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ABOUT COVER

Editorial board member of *World Journal of Hepatology* . Koo Jeong Kang, MD, PhD, Professor, Division of HBP Surgery, Department of Surgery, Keimyung University Dong-San Hospital, Daegu, South Korea. As a hepatobiliary surgeon, he has been dedicated in hepatobiliary and pancreatic surgery including HCC, intrahepatic cholangiocarcinoma, hilar cholangiocarcinoma, cancer of gallbladder, pancreatic cancer and liver transplantation including LDLT, laparoscopic as well as open. Initially as a HBP surgeon, his research interests was hepatic ischemia/reperfusion injury to prevent hepatic injury both clinical but experimental research to establish safe liver surgeries. And he interested in the clinical significance of anatomical resection of the liver cancer, systematic segmentectomy or Glissonean pedicle approach according to the size and location of the tumor.

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REVIEW

Oxidative stress in alcohol-related liver disease

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Abstract

Alcohol consumption is one of the leading causes of the global burden of disease and results in high healthcare and economic costs. Heavy alcohol misuse leads to alcohol-related liver disease, which is responsible for a significant proportion of alcohol-attributable deaths globally. Other than reducing alcohol consumption, there are currently no effective treatments for alcohol-related liver disease. Oxidative stress refers to an imbalance in the production and elimination of reactive oxygen species and antioxidants. It plays important roles in several aspects of alcohol-related liver disease pathogenesis. Here, we review how chronic alcohol use results in oxidative stress through increased metabolism *via* the cytochrome P450 2E1 system producing reactive oxygen species, acetaldehyde and protein and DNA adducts. These trigger inflammatory signaling pathways within the liver leading to expression of pro-inflammatory mediators causing hepatocyte apoptosis and necrosis. Reactive oxygen species exposure also results in mitochondrial stress within hepatocytes causing structural and functional dysregulation of mitochondria and upregulating apoptotic signaling. There is also evidence that oxidative stress as well as the direct effect of alcohol influences epigenetic regulation. Increased global histone methylation and acetylation and specific histone acetylation inhibits antioxidant responses and promotes expression of key pro-inflammatory genes. This review highlights aspects of the role of oxidative stress in disease pathogenesis that warrant further study including mitochondrial stress and epigenetic regulation. Improved understanding of these processes may identify novel targets for therapy.

Key words: Alcohol-related liver disease; Alcoholic hepatitis; Oxidative stress; Reactive

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Core tip: Alcohol is a global health problem with alcohol-related liver disease forming a significant proportion of alcohol-attributable deaths. However, there are no effective treatments for alcohol-related liver disease. Oxidative stress plays multiple roles in disease pathogenesis, which if better understood may yield new therapeutic targets. Here, we review the current literature on how alcohol consumption leads to oxidative stress and how this results in hepatocyte apoptosis and necrosis through its contribution to mitochondrial stress, dysregulation of cell signalling pathways and epigenetic regulation.

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INTRODUCTION

Europe has the highest per capita alcohol consumption and alcohol-related loss of disability adjusted life years globally[1]. The European Union is the heaviest drinking region of the world, with 11 liters of pure alcohol drunk per adult each year[1]. In the last decade, this trend continues to rise in central and northern Europe. Alcohol is a dose-related risk factor for more than 200 diseases[1]. It causes 5.9% of all deaths globally and more than 25% of deaths in the age group 20-39 years[1].

Heavy alcohol use is the cause of alcohol-related liver disease (ALD). Therefore, it is not surprising that the incidence of ALD is on a rising trajectory[2]. The scale of ALD is estimated to burden the United Kingdom national health system with health-related costs of over £3.5 billion per year[2]. In Europe, the cost of treating ALD is estimated to be €17 billion, together with €5bn spent on treatment and prevention of harmful alcohol use and alcohol dependence[3]. Worldwide, alcohol-related liver cirrhosis deaths account for approximately 10% of all alcohol-attributable deaths resulting in the loss of 22.2 million disability-adjusted life years annually[4].

The ALD spectrum ranges from simple steatosis to steatohepatitis, fibrosis, and cirrhosis. While alcohol-related cirrhosis is no longer considered a completely irreversible condition, no effective anti-fibrotic therapies are currently available. Cirrhosis can be divided into compensated and decompensated stages, with differentiating clinical features and prognosis. The median survival of patients with compensated liver disease is approximately 6.5 years but only 2.5 years in those with decompensated cirrhosis[4]. Once a complication of cirrhosis develops, the 5-year survival rate decreases to less than 20%[4].

Alcoholic hepatitis (AH) is an acute inflammatory condition that occurs on the background of ALD. Severe AH has a mortality rate of 30% within 3 mo[5] but even non-severe AH has a significant 7% mortality within 3 mo[6]. The established treatment for AH is corticosteroids, which improve short-term survival but do not affect long-term survival[5].

The molecular mechanisms underlying ALD pathogenesis are complex and have not been fully elucidated. However, there is emerging evidence that oxidative stress plays a role in mediating the inflammatory response and in directly causing liver damage. Oxidative stress represents the body's imbalance in the production and the elimination of reactive species (including reactive oxygen and nitrogen species) as well as decreased production of antioxidants[7]. Here, we review the role of oxidative stress in ALD focusing on its effect on mitochondrial stress, cell signaling and epigenetic regulation.

LITERATURE SEARCH

Comprehensive searches of MEDLINE, EMBASE, PubMed and TRIPS from their commencement to June 2019 were conducted. The search strategy included subject

headings and keywords related to "alcohol" and "oxidative stress" and "liver". The reference list of all included studies was screened for eligibility. This review included all study types in humans and animals. Studies published in all languages were considered. One author independently screened titles and abstracts and subsequently reviewed full-texts of retrieved studies for eligibility.

ALCOHOL METABOLISM

Alcohol (ethanol) is metabolized by three major pathways (Figure 1)[7]. The primary pathway is initiated by alcohol dehydrogenase (ADH), a NAD^+ requiring enzyme expressed at high levels in hepatocytes, which oxidizes ethanol to acetaldehyde[7]. In a normal liver, acetaldehyde enters the mitochondria and is quickly metabolized to acetate by aldehyde dehydrogenase (ALDH). Acetate is then broken down to carbon dioxide and water for elimination[8]. In chronic alcohol users, the ADH/ALDH pathway becomes saturated and reactive aldehydes are produced from the metabolism process such as malondialdehyde-acetaldehyde (MAA), 4-hydroxy-2-nonenal (HNE) and lipid hydroperoxides which can bind to proteins to produce protein adducts[8].

These protein adducts are capable of provoking an immune response. *In vitro* experiments showed that the viability of antigen-presenting cells, lymphocytes, and hepatocytes was decreased on incubation with an MAA hen egg lysosome adduct[9]. Circulating antibodies against MAA protein adducts were increased in patients with ALD and AH and correlated with the severity of liver injury[9].

The second major pathway to metabolise ethanol is the microsomal ethanol oxidizing system (MEOS), which involves an NADPH-requiring enzyme, the cytochrome P450 enzyme CYP2E1[10], which is induced by chronic alcohol exposure[11,12]. The increase of CYP2E1 after alcohol intake is due to stabilization of CYP2E1 rather than to a de novo synthesis[11]. The MEOS pathway metabolises ethanol to acetaldehyde by converting $NADPH^+$ and O_2 to NADP and H_2O resulting in the generation of reactive oxygen species (ROS). CYP2E1 plays a role in lipid peroxidation, protein oxidation, and protein nitration (Figure 1)[11]. It is also known to promote hepatic carcinogenesis by oxidizing DNA in alcohol-exposed rodents[13].

Ethanol metabolism through CYP2E1 not only produces acetaldehyde but also generates ROS including H_2O_2, hydroxyl (OH^-) and carbon centered OH^- (Figure 1)[14]. These ROS may be neutralized by a potent antioxidant defense system[14]. However, chronic alcohol consumption disrupts this system; depletion of mitochondrial glutathione (GSH) is observed in patients with alcohol dependence[15], which impairs hepatocyte tolerance to tumour necrosis factor alpha (TNF-α) resulting in an increased likelihood of cell death[16]. ROS increases and activates c-Jun N-terminal kinase (JNK) with consecutive expression of the activator protein 1 (AP-1) transcription factor leading to cellular hyper-regeneration, and lipid peroxidation. Lipid peroxidation products such as malondialdehyde and HNE are generated. HNE can bind to adenosine and cytosine forming highly carcinogenic exocyclic etheno DNA adducts[17]. These DNA adducts have been identified in the livers of patients with ALD and other types of liver disease associated with inflammation and oxidative stress like viral hepatitis[18].

