-

Table 1 Laboratory data on admission

Blood chemistry		Arterial blood gas		Urinary chemistry	
Total protein	6.3 g/dL	pH	7.375	Urinary Na	0.78 g/d
Albumin	3.9 g/dL	PaO2	86.8 mmHg	Urinary K	0.20 g/d
AST	39 IU/L	PaCO2	37.1 mmHg	Urinary Cl	0.67 g/d
ALT	22 IU/L	HCO3-	21.2 mmol/L	Urinary Ca	0.14 g/d
ALP	3410 IU/L	Anion gap	11.3 mmol/L	Uurinary phosphate	0.43 g/d
γ-GTP	116 IU/L	Base excess	- 3.0 mmol/L	% TRP	53.7 %
Total bilirubin	0.6 mg/dL			Urinary creatinine	0.45 g/d
Direct bilirubin	0.2 mg/dL			Ccreatinine clearance	37.9 mL/min
Urea nitrogen	36 mg/dL			Uurinary glucose	2.57 g/d
Creatinine	1.1 mg/dL			Urinary protein	1.44 g/d
Uric acid	1.7 mg/dL			Urinary NAG	49.0 U/L
Plasma glucose	90 mg/dL			Urinary BMG	129, 220 μg/L
Na	142 mEq/L			Generalized aminoaciduria	(+)
K	3.5 mEq/L				
Cl	113 mEq/L				
Ca	8.0 mg/dL				
P	1.4 mg/dL				
Mg	2.1 mg/dL				
Prothrombin time (INR)	0.96				
Serum BMG	3.5 mg/L				
Bone-type ALP	551 μg/L				
Intact-PTH	85 pg/mL				
1, 25-dihydroxy vitamin D3	21.3 pg/mL				

AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase; γ-GTP: γ-glutamyl transferase; P: phosphate; BMG: β2-microglobulin; %TRP: % tublar reabsorption of phosphate; NAG: N-acetyl-β-D-glucosaminidase; Intact-PTH: intact parathyroid hormone.

neralized dysfunction of the proximal renal tubule, leading to impaired reabsorption of amino acids, glucose, urate, and phosphate[11]. The chronic loss of phosphate and the adequate synthesis of 1, 25-dihydroxy vitamin D3 together produce phosphate depletion and failure to properly mineralize bone. Recently, patients with acquired osteomalacia have been reported during treatment of human immunodeficiency virus (HIV) infection using nucleotide analogues such as tenofovir and ADV[12-16]. We report herein a case of osteomalacia that developed during ADV therapy after living donor-related liver transplantation (LDLT).

CASE REPORT

A 48-year-old Japanese man was diagnosed with HBV-related decompensated liver cirrhosis in January 2000 and began undergoing LAM therapy at 100 mg/d. The serum HBs antigen (HBsAg) and HBe antigen (HBeAg) were positive, anti-HBe antibody was negative, and HBV-DNA was detected at 8.0 log genome equivalent (LGE)/mL by transcription-mediated amplification (TMA) before the treatment with LAM. Three months later, his serum HBV-DNA decreased to < 3.7 LGE/mL with a favorable initial response. The response persisted up to February 2001, at which time LAM-resistant mutants (YIDD) emerged with elevated transaminases [alanine aminotransferase (ALT) 374 IU/L (normal; 7-47 IU/L) and aspartate aminotransferase (AST) 444 IU/L (normal; 12-31 IU/L)]. In March 2002, the patient was started on ADV therapy at 10 mg/d combined with LAM, and the antiviral response was progressive, but slow. Although the HBV-DNA was decreased to 4.0 LGE/mL after 2 mo, liver function was not favorably improved. In accordance with our hospital policy, the patient could then be accepted as a liver transplant recipient. His wife volunteered to undergo right hepatectomy for living donation, and he underwent LDLT in May 2002. Serum HBsAg and HBV-DNA were negative, but anti-HBc antibody was positive in the donor. The anti-HBV prophylactic regimen consisted of an intravenous injection of HBIG and peroral administration of LAM (100 mg/d) plus ADV (10 mg/d). Intravenous HBIG was given at a dose of 10 000 IU during the anhepatic phase, and then daily for 6 d. Repeated doses were given to maintain anti-HBs titers above 500 IU/L for the initial 6 mo. The patient remained well with a combination of LAM, ADV, and periodic doses of HBIG injection to maintain anti-HBs greater than 200 IU/L. Anti-HBsAb was always positive, and HBsAg and HBV-DNA were not detected in his serum during observation.

Postoperative immunosuppression consisted of tacrolimus (target trough level 10-15 ng/mL) and steroids (intravenous methylprednisolone 500 mg/d tapered to oral prednisolone 20 mg/d from day 7). The tacrolimus trough level was lowered to 5 to 10 ng/mL, and the dosage of prednisolone was reduced to 2.5 mg/d during the 6th mo after transplantation.

The patient began to complain of right ankle pain in November 2003. His bone pain gradually increased and involved his knees and shoulders. He also began to experience weakness in his leg muscles, with difficulty in walking in July 2004. A neurological evaluation, an electromyogram (EMG), and a muscle biopsy were performed in February 2005, which revealed almost normal

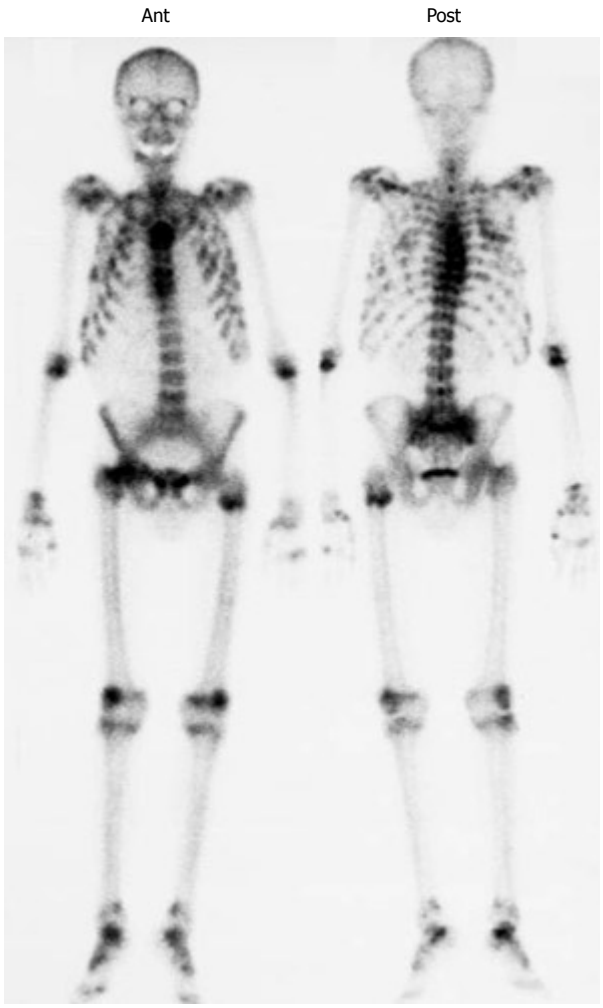

Figure 1 Whole body bone scintigraphy shows multiple foci of increased radiotracer uptake in the thoracic spine, the sacroiliac region, the rib cage, the shoulders, the knees, and the ankles.

muscular fibers. He was admitted for further examination in May 2006. The laboratory data obtained on admission are shown in Table 1. He demonstrated persistent hypophosphatemia with a phosphate level of 1.4 mg/dL (normal range; 2.4-5.1 mg/dL). In addition, he showed significantly elevated levels (3410 IU/L) of alkaline phosphatase (ALP) (normal range; 116-280 IU/L). His serum creatinine level was 1.1 mg/dL and calculated creatinine clearance was 37.9 mL/min, indicating moderate renal insufficiency. Urinalysis and 24-h urine collection confirmed phosphate wasting, which can be caused by impairment of proximal renal tubular reabsorption of phosphate. Although his serum level of intact parathyroid hormone (intact-PTH) was slightly increased, his serum level of 1, 25-dihydroxy vitamin D3 was 21.3 pg/mL (normal range; 20-60 pg/mL). Indications were that the impairment of reabsorption of phosphate could be mainly brought about by the proximal renal tubular injury, and not by vitamin D deficiency, in this patient.