The other two most prevalent DNA adducts are N2-ethyldeoxyguanosine (N2-Et-dG), and 1,N(2)-propano-2′-deoxyguanosine (PdG). N2-Et-dG is detectable in livers of alcohol-exposed mice and leukocytes of human alcohol misusers[19]. PdG, on the other hand, is distinguished by its genotoxic and mutagenic effects which impair DNA replication, thereby triggering cell death. These two major acetaldehyde-DNA adducts also promote carcinogenesis by initiating replication errors and mutations in oncogenes/onco-suppressor genes[19].

A third minor pathway for ethanol metabolism involves catalase, a peroxisomal enzyme (Figure 1)[20], which requires the presence of H_2O_2, a breakdown product of fatty acids. Catalase located in the peroxisomes of the hepatocyte plays only a minimal role in alcohol metabolism due to low hepatic production of H_2O_2. Under normal conditions, ADH metabolizes about 75%-80% of the ethanol entering the liver and MEOS the remainder. Hepatic ADH and hepatic catalase activities remain unchanged following chronic alcohol consumption, whereas hepatic MEOS activity strikingly increases and is responsible for the enhanced alcohol metabolism found after chronic alcohol consumption[11,12].

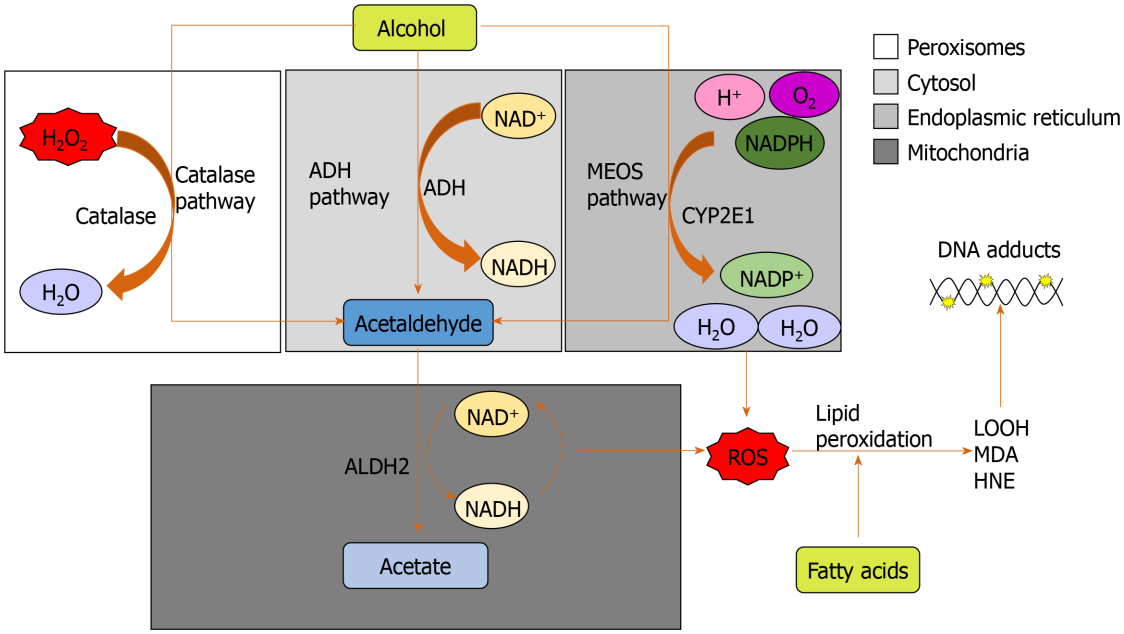

Figure 1 The three major pathways of alcohol metabolism. The primary pathway is initiated by alcohol dehydrogenase, a NAD^+ requiring enzyme expressed at high levels in hepatocytes, which oxidizes ethanol to acetaldehyde. The second major pathway, the microsomal ethanol oxidizing system pathway, involves the NADPH-requiring enzyme cytochrome P450 enzyme 2E1, which is induced by chronic alcohol exposure. The third pathway for ethanol metabolism is carried out by catalase, a peroxisomal enzyme. ADH: Alcohol dehydrogenase; ALDH2: Aldehyde dehydrogenase; CYP2E1: Cytochrome P450 enzyme 2E1; HNE: 4-hydroxy-2-nonenal; LOOH: Lipid hydroperoxides; MDA: Malondialdehyde; MEOS: Microsomal ethanol oxidizing system; ROS: Reactive oxygen species.

MITOCHONDRIAL STRESS

Chronic alcohol consumption results in structural and functional abnormalities in hepatic mitochondria, including enlarged morphology[21,22], mitochondrial DNA (mtDNA) damage[23], reductions in hepatic ATP levels[24] and mitochondrial protein synthesis[25] (Figure 2). This can result in hepatocellular apoptosis and associated necrosis[26]. Chronic alcohol metabolism and associated mitochondrial dysfunction has been implicated in increasing ROS production and accumulation in hepatic mitochondria.

In humans, *in vivo* measurement of ROS is complicated due to their rapid reactions with surrounding molecules[27]. Surrogate measures of mitochondrial-derived ROS include urinary isoprostane levels[28,29], NADH delivery to the respiratory chain[30], lipid peroxidation[31,32] and HNE levels and associated adducts[17,33]. The greatest indicator of ROS overproduction is the increase in hepatic CYP2E1 levels[32,34-37]. The respiratory chain has also been implicated in mitochondrial ROS overproduction in response to chronic alcohol consumption. Excessive levels of reducing equivalents (*e.g.*, NADH), produced by alcohol and ADH entering the mitochondrial respiratory chain, lead to electron transport chain reduction, facilitating superoxide anion formation[38,25].

Cell death can be triggered through ROS-induced release of apoptosis signal-regulating kinase 1 (ASK1) (a member of the mitogen-activated protein kinase [MAPK] family), resulting in the cleavage of pro-caspase-3 to active caspase-3, which promotes cellular apoptosis[39-41]. Additionally, cytosolic ASK1 activates MAPK kinase 4 and JNK resulting in increased mitochondrial permeability, mediated by SAB protein, and thus hepatocyte cell death[40,41](Figure 2).

Reactive nitrogen species (RNS) also contribute to mitochondrial damage[22,38]. Alcohol-mediated overproduction of the superoxide anion can result in the generation of RNS, such as peroxynitrite, *via* interaction with nitric oxide, culminating in mitochondrial protein damage[25]. Numerous mitochondrial-localized enzymes involved in respiration and cellular energetic processes are inactivated in this way, including NADH dehydrogenase, succinate dehydrogenase, cytochrome *c* reductase and ATP synthase[42].

To limit oxidative damage following alcohol consumption, hepatic mitochondria have various adaptive mechanisms to prevent functional and structural impairments. Uncoupling proteins (UCPs), specifically UCPs 1-3, reduce ROS production by the uncoupling of mitochondrial oxidative phosphorylation[43], a process observed in patients with non-alcoholic fatty liver disease (NAFLD)[44]. Furthermore, there is

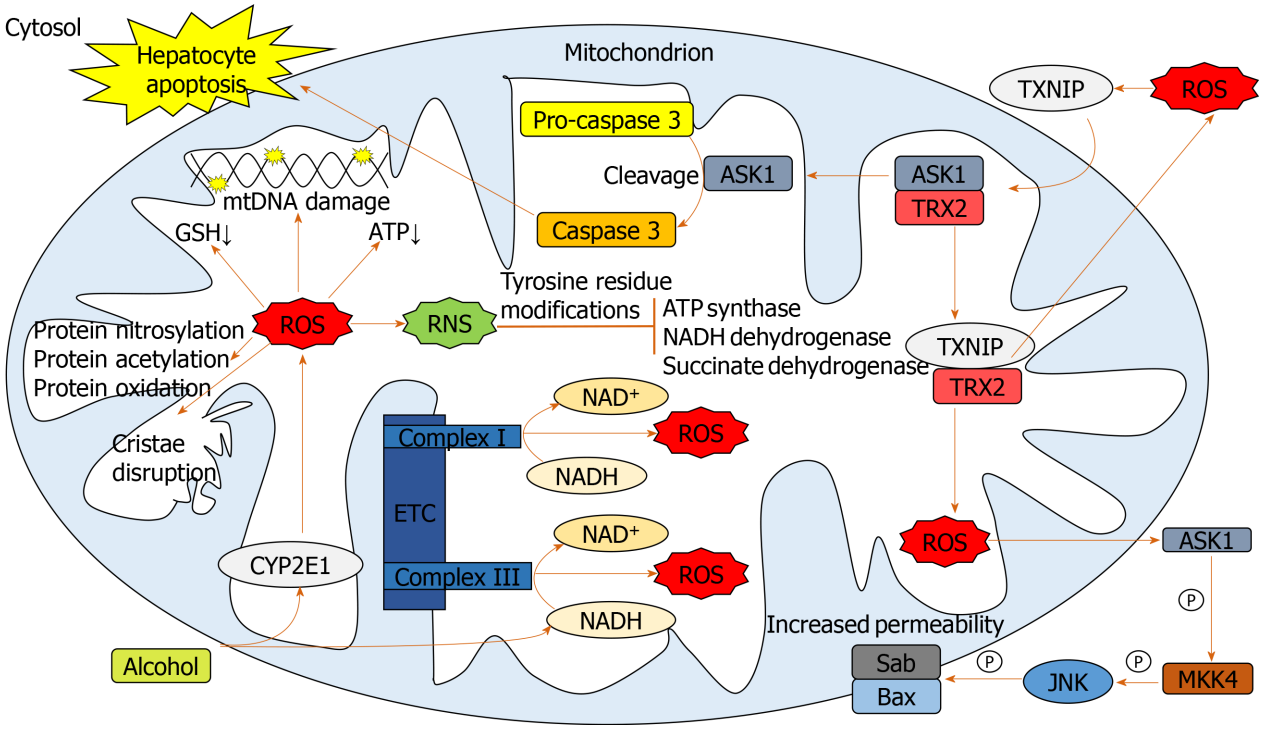

Figure 2 Pathways involved in mediating mitochondrial oxidative stress. Alcohol elevates mitochondrial cytochrome p450 2E1 and NADH levels facilitating reactive oxygen species (ROS) upregulation. Elevated ROS damages mitochondrial DNA, proteins and cristae and causes a reduction in mitochondrial ATP and glutathione. ROS-activated thioredoxin-interacting protein translocates to mitochondria binding thioredoxin 2, indirectly producing further ROS through inhibiting its antioxidant activity. Apoptosis signal-regulating kinase 1 liberated from thioredoxin 2, facilitates cleavage of pro-caspase 3 to caspase 3 leading to hepatocellular apoptosis. Mitochondrial ROS activates cytosolic apoptosis signal-regulating kinase 1 leading to downstream opening of the mitochondrial transition pore through mitogen-activated protein kinase kinase 4 and c-Jun N-terminal kinase activation. ROS form reactive nitrogen species which inhibit mitochondrial enzymes. ASK1: Apoptosis signal-regulating kinase 1; BAX: Bcl-2-associated X protein; CYP2E1: Cytochrome p450 2E1; ETC: Electron transport chain; GSH: Glutathione; JNK: C-Jun N-terminal kinase; MKK4: Mitogen-activated protein kinase kinase 4; mtDNA: Mitochondrial DNA; ROS: Reactive oxygen species; RNS: Reactive nitrogen species; SAB: SH3 domain-binding protein that preferentially associates with Btk; TRX2: Thioredoxin 2; TXNIP: Thioredoxin-interacting protein.

mitochondrial upregulation of enzymatic antioxidants catalase, glutathione transferase and heme oxygenase-1 and a marked increase in GSH[45,46]. However, mitochondrial GSH depletion was observed in patients with alcohol dependence and ALD[15,16] suggesting that chronic alcohol exposure downregulates GSH expression.

Manganese-dependent superoxide dismutase (MnSOD) detoxifies mitochondrial superoxide[47], but its response to alcohol is poorly documented. Increased mitochondrial localization of MnSOD was associated with more severe forms of ALD[48], which may be mediated by increased hydroxyl radical generation[22]. Thus, overexpression of MnSOD may be hepatotoxic rather than hepatoprotective.

S-adenosylmethionine (SAMe) has been implicated in regulating mitochondrial function, following alcohol consumption in a variety of animal models[49]. SAMe binds and inactivates the catalytic activity of CYP2E1[50], limiting alcohol-dependent increases in mitochondrial production of superoxide[49]. SAMe also increases synthesis and availability of glutathione[51] and maintains mitochondrial respiration rate and mtDNA integrity[38]. Although greater SAMe levels have been observed in the serum of ALD patients compared to healthy subjects[52], a reduction in hepatic SAMe levels was observed in patients with AH[53], suggesting the acute inflammatory state leads to hepatic SAMe depletion. SAMe has been evaluated as a treatment for AH in a recent phase 2 randomized controlled clinical trial. SAMe with prednisolone improved 6-mo survival compared to prednisolone treatment alone[54]. Although these preliminary results are encouraging, a definitive study has yet to be undertaken.

CELL SIGNALING PATHWAYS

Lipopolysaccharide (LPS) plays a key role in the pathogenesis of ALD, with higher circulating LPS levels in alcohol dependent patients[55,56]. In AH, LPS predicts organ failure, mortality[57] and infection[58]. Alcohol exposure increases gut permeability, mediating translocation of LPS from the lumen of the intestine to the portal vein into

the liver[55]. LPS binds to Toll-like receptor 4 (TLR4) expressed on a wide variety of immune and parenchymal cells including Kupffer cells, hepatocytes, endothelial cells and hepatic stellate cells, initiating one of the primary signaling cascades associated with liver damage[59,60]. LPS-mediated cell signaling results in transcription of pro-inflammatory genes through nuclear factor-κB (NF-κB) and interferon regulatory factor 3 DNA binding[59,61].

Upon LPS stimulation of the TLR4 complex, NADPH oxidase (NOX) 4 interacts with the COOH-terminal region of TLR4 resulting in ROS generation in neutrophils and monocytes[62,63], which directly activates NF-κB[62,64]. ROS-mediated activation and potential regulation of NF-κB activity occurs by several mechanisms: IκB*a* phosphorylation; S-glutathionylation of IKKβ; disruption of IκB ubiquitination and degradation; NF-κB inducing kinase (NIK) activation and phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt) stimulation[65] (Figure 3). ROS both negatively and positively regulates NF-κB, with oxidative stress in the early phase being a positive regulator, compared to a negative regulator in the late phase[66]. Diphenyliodonium (DPI), an inhibitor of NOX, used as a pre-treatment in alcohol-fed rats, results in normalized ROS production, and inhibition of TNF-α production in Kupffer cells[59,67]. Treatment of alcohol-fed rats with the antioxidant dilinoleoyl-phosphatidylcholine, also inhibited TNF-α production in Kupffer cells and LPS-induced NF-κB activation[68].

Diphenyliodonium and dilinoleoyl-phosphatidylcholine reduce extracellular signal-regulated protein kinase (ERK)1/2 activation[67,68]. LPS-induced activation of ERK1/2 results in transcription of early growth response protein 1 (Egr-1), involved in binding to the TNF-α promoter and increasing TNF-α expression[59]. Egr-1 deficient mice are protected from chronic alcohol-induced liver injury in association with decreased TNF-α messenger RNA (mRNA) levels[69].

LPS activates other MAPKs including p38 and JNK[59], involved in TNF-α production[70]. p38 has been implicated in maintaining the stability of TNF-α mRNA[59,71]. In response to acute alcohol exposure, the JNK pathway has been associated with increased hepatic mitochondrial ROS production[72], increased JNK phosphorylation and AP-1 binding in monocytes[73]. ROS is likely to activate JNK through interaction with upstream MEKK1[65] and by inactivating JNK inhibitor dual specificity protein phosphatase 1[40,74]. ROS have also been associated with activation of cytosolic ASK1[40] (Figure 3). Clinical trials of ASK1 inhibitors as a treatment for inflammatory liver disease are ongoing with a suggestion of reduced fibrosis in patients with NAFLD[75] but no efficacy seen in AH[76].