Radiographic studies including X-rays, MRI, and bone scans were performed. ^{99m}Tc-HMDP whole-body bone scintigraphy showed multiple foci of increased radiotracer uptake in the thoracic spine, the sacroiliac region, the rib cage, the shoulders, the knees, and the ankles (Figure 1). X-rays and MRI findings showed pseudofractures (Looser's zones) in the right femoris, which could indicate osteomalacia.

The highest plasma level of ADV in the patient was 38 ng/mL, after administration of ADV at 10mg/day, which was approximately 3 times higher than that in patients with normal renal function. We also found that ADV contributed to the elevation of plasma tacrolimus levels in this patient, as the trough levels of tacrolimus with administration of ADV were 1.5 times higher than those without ADV. These results suggest that ADV could affect the metabolism of tacrolimus, and cause increases in the plasma levels of tacrolimus. In this context, high levels of both ADV and tacrolimus could contribute to nephrotoxicity and hypophosphatemia.

The patient was initially treated with phosphate supplementation and decreasing doses of ADV (10 mg/every other day → 5mg/every other day → 2.5mg/every day). After switching from ADV plus LAM to entecavir hydrate (ETV) at 1 mg/every other day, several laboratory parameters improved, including serum levels of phosphorus and ALP. He was maintained on phosphate replacement, and his bone pain also decreased dramatically (Figure 2).

DISCUSSION

We report herein a case with renal tubular injury, hypophosphatemia, and osteomalacia all of which developed during ADV therapy after LDLT. ADV is known to cause renal tubulopathy in patients with HIV or HBV infection[13-14], as is tenofovir, a major antiretroviral medication[15-16]. The pathophysiology of the proximal tubular dysfunction caused by ADV is thought to be due to the concentration of ADV in the mitochondria[17], and, consequently, mitochondrial toxicity and the inhibition of several ATP-dependent critical transporters in proximal tubular cells[18]. Although every-other-day administration of ADV (10 mg/2 d) is generally recommended when a patient has moderate levels of renal dysfunction, we recommend complete cessation of ADV treatment and a change to another antiviral reagent such as ETV[19-20], as the continued every-other-day administration of ADV may continue to injure the proximal tubular cells. It is unclear why, if all patients accumulate ADV in the proximal tubule, that only a small percentage of patients experience the renal complications seen in this case.

Tacrolimus is a calcineurin-inhibitor (CNI), which are immunosuppressive agents for liver transplantation[21]. Renal dysfunction is common after liver transplantation[22-25], and it has been reported to be associated with high levels of CNI[26]. ADV contributed to the elevation of plasma tacrolimus levels in this patient, and this elevation may have been associated with his renal dysfunction. In cases such as this one, tacrolimus levels should be reduced as far as possible and the interaction between tacrolimus and ADV should be given strong consideration in liver-transplant patients with HBV infection.

Although the patient had typical clinical features such as bone pain, hypophosphatemia, and elevated serum