ROS-mediated S-glutathionylation results in decreased expression of downstream antioxidants such as MnSOD, catalase and Sestrin3 *via* the PI3K/AKT pathway[77]. Akt has also been implicated in increasing oxygen consumption, resulting in elevated mitochondrial generation of H_2O_2, facilitating further oxidative damage[78,79].

The net result of these alcohol-induced cell signaling pathways is the increased production of pro-inflammatory cytokines through upregulation of transcription factors such as AP-1 and NFκB. TNF-α, a key pro-inflammatory cytokine, is highly elevated in patients with ALD and AH[80-82], with observed TNF-α gene expression increasing in ALD patients[83]. TNF-α induces apoptosis through interaction with TNF-α receptor 1 (TNFR1), initiating a cell-death cascade *via* activation of caspases[84]. In ALD, TNF-α-induces mitochondrial peroxidation[55], which is worsened following depletion of GSH[15,85].

TNF-α exacerbates oxidative damage and inflammation *via* a positive feedback loop. Through association with TNFR1, TNF-α stimulates the association of complex I[86], which culminates in MAPK activation (JNK, p38 and ERK). Complex I also directly contributes to ROS accumulation through generation of superoxide, capable of causing further oxidative damage and eventual TNF-α, perpetuating the cycle[40,87,88].

Soluble inflammatory mediators including interleukins have been implicated in ALD[60,89,90] and are associated with outcome in patients with AH[91]. Elevated serum IL-6 levels have recently been identified as a predictor of mortality in severe AH patients[64]. Hepatic upregulation of IL-6 and IL-1β in ALD, results in the differentiation of naïve $CD4^+$ cells into IL-17-producing T-helper 17 cells (Th17) (Figure 4), resulting in elevated hepatic and serum levels of IL-17 observed in ALD patients[64,92]. IL-17 has a multitude of pro-inflammatory downstream effects, including inducing neutrophil recruitment to the liver; stimulating IL-8 and CXCL1 production by hepatic stellate cells[93] and CXCL4, 5 and 6 expression[92,93]. IL-6 and interferon (IFN)-γ are involved in JAK/STAT activation promoting hepatic regeneration[59,94]. Conversely, despite upregulation of IL-6 in ALD patients, downregulation of STAT activation has been observed in human monocytes with chronic alcohol exposure[95].

Inflammasomes propagate IL-1β and IL-18 signals, important in the regulation of hepatic inflammation[94]. ROS mediates IL-1β and IL-18 signaling *via* inflammasome

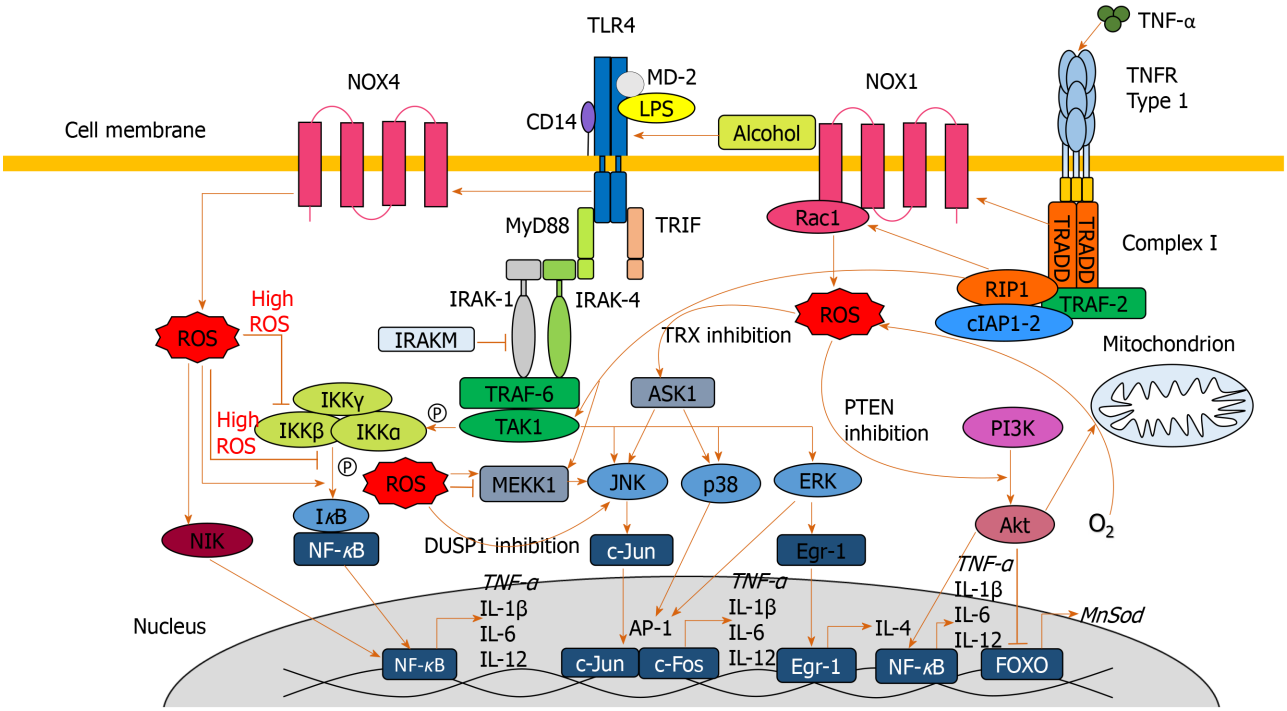

Figure 3 Signaling pathways involved in exacerbating oxidative damage and liver injury. Lipopolysaccharide, alcohol and extracellular reactive oxygen species (ROS) are all capable of activating toll-like receptor 4 leading to myeloid differentiation primary response 88 (MyD88) activation. MyD88 association with interleukin-1 receptor-associated kinase 1-4 results in activation of the tumor necrosis factor receptor-associated factor 6/transforming growth factor beta-activated kinase 1 complex, which activates MAPKs c-Jun N-terminal kinase, p38 and extracellular signal-regulated protein kinase, facilitating transcription factors activator protein 1 and early growth response protein 1 to translocate to the nucleus and upregulate pro-inflammatory mediators. Tumor necrosis factor receptor-associated factor 6/transforming growth factor beta-activated kinase 1-mediated phosphorylation of the IKKα-β-γ complex leads to IκB phosphorylation and nuclear factor κB (NF-κB) translocation to the nucleus to upregulate pro-inflammatory cytokines. MyD88 signaling also activates NADPH oxidase 4 to produces ROS. ROS are also produced by the NADPH oxidase 1/ras-related C3 botulinum toxin substrate 1 complex which is activated upstream by tumour necrosis factor alpha interacting with tumour necrosis factor alpha receptor type 1, at the cell surface, which activates complex I. ROS upregulate NF-κB translocation to the nucleus through IκB phosphorylation, nuclear factor κB inducing kinase activation and indirect protein kinase B activation. At high concentrations, ROS inhibit NF-κB activation through inhibition of IκB phosphorylation and S-glutathionylation of IKKβ. ROS inhibit dual specificity protein phosphatase 1 and thioredoxin to further upregulate the c-Jun N-terminal kinase pathway. ROS inactivation of phosphatase and tensin homolog facilitates phosphoinositide 3-kinase to produce protein kinase B, which elevates ROS levels via increased oxygen consumption, and inactivates forkhead box protein O and downstream antioxidant expression. AKT: Protein kinase B; AP-1: Activator protein 1; ASK1: Apoptosis signal-regulating kinase 1; DUSP1: Dual specificity protein phosphatase 1; Egr-1: Early growth response protein 1; ERK: Extracellular signal-regulated protein kinase; FOXO: Forkhead box protein O; IAP: Inhibitor of apoptosis; IFN: Interferon; IL: Interleukin; IRAK: Interleukin-1 receptor-associated kinase 1; JNK: C-Jun N-terminal kinase; LPS: Lipopolysaccharide; MEKK1: Mitogen-activated protein kinase kinase kinase 1; MyD88: Myeloid differentiation primary response 88; NF-κB: Nuclear factor κB; NIK: Nuclear factor κB inducing kinase; NOX: NADPH oxidase; MnSOD: Manganese-dependent superoxide dismutase; PI3K: Phosphoinositide 3-kinase; PTEN: Phosphatase and tensin homolog; Rac1: Ras-related C3 botulinum toxin substrate 1; ROS: Reactive oxygen species; TAK1: Transforming growth factor beta-activated kinase 1; TLR4: Toll-like receptor 4; TNF-α: Tumour necrosis factor alpha; TNFR1: Tumour necrosis factor alpha receptor 1; TRADD: Tumour necrosis factor alpha receptor 1-associated death domain protein; TRAF: Tumor necrosis factor receptor-associated factor; TRIF: TIR-domain-containing adapter-inducing interferon-β; TRX: Thioredoxin; TXNIP: Thioredoxin-interacting protein.

NLRP3 activation[96,97] and inhibition of antioxidant molecules[41] (Figure 4). Increased production of IL-1β is critical in Th17 differentiation[64,92,98], while IL-18 activates natural killer T-cells (NKTs) to produce IFN-γ[99]. Anti-IL-18 antibodies reduce activation of NF-κB and AP-1, inflammation, liver damage and mortality in animal models[99,100]. IL-1β has also been identified as an activator of MAPKs, including p38, JNK, MEKK1 and IKKβ, involved in mediating upregulation of itself and other pro-inflammatory cytokines[101], creating another positive feedback loop.

TRACE ELEMENTS

Trace elements are a group of naturally occurring minerals that are nutritionally fundamental to basic cellular and immunological functions[102]. An essential role of the these molecules, including zinc, copper, selenium and manganese, is to act as cofactors of anti-oxidant enzymes, making their role imperative in the context of oxidative stress[103,104]. Manganese, copper and zinc are part of the SOD enzyme group that catalyze the breakdown of highly reactive superoxide radicals to H_2O_2 or $O_2^{\cdot}$. Selenium

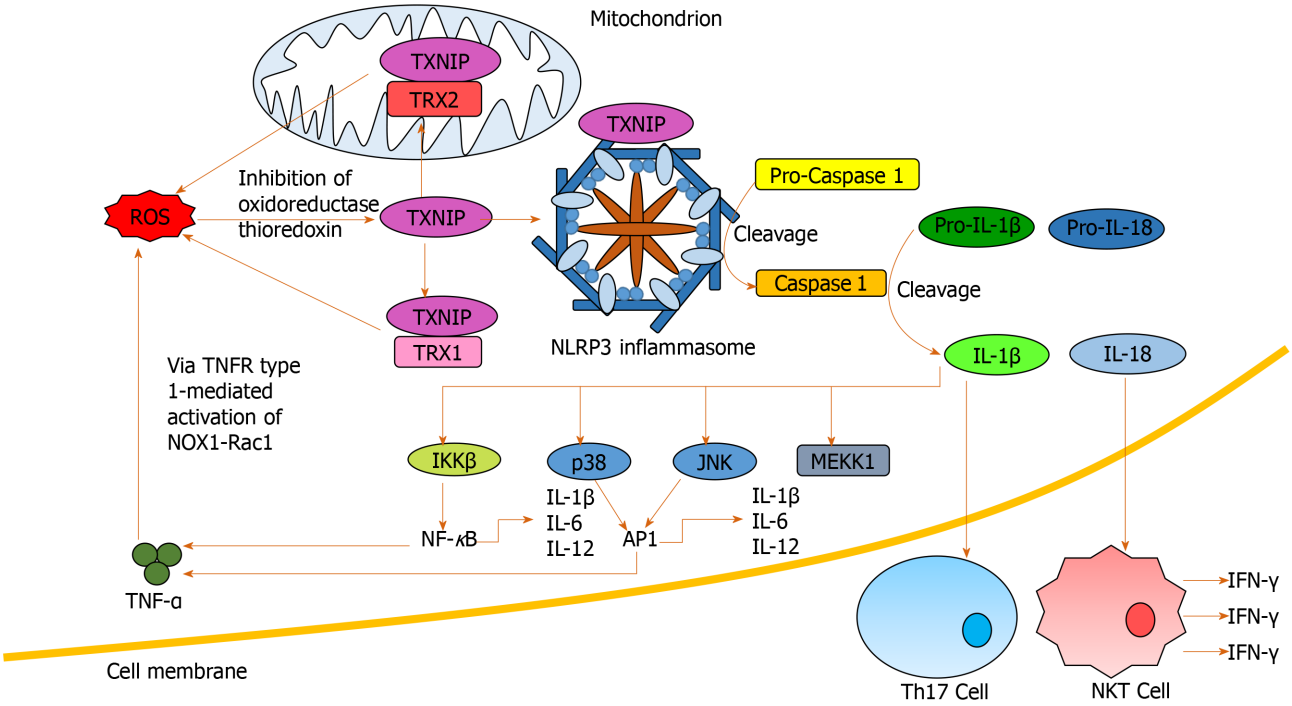

Figure 4 Nucleotide-binding domain, leucine-rich-containing family, pyrin domain-containing-3 inflammasome activation and downstream signaling. Reactive oxygen species (ROS) activate thioredoxin-interacting protein *via* inhibition of oxidoreductase thioredoxin. Thioredoxin-interacting protein both binds and activates nucleotide-binding domain, leucine-rich-containing family, pyrin domain-containing-3 inflammasomes and interacts with thioredoxin 1 and 2 to indirectly promote further ROS generation through inhibiting their antioxidant activity. Activated nucleotide-binding domain, leucine-rich-containing family, pyrin domain-containing-3 inflammasomes facilitate pro-caspase 1 cleavage to caspase 1, which facilitates pro- interleukin (IL)-1β and pro-IL-18 cleavage to IL-1β and IL-18 respectively. IL-18 induces interferon-γ production by natural killer T-cells. IL-1β induces generation of T-helper 17 cells in addition to nuclear factor κB and activator protein 1 activation through IKKβ, p38, c-Jun N-terminal kinase and mitogen-activated protein kinase kinase kinase 1 stimulation. Activating nuclear factor κB and activator protein 1 results in pro-inflammatory cytokine release, indirectly inducing further ROS accumulation. AP-1: Activator protein 1; IFN: Interferon; IL: Interleukin; JNK: C-Jun N-terminal kinase; MEKK1: Mitogen-activated protein kinase kinase kinase 1; NF-κB: Nuclear factor κB; NKT: Natural killer T-cell; NLRP3: Nucleotide-binding domain, leucine-rich-containing family, pyrin domain-containing-3; NOX: NADPH oxidase; Rac1: Ras-related C3 botulinum toxin substrate 1; ROS: Reactive oxygen species; Th17: T-helper 17 cells; TNF-α: Tumour necrosis factor alpha; TNFR1: Tumour necrosis factor alpha receptor 1; TRX: Thioredoxin; TXNIP: Thioredoxin-interacting protein.

is a component of the active site of glutathione peroxides (GPx), the main function of which is the neutralization of hydrogen peroxide[105]. These enzyme systems are crucial in counterbalancing the oxidative stress state and are impaired in chronic liver disease[106].

Reduced serum levels of trace elements have been confirmed in patients with liver disease, including ALD, and correlate with severity[107-110]. Decreased zinc is associated with liver cirrhosis in alcohol dependent individuals[111] and reduced serum levels of zinc, copper and iron have been observed when compared with healthy controls[112]. Zinc is a crucial trace element involved in multiple cellular and metabolic pathways[113] as well as acting as a cofactor for ALDH. Deficiency or abnormality in zinc function is implicated multiple pathologies, including liver disease (both acute and chronic)[114,115] and is associated with immune dysfunction evidenced by increased inflammation and aberrant immune cell activation[116]. Zinc deficiency in endothelial cells results in increased oxidative stress and decreased inflammatory regulation which is corrected or partially ameliorated by zinc supplementation[117,118]. In alcohol-fed mice, zinc deficiency worsens the balance between hepatic pro- and antioxidant enzymes[119] and is associated with accumulation of ROS in gut epithelial cells and disruption of tight junctions[120]. Given zinc's influence on antioxidant responses, gut integrity and immune function, a trial of zinc supplementation to improve clinical outcomes in patients with ALD cirrhosis is ongoing (NCT02072746). Preliminary reports suggest zinc supplementation is associated with a reduction in liver inflammation and improvement in immune function[121].