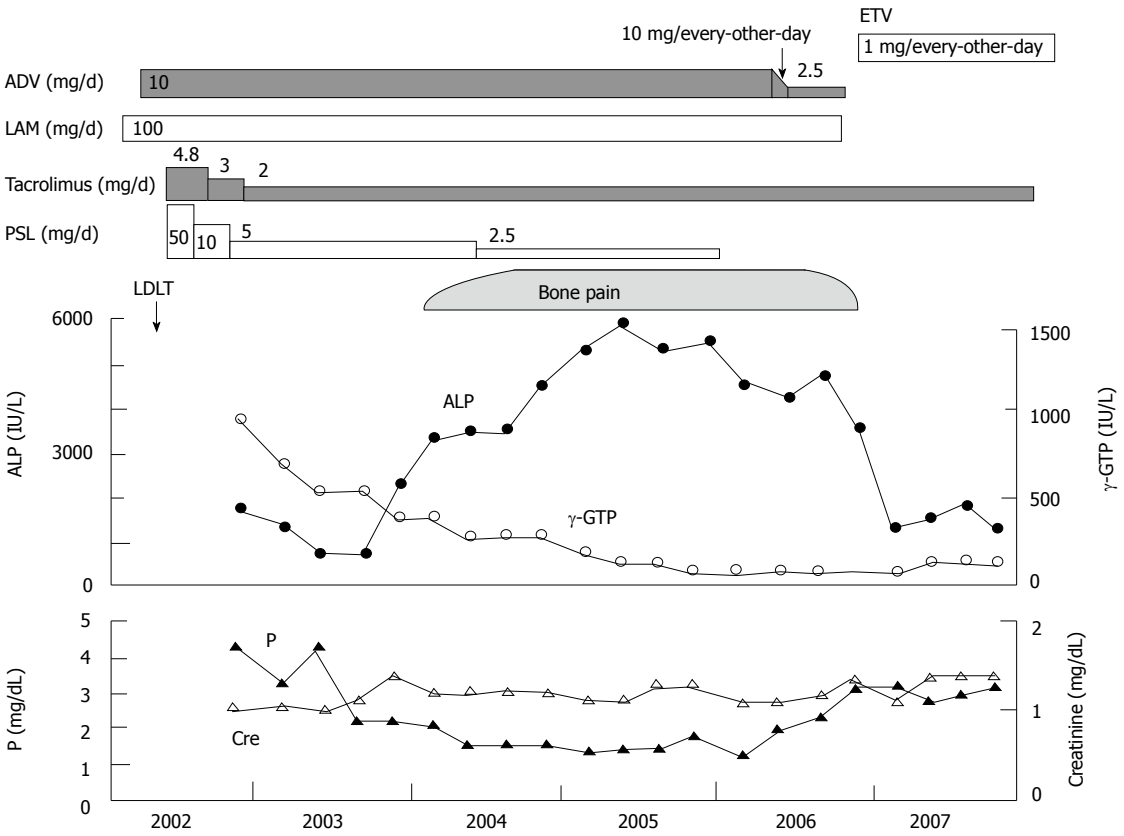

Figure 2 Clinical course of the present case. After switching from ADV plus LAM to ETV, serum levels of P and ALP improved, and the patient's bone pain also decreased dramatically. ADV: adefovir dipivoxil; LAM: lamivudine; ETV: entecavir hydrate; PSL: prednisolone; P: phosphate; APL: alkaline phosphatase; γ-GTP: γ-glutamyl transferase; LDLT: living donor-related liver transplantation.

APL levels for osteomalacia, the diagnosis of osteomalacia was delayed. It was initially difficult to distinguish bone-derived ALP and liver-derived ALP because the patient had persistently high levels of serum ALP and γ-glutamyl transferase (γ-GTP) associated with chronic rejection after LDLT.

Hypophosphatemic osteomalacia is a potential adverse effect of ADV[13], and patients treated with ADV should be monitored by measuring serum ALP and phosphorus levels. If patients develop bone pain or myopathy in response to ADV treatment, serum hypophosphatemia and phosphate wasting into urine should be confirmed. Initial treatment with phosphate supplementation and decreasing doses of ADV should be performed. Discontinuing administration of ADV and switching to ETV may be recommended for patients after LT, because these patients often have renal insufficiency associated with the use of CNI.

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CASE REPORT

Simple nucleos(t)ides as HBV prophylaxis regime of post-liver transplantation: Six-year followed up

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Abstract

A combination of nucleos(t)ides and hepatitis B immunoglobulin (HBIg) has been found to be effective for the prevention of hepatitis B viral (HBV) reinfection after liver transplantation (LT), but its administration is costly, and not always available. We report the case of a male, 33-year-old cirrhotic patient who has tested positive for serum HBsAg, and HBeAg, with 9.04×10^7 copies/mL of HBV DNA. He suffered from acute liver failure and was near death before undergoing emergency LT. No HBIg was available at the time, so only lamivudine was used. He routinely received immunosuppression medication. Serum HBV DNA and HBsAg still showed positive post-LT, and the graft re-infected. Hepatitis B flared three months later. Adefovir dipivoxil was added to the treatment, but in the 24th mo of treatment, the patient developed lamivudine resistance and a worsening of the hepatitis occurred shortly thereafter. The treatment combination was then changed to a double dosage of entecavir and the disease was gradually resolved. After 60-mo of post-LT nucleos(t)ide analogue therapy, anti-HBs seroconverted, and the antiviral was stopped. By the end of a 12-mo follow-up, the patient had achieved sustained recovery. In conclusion, the case seems to point to evidence that more potent and less resistant analogues like entecavir might fully replace HBIg as an HBV prophylaxis and treatment regimen.