Antioxidant therapy may also have a benefit in the treatment of AH. An antioxidant cocktail (including zinc and selenium) in combination with steroids for the treatment of severe AH correlated with a significant reduction in serum biomarkers, improved short-term prognosis and reduced length of stay in hospital[122]. However, a subsequent

study of a complex regimen of N-acetylcysteine (NAC) followed by antioxidant therapy, alone or in conjunction with steroids, reduced renal injury but resulted in no survival benefit over 6 mo[121]. Another clinical trial of steroids combined with NAC in AH showed reduced infection rate but not mortality at 6 mo[123]. Antioxidants have also been shown to have a protective effect in patients with NAFLD by reducing serum levels of alanine transaminase (ALT) and spleen size, a finding that likely correlates with an improvement of fatty infiltration[122]. These findings suggest that targeting or counterbalancing oxidative stress in ALD patients may improve patient outcomes.

EPIGENETICS

Lifestyle and environmental factors can modify gene expression without altering the DNA sequence, which gets transmitted to the next generation of cells after mitotic division, termed epigenetics[123]. Epigenetic regulation includes both DNA and histone protein modifications as well as action through non-coding micro RNAs[123]. DNA methylation is the most abundant epigenetic modification that directly affects the function of a gene in eukaryotes[124]. Acetylation and deacetylation are modifications in histone proteins carried out by two enzyme families, histone deacetylases (HDACs) and histone acetyl transferase (HAT)[124]. Histone modifying enzymes contribute to the activation or inactivation of transcription by catalyzing the unfolding or further compaction, respectively, of chromatin structure[124].

Excessive ROS is involved in epigenetic gene activation or silencing by changing DNA methylation levels[125]. ROS production induces alterations in DNA methylation patterns and global histone acetylation, which then lead to aberrant gene expression, and may contribute to the process of carcinogenesis[124]. The reduction of global histone acetylation in short term oxidative stress might be due to an immediate increase of class I/II HDAC activity by an unknown mechanism[126,127]. Class III HDAC (Sirtuin NAD^+-dependent family of protein deacetylases) has been hypothesized to be upregulated under oxidative stress because NAD^+ levels increase in the mitochondria under oxidative stress conditions but direct evidence is lacking[126].

Alcohol consumption increases gene-selective acetylation of histone H3 at lysine 9 (H3K9), levels of enzymes mediating histone acetylation, and results in a generalized increase in DNA methylation[126,127]. These epigenetic-mediated effects of alcohol consumption regulate the inflammatory response, through key pro-inflammatory cytokines, such as TNF-α, which is silenced by H3K9 methylation and activated by H3K9 acetylation[128]. In a macrophage cell line, alcohol treatment resulted in global increased histone H3 and H4 acetylation and specifically increased acetylation of pro-inflammatory gene histones[129].

Oxidative stress itself is an important regulator of epigenetic processes by inhibition of HDAC expression[130]. This takes place *via* activation of PI3Kδ, a signalling molecule controlling many inflammatory signalling pathways[131]. Drugs that inhibit PI3Kδ (*e.g.*, theophylline, nortriptyline and specific inhibitors) reduce oxidative stress in *in vitro* and *in vivo* models of lung disease[132]. In patients with AH, there is *in vitro* evidence that theophylline can enhance response to corticosteroid treatment, which may be mediated by its epigenetic effects[133]. Targeting epigenetic regulation has recently been shown to have a beneficial effect in patients with AH; a novel sulphated oxysterol, DUR-928, was well tolerated and improved liver biochemistry in a small phase 2 clinical trial in AH[134].

Activation of the transcription factor Nrf2 is central to cellular defence against ROS[135]. Its negative regulator, kelch-like ECM-associated protein 1 (Keap1), promotes proteasomal degradation of Nrf2. ROS decouples Nrf2 from Keap1, allowing it to translocate to the nucleus to bind to antioxidant response elements (AREs), initiating a range of antioxidant processes[135,136]. Both Nrf2 and Keap1 expression are influenced by epigenetics with evidence of DNA hypermethylation in the Nrf2 promoter[135,136] and Keap1 promoter[137]. Histone acetylation and deacetylation also modify ARE-dependent gene expression with Class 1 HDACs reducing Nrf2[138]. Conversely, HDAC inhibitors restore Nrf2 expression and antioxidant responses. Targeting epigenetic regulation of Nrf2/Keap1 to ameliorate oxidative stress induced inhibition of antioxidant responses is an appealing strategy[138]. However, much of this work has been performed in cancer cell lines and needs further investigation in the context of ALD.

IMPLICATIONS FOR THERAPY OF ALD

An improved understanding of the detailed mechanisms by which oxidative stress influences liver damage in patients with ALD may yield new targets for therapy. Current data from pre-clinical and clinical studies suggest potential new avenues for therapy of ALD.

MITOCHONDRIAL STRESS

Chronic alcohol consumption results in significant mitochondrial ROS generation leading to morphological and functional changes. Preventing ROS generation may ameliorate this process. Pre-clinical and early phase clinical studies have shown promise of this approach with SAMe. A systematic review and meta-analysis of 11 randomized controlled trials of SAMe treatment for chronic liver disease concluded that it improved liver biochemistry (bilirubin and AST) and had a good safety profile but did not affect mortality[139]. Long term SAMe treatment in patients with ALD does not appear to be clinically effective with no reduction in adverse events or mortality in the two included studies performed in patients with ALD[140,141]. However, short term treatment of the acute mitochondrial stress seen in AH may be a better strategy for the use of SAMe. A phase 2 clinical trial of SAMe with prednisolone for the treatment of severe AH demonstrated improved response rate measured by Lille score and a reduction in hepatorenal syndrome[54]. However, there was no statistically significant difference in 28-d mortality. It may yet prove to be an effective adjunct to anti-inflammatory therapy for AH.

UCPs are strongly associated with mitochondrial stress in ALD. Overexpression of UCP2 reduces apoptosis and oxidative stress *in vitro*[142]. Hepatocellular downregulated mitochondrial carrier protein (HDMCP) expression induced uncoupling and reduced steatosis in an animal model of NAFLD[143]. However, such an approach may promote hepatocyte necrosis and increase the risk of hepatocellular carcinoma[144]. Further studies in this area are required to determine whether targeting UCPs would be a beneficial therapeutic strategy.

ANTIOXIDANT THERAPY

NAC, an antioxidant therapy that provides cysteine for glutathione synthesis, has been tested in patients with AH. Although initial trials did not demonstrate a survival benefit[145,146]. a more recent study of NAC in combination with prednisolone, showed a reduction in infective events and 1-month mortality[147]. Therefore, NAC has been suggested for the treatment of AH in clinical practice guidelines, with the caveat that a definitive randomized controlled trial is still required[148].

Deficiency of key trace elements is associated with oxidative stress, which is ameliorated by supplementation. Antioxidant therapy including zinc and other trace elements has shown clinical benefit in patients with AH[122]. However, interpretation is hampered by use of a variety of antioxidants at differing concentrations and durations[145,146]. A trial of long-term zinc supplementation in ALD patients has demonstrated improvements in short-term immune function[149] with long-term clinical outcomes due to be reported shortly. Improved understanding of the role of trace elements in ALD and the optimal formulation and duration of treatment is required.

EPIGENETIC REGULATION

Oxidative stress reduces HDAC expression *via* PI3Kδ activation resulting in increased expression of pro-inflammatory genes. Studies targeting HDACs have yet to be performed in patients with ALD. Although *in vitro* studies suggest an antioxidant effect of HDAC inhibition with upregulation of Nrf2 expression[138], HDAC inhibitors approved for use in the treatment of cancer induce cell cycle arrest, apoptosis and oxidative stress in cancer cells which overexpress HDAC[150]. The effect of HDAC inhibitors in the context of ALD requires careful *in vitro* confirmation before clinical translation. However, targeting PI3Kδ is a more appealing strategy with evidence from the respiratory field that specific inhibitors reduce oxidative stress *in vitro* and *in vivo*[132].

CONCLUSION

Alcohol is a major global healthcare and economic burden and is a growing cause of chronic liver disease. However, there are currently no effective therapies to treat ALD. Oxidative stress is involved in multiple aspects of ALD pathogenesis (Figure 5). Chronic alcohol consumption results in the saturation of the ADH pathway and increased CYP2E1-mediated alcohol metabolism. This leads to the generation of reactive species including MAA, HNE, lipid hydroperoxides, RNS and ROS, which cause hepatic damage *via* lipid and protein peroxidation, adduct formation and cellular hyper-regulation. Similar damage occurs in hepatic mitochondria with ROS inducing structural and functional damage. ROS cause oxidative damage through multiple mechanisms: Promoting cell death *via* protein mediators, increasing and sustaining the upregulation of pro-inflammatory mediators, as well as inducing multiple epigenetic modifications.