Key words: Chronic hepatitis B; Hepatitis B immunoglobulin; Liver transplantation; Nucleos(t)ides

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Luo KX, Zhou FY, Liu DL, Feng XR. Simple nucleos(t)ides as HBV prophylaxis regime of post-liver transplantation: Six-year followed up. *World J Hepatol* 2010; 2(12): 447-450 Available from: URL: http://www.wjgnet.com/1948-5182/full/v2/i12/447.htm DOI: http://dx.doi.org/10.4254/wjh.v2.i12.447

INTRODUCTION

Because of immunosuppression usage, liver transplantation (LT) due to hepatitis B virus (HBV)-related diseases is often followed by HBV re-infection of the allograft which is associated with severe liver damage, and often progresses to graft loss[1]. However, prophylaxis strategies have greatly advanced in the past few years. Initially, long-term intravenous (IV) high-dose hepatitis B immunoglobulin (HBIg) was used, but it was very expensive, and the recurrence rate still remained high[2]. Since lamivudine (LMV) has been introduced in treatment of chronic hepatitis B, it has also been used in HBV prophylaxis post-LT. The LMV and HBIg combination reduces HBV recurrence rates to less than 5% in 5 years[3], and thus a new era of LT for HBV-related disease had begun. Recently,

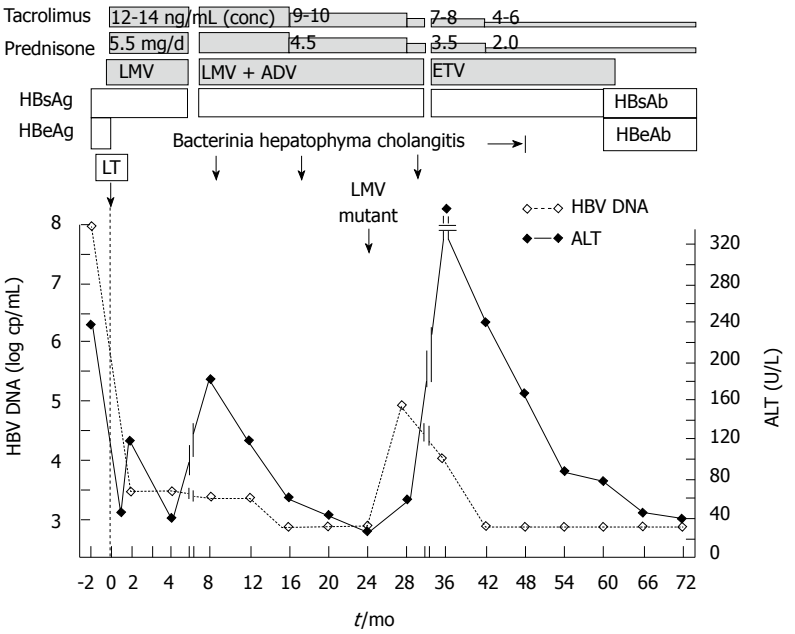

Figure 1 Clinical course and virological features of the patient. Conc: concentration; LMV: lamivudine; ADV: adefovir dipivoxil; ETV: entecavir; LT: liver transplantation.

high-dose IV HBIg in combination has been replaced by low-dose intramuscular (IM) HBIg, which has achieved similar results[4,5]; hence the latter regimen has been widely adopted in most hepatology departments. However, a low-dose HBIg regimen is still costly and not always available, and strategies using HBIg-free nucleos(t)ide analogues (NAs) have been tried instead[6]. Very recently, a multicenter randomized study of adefovir dipivoxil (ADV) substitution for low-dose IM HBIg showed no HBV recurrence for at least 12-mo post-LT[7]. In the following case study, a cirrhotic patient with high HBV levels and fatal liver failure received an emergency orthotopic LT. No HBIg was available; he received only NAs treatment. He achieved a sustained recovery even after the antivirals were discontinued, and even showed that HBs had seroconverted. HBIg-free NAs regimens are widely used in China to prevent LT re-infection, and the following case may be a typical one.

CASE REPORT

A 33-year-old male patient had been HBsAg-positive since childhood, but had had no regular examinations. At this time he had lassitude, lack of appetite, and dark yellow urine for 6 wk. In mid-October of 2003, he was admitted to a local primary hospital, and only treated with conventional herbal medicine to alleviate his symptoms and lower the serum transaminase. His symptoms worsened in a week and he went into a coma for 3 d. He was then transferred to the hepatology unit at Nanfang Hospital on October 20th, 2003. Examination revealed a temperature 38.2℃, blood pressure of 96/60 mmHg, pulse rate at 90/min, and respiration rate at 32/min. The patient was heavy jaundiced and was in a deep coma. Heart and lungs were normal, liver and spleen not palpable, and the abdomen had swelling with ascites. Virological tests were positive for serum HBsAg and HBeAg (EIA, Abbott Lab, Chicago, IL, USA), and 9.04×10^7 copies/mL of HBV DNA (Fluorescent quantitative PCR-based assay with a Roche Amplicor machine using a locally licensed kit). There was no evidence of hepatitis C, hepatitis D, or human immunodeficiency virus infections. Biochemical tests revealed alanine transaminase (ALT) 120 U/L, aspartate transaminase (AST) 157 U/L, albumin 32 g/L, total bilirubin 211 μmol/L, direct bilirubin 110 μmol/L, prothrombin time 49 (normal < 13) sec, alfa fetal protein 172 (normal < 10.9) ng/mL, serum urea and creatinine normal.

Emergency management included tracheal incubation ventilation, artificial liver support and plasmapheresis, and LMV 100 mg was administered daily by gastric tube. Two days after admission, the patient received an orthotopic LT.

The explant liver was tenacious with grey yellow cross sections. Microscopy showed hepatocyte ballooning degeneration and confluent necrosis, diffusing pseudo-lobule with generously fibrous connective tissue.

The virological features and the clinical course are shown in Figure 1. Immunosuppressive therapy began with 15 mg of corticosteroid daily in the first week, which was then lowered to 5.5 mg daily, and tacrolimus was adjusted to maintain a serum level of 12-14 ng/mL during the first year. The dosages of both drugs were decreasedannually. There was no HBIg available, and simple LMV use continued, and we had no choice but to use LMV as the HBV prophylaxis and treatment regimen at that time. Serum HBV DNA was 1.72×10^4 copies/mL and HBeAg cleared at the second week of treatment, but HBsAg was still positive. At the end of the first month post-LT, HBV DNA was 7.68×10^3 copies/mL. The patient's liver chemistry gradually returned to normal.

Three months later, the patient complained of lassitude and nausea. His ALT was at 121 U/L and HBV DNA was 5.25×10^3 copies/mL. There was no evidence

of cytomegalovirus infection. Histological examination of the biopsied transplanted liver revealed moderate inflammation and mild fibrosis. ADV 10 mg/d was added to the treatment regime not long after the hepatitis flared, but serum HBV DNA was still detectable, and the ALT levels remained abnormal during 10 mo of combined LMV and ADV therapy.

Then in the following 2 years (from June 2004 to December 2006) the patient suffered bacteremia of aerugo pseudo-monosporangium, hepatophyma and cholangitis successively. He received multiple treatments, hepatotoxic antibiotics were carefully avoided, and the infections were finally cured.

Unfortunately, even combined with ADV, the LMV resistance developed 24-mo into treatment, and t mutations of rtM204V and rtL180M were detected by means of sequencing. The HBV DNA had increased to 1.01 $\times$ 10^5 copies/mL and acute exacerbation occurred, with ALT elevating up to 468 U/L. Antivirals were switched to 1.0 mg of entecavir daily. HBV DNA was undetectable 12-mo after and HBsAg was negative 60-mo after LT and NAs therapy. On December of 2008, anti-HBs sero-converted, and all liver function tests were normal. After another year, , with antivirals being discontinued for 12 mo, the patient had achieved a sustained recovery.

DISCUSSION

The patient with HBV-related cirrhosis and liver failure was near death, characterized by intense ascites, severe encephalopathy, and markedly prolonged prothrombin time. With liver disease at such an advanced stage, emergency LT might be seen to be the only treatment option[8]. LMV was used simply to prevent graft reinfection, but HBIg was not available in an urgent situation. He had high levels of serum HBV DNA pre-LT, and even after the removal of the infected liver, HBV usually still replicated persistently outside the liver, which has been shown to be common in such patients[9]. Thus, about three months later, the graft liver reinfected and hepatitis B flared. Without effective treatment, his prospect of survival was low. ADV was then added his treatment regime, but low virus replication and mild hepatitis still persisted for more than a year, suggesting that in immunosuppressive conditions, the drug combination was still not strong enough[10]. In addition, LMV resistance occurred even with ADV treatment, resulting in HBV rebound and worsening of the disease symptoms, which is uncommon in a non-transplant patient[11]. After changing to entecavir with double the normal dosage, and decreasing the immunosuppression to minimal maintaining doses, the patient's clinical status substantially improved. In the fifth year of NAs therapy post-LT, the patient had achieved anti-HBs seroconversion and even having been off antivirals for 12 mo, remained well.

Currently, combined NAs and IM low-dose HBIg therapy is widely adopted as the most effective strategy against HBV recurrence post-LT[5,6,12]. Free-HBIg NAs therapy has also been investigated and some facts may give new insights. When NAs are used as a part of a prophylaxis regimen, the effect of low-dose IM HBIg is equivalent to that of high-dose IV HBIg[5,6], and maintaining LMV treatment alone always results in a low risk of HBV recurrence, regardless of HBIg discontinuance.Moreover, even one week of HBIg combined with lamivudine regimen at the beginning of the treatment had an equivalent effect, compared with a long-term high-dose HBIg regimen for preventing hepatitis B recurrence[13]. It is suggested that with adequate treatment of potent NAs, concomitant indefinite passive immunization may not be essential[14]. Some studies have compared complete HBIg-free NAs monotherapy (without a short initial HBIg phase) with combined therapy, describing 2-4 year recurrence rates of about 15%-40%, higher than those of the combination therapy[15-19].

In fact, a major factor for recurrence was related to the high resistance to LMV[20], therefore the inclusion of ADV might be a better strategy[7,10]. However, because of the negative influence of immune suppression, the potential for NAs resistance and HBV reinfection in the long-term cannot be excluded. The LT recipients treated with NAs may have higher resistance rates in shorter periods, and fewer viral clearance effects even for lower viremia, and these affect the progression of the disease[20]. Therefore, NAs of more potency and less resistance (telbivudine, entecavir, and tenofovir) are to be preferred. Although there are few studies of these newer antivirals being used as HBV prophylaxis updates[21,22], in accordance with wide use in patients with chronic hepatitis B, they should still replace LMV within prophylaxis regimens in LT patients. With the properties they have, it could be expected that more successful HBIg-free regimens could be established, but most up- to- date studies have been limited by short-term follow-ups. To date, no reliable conclusions have been drawn as to whether treatment based on combination medication is superior to NAs monotherapy. It is therefore important that more control studies with long-term follow-ups are undertaken.

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March 04-06
Bethesda, MD, United States
8th International Symposium on Targeted Anticancer Therapies

March 05-07
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26th Pakistan Society of Gastroenterology & Endoscopy Meeting

March 12-14
Bhubaneswar, India
18th Annual Meeting of Indian National Association for Study of the Liver

March 25-28
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San Diego, California, United States
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6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

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9 Outreach: Bringing HIV-positive individuals into care. *HRSA Careaction* 2002; 1-6 [PMID: 12154804]

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10 **Sherlock S**, Dooley J. Diseases of the liver and billiary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

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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

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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

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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/index.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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