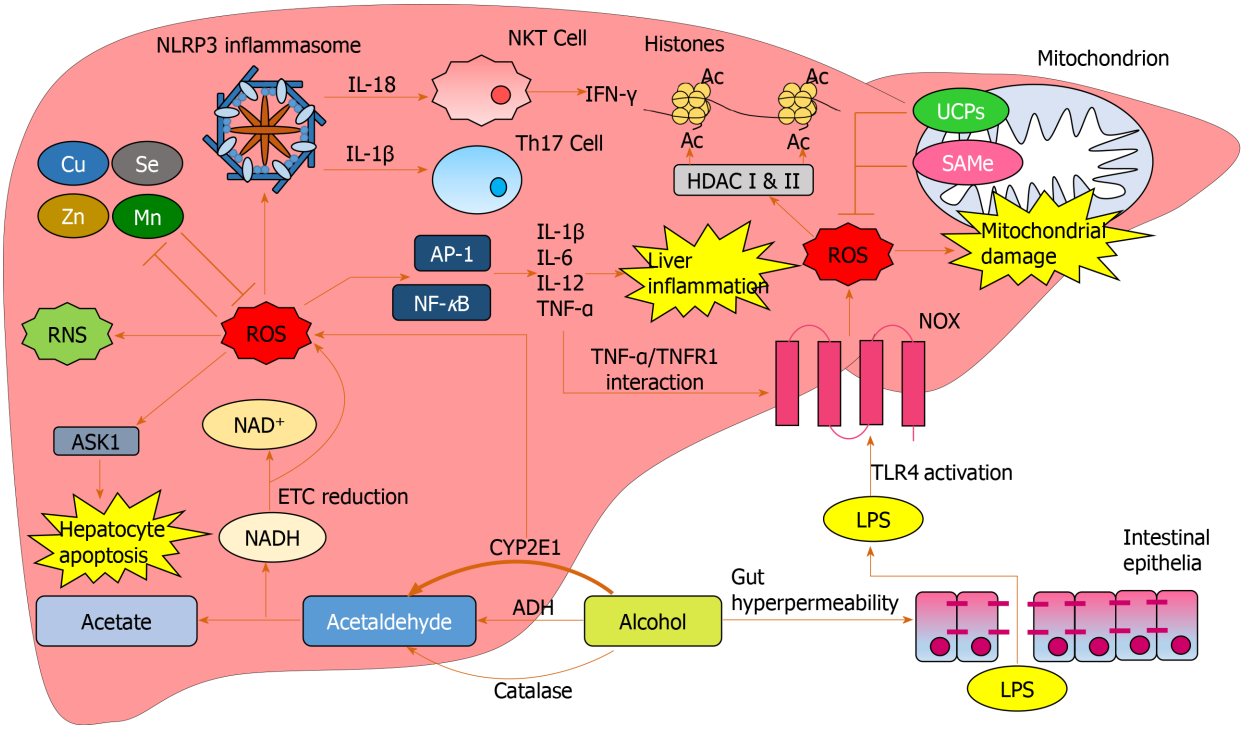

Figure 5 Reactive oxygen species-mediated oxidative damage in the liver. Increased cytochrome p450 2E1-mediated alcohol breakdown and electron transport chain reduction results in overproduction of reactive oxygen species (ROS). Excess alcohol causes gut hyperpermeability resulting in tight junction disruption and an excess of lipopolysaccharide translocation from the gut to the liver. Lipopolysaccharide activates NADPH oxidase *via* toll-like receptor 4 activation resulting in further ROS production. Excess ROS produce RNS and reduce antioxidant cofactors such as Mn and Zn. ROS induce hepatocyte damage through activation of apoptosis signal-regulating kinase 1. Nucleotide-binding domain, leucine-rich-containing family, pyrin domain-containing-3 inflammasomes are activated by ROS, inducing T-helper 17 generation and natural killer T cell-mediated interferon-γ production through interleukin expression. ROS upregulate transcription factors activator protein 1 and nuclear factor κB resulting in pro-inflammatory cytokine expression causing downstream liver inflammation. Tumour necrosis factor alpha further upregulates ROS through activating NADPH oxidase *via* tumour necrosis factor alpha receptor 1. ROS cause an array of functional and structural mitochondrial damage, which is initially impeded by uncoupling proteins and SAMe expression. ROS mediates epigenetic alterations through interacting with HDACs which mediate histone acetylation. Ac: Acetylation; ADH: Alcohol dehydrogenase; AP-1: Activator protein 1; ASK1: Apoptosis signal-regulating kinase 1; Cu: Copper; CYP2E1: Cytochrome p450 2E1; ETC: Electron transport chain; HDAC: Histone deacetylases; IFN: Interferon; IL: Interleukin; LPS: Lipopolysaccharide; Mn: Manganese; NF-κB: Nuclear factor κB; NKT: Natural killer T-cell; NOX: NADPH oxidase; ROS: Reactive oxygen species; SAMe: *S*-adenosylmethionine; Se: Selenium; Th17: T-helper 17 cells; TLR4: Toll-like receptor 4; TNF-α: Tumour necrosis factor alpha; TNFR1: Tumour necrosis factor alpha receptor 1; UCP: Uncoupling protein; Zn: Zinc.

REFERENCES

1 **Pimpin L**, Cortez-Pinto H, Negro F, Corbould E, Lazarus JV, Webber L, Sheron N; EASL HEPAHEALTH Steering Committee. Burden of liver disease in Europe: Epidemiology and analysis of risk factors to identify prevention policies. *J Hepatol* 2018; **69**: 718-735 [PMID: 29777749 DOI: 10.1016/j.jhep.2018.05.011]

2 **Bhattacharya A**. Which cost of alcohol? What should we compare it against? *Addiction* 2017; **112**: 559-565 [PMID: 26970215 DOI: 10.1111/add.13335]

3 **Anderson P**, Baumberg B. Alcohol in Europe-Public Health Perspective: Report summary. *Drugs Educ Prev Policy* 2006; **13**: 483-488 [DOI: 10.1080/09687630600902477]

4 **Ohashi K**, Pimienta M, Seki E. Alcoholic liver disease: A current molecular and clinical perspective. *Liver Res* 2018; **2**: 161-172 [PMID: 31214376 DOI: 10.1016/j.livres.2018.11.002]

5 **Philips CA**, Augustine P, Yerol PK, Rajesh S, Mahadevan P. Severe alcoholic hepatitis: current perspectives. *Hepat Med* 2019; **11**: 97-108 [PMID: 31496843 DOI: 10.2147/HMER.S197933]

6 **Bennett K**, Enki DG, Thursz M, Cramp ME, Dhanda AD. Systematic review with meta-analysis: high mortality in patients with non-severe alcoholic hepatitis. *Aliment Pharmacol Ther* 2019; **50**: 249-257 [PMID: 31231848 DOI: 10.1111/apt.15376]

7 **Li S**, Tan HY, Wang N, Zhang ZJ, Lao L, Wong CW, Feng Y. The Role of Oxidative Stress and Antioxidants in Liver Diseases. *Int J Mol Sci* 2015; **16**: 26087-26124 [PMID: 26540040 DOI: 10.3390/ijms161125942]

8 **Cederbaum AI**, Lu Y, Wu D. Role of oxidative stress in alcohol-induced liver injury. *Arch Toxicol* 2009; **83**: 519-548 [PMID: 19448996 DOI: 10.1007/s00204-009-0432-0]

9 **Willis MS**, Klassen LW, Tuma DJ, Sorrell MF, Thiele GM. Adduction of soluble proteins with malondialdehyde-acetaldehyde (MAA) induces antibody production and enhances T-cell proliferation. *Alcohol Clin Exp Res* 2002; **26**: 94-106 [PMID: 11821659 DOI: 10.1111/j.1530-0277.2002.tb02436.x]

10 **Lieber CS**, Rubin E, DeCarli LM. Hepatic microsomal ethanol oxidizing system (MEOS): differentiation from alcohol dehydrogenase and NADPH oxidase. *Biochem Biophys Res Commun* 1970; **40**: 858-865 [PMID: 4395603 DOI:

10.1016/0006-291X(70)90982-4]
11 **Pérez MJ**, Cederbaum AI. Metallothionein 2A induction by zinc protects HEPG2 cells against CYP2E1-dependent toxicity. *Free Radic Biol Med* 2003; **34**: 443-455 [PMID: 12566070 DOI: 10.1016/S0891-5849(02)01302-3]
12 **Weltman MD**, Farrell GC, Hall P, Ingelman-Sundberg M, Liddle C. Hepatic cytochrome P450 2E1 is increased in patients with nonalcoholic steatohepatitis. *Hepatology* 1998; **27**: 128-133 [PMID: 9425928 DOI: 10.1002/hep.510270121]
13 **Lieber CS**, Seitz HK, Garro AJ, Worner TM. Alcohol-related diseases and carcinogenesis. *Cancer Res* 1979; **39**: 2863-2886 [PMID: 221110]
14 **Seitz HK**, Stickel F. Risk factors and mechanisms of hepatocarcinogenesis with special emphasis on alcohol and oxidative stress. *Biol Chem* 2006; **387**: 349-360 [PMID: 16606331 DOI: 10.1515/BC.2006.047]
15 **Albano E**. Oxidative mechanisms in the pathogenesis of alcoholic liver disease. *Mol Aspects Med* 2008; **29**: 9-16 [PMID: 18045675 DOI: 10.1016/j.mam.2007.09.004]
16 **Fernandez-Checa JC**, Kaplowitz N. Hepatic mitochondrial glutathione: transport and role in disease and toxicity. *Toxicol Appl Pharmacol* 2005; **204**: 263-273 [PMID: 15845418 DOI: 10.1016/j.taap.2004.10.001]
17 **Setshedi M**, Wands JR, Monte SM. Acetaldehyde adducts in alcoholic liver disease. *Oxid Med Cell Longev* 2010; **3**: 178-185 [PMID: 20716942 DOI: 10.4161/oxim.3.3.12288]
18 **Nair J**, Srivatanakul P, Haas C, Jedpiyawongse A, Khuhaprema T, Seitz HK, Bartsch H. High urinary excretion of lipid peroxidation-derived DNA damage in patients with cancer-prone liver diseases. *Mutat Res* 2010; **683**: 23-28 [PMID: 19822158 DOI: 10.1016/j.mrfmmm.2009.10.002]
19 **Abdelmegeed MA**, Choi Y, Ha SK, Song BJ. Cytochrome P450-2E1 promotes aging-related hepatic steatosis, apoptosis and fibrosis through increased nitroxidative stress. *Free Radic Biol Med* 2016; **91**: 188-202 [PMID: 26703967 DOI: 10.1016/j.freeradbiomed.2015.12.016]
20 **Handler JA**, Thurman RG. Hepatic ethanol metabolism is mediated predominantly by catalase-H2O2 in the fasted state. *FEBS Lett* 1988; **238**: 139-141 [PMID: 3169246 DOI: 10.1016/0014-5793(88)80243-6]
21 **Nassir F**, Ibdah JA. Role of mitochondria in alcoholic liver disease. *World J Gastroenterol* 2014; **20**: 2136-2142 [PMID: 24605012 DOI: 10.3748/wjg.v20.i9.2136]
22 **Das SK**, Vasudevan DM. Alcohol-induced oxidative stress. *Life Sci* 2007; **81**: 177-187 [PMID: 17570440 DOI: 10.1016/j.lfs.2007.05.005]
23 **Venkatraman A**, Landar A, Davis AJ, Chamlee L, Sanderson T, Kim H, Page G, Pompilius M, Ballinger S, Darley-Usmar V, Bailey SM. Modification of the mitochondrial proteome in response to the stress of ethanol-dependent hepatotoxicity. *J Biol Chem* 2004; **279**: 22092-22101 [PMID: 15033988 DOI: 10.1074/jbc.M402245200]
24 **Grattagliano I**, Russmann S, Diogo C, Bonfrate L, Oliveira PJ, Wang DQ, Portincasa P. Mitochondria in chronic liver disease. *Curr Drug Targets* 2011; **12**: 879-893 [PMID: 21269263 DOI: 10.2174/138945011795528877]
25 **García-Ruiz C**, Kaplowitz N, Fernandez-Checa JC. Role of Mitochondria in Alcoholic Liver Disease. *Curr Pathobiol Rep* 2013; **1**: 159-168 [PMID: 25343061 DOI: 10.1007/s40139-013-0021-z]
26 **Hoek JB**, Cahill A, Pastorino JG. Alcohol and mitochondria: a dysfunctional relationship. *Gastroenterology* 2002; **122**: 2049-2063 [PMID: 12055609 DOI: 10.1053/gast.2002.33613]
27 **Beier JI**, McClain CJ. Mechanisms and cell signaling in alcoholic liver disease. *Biol Chem* 2010; **391**: 1249-1264 [PMID: 20868231 DOI: 10.1515/BC.2010.137]
28 **Meagher EA**, Barry OP, Burke A, Lucey MR, Lawson JA, Rokach J, FitzGerald GA. Alcohol-induced generation of lipid peroxidation products in humans. *J Clin Invest* 1999; **104**: 805-813 [PMID: 10491416 DOI: 10.1172/JCI5584]
29 **Morrow JD**. The isoprostanes: their quantification as an index of oxidant stress status in vivo. *Drug Metab Rev* 2000; **32**: 377-385 [PMID: 11139135 DOI: 10.1081/DMR-100102340]
30 **Mantena SK**, King AL, Andringa KK, Landar A, Darley-Usmar V, Bailey SM. Novel interactions of mitochondria and reactive oxygen/nitrogen species in alcohol mediated liver disease. *World J Gastroenterol* 2007; **13**: 4967-4973 [PMID: 17854139 DOI: 10.3748/wjg.v13.i37.4967]
31 **Ekström G**, Ingelman-Sundberg M. Rat liver microsomal NADPH-supported oxidase activity and lipid peroxidation dependent on ethanol-inducible cytochrome P-450 (P-450IIE1). *Biochem Pharmacol* 1989; **38**: 1313-1319 [PMID: 2495801 DOI: 10.1016/0006-2952(89)90338-9]
32 **Seth D**, Haber PS, Syn WK, Diehl AM, Day CP. Pathogenesis of alcohol-induced liver disease: classical concepts and recent advances. *J Gastroenterol Hepatol* 2011; **26**: 1089-1105 [PMID: 21545524 DOI: 10.1111/j.1440-1746.2011.06756.x]
33 **Roede JR**, Jones DP. Reactive species and mitochondrial dysfunction: mechanistic significance of 4-hydroxynonenal. *Environ Mol Mutagen* 2010; **51**: 380-390 [PMID: 20544880 DOI: 10.1002/em.20553]
34 **Niemelä O**, Parkkila S, Juvonen RO, Viitala K, Gelboin HV, Pasanen M. Cytochromes P450 2A6, 2E1, and 3A and production of protein-aldehyde adducts in the liver of patients with alcoholic and non-alcoholic liver diseases. *J Hepatol* 2000; **33**: 893-901 [PMID: 11131450 DOI: 10.1016/S0168-8278(00)80120-8]
35 **Takahashi T**, Lasker JM, Rosman AS, Lieber CS. Induction of cytochrome P-4502E1 in the human liver by ethanol is caused by a corresponding increase in encoding messenger RNA. *Hepatology* 1993; **17**: 236-245 [PMID: 8428720 DOI: 10.1002/hep.1840170213]
36 **Yun JW**, Son MJ, Abdelmegeed MA, Banerjee A, Morgan TR, Yoo SH, Song BJ. Binge alcohol promotes hypoxic liver injury through a CYP2E1-HIF-1α-dependent apoptosis pathway in mice and humans. *Free Radic Biol Med* 2014; **77**: 183-194 [PMID: 25236742 DOI: 10.1016/j.freeradbiomed.2014.08.030]
37 **Adam-Vizi V**. Production of reactive oxygen species in brain mitochondria: contribution by electron transport chain and non-electron transport chain sources. *Antioxid Redox Signal* 2005; **7**: 1140-1149 [PMID: 16115017 DOI: 10.1089/ars.2005.7.1140]
38 **Manzo-Avalos S**, Saavedra-Molina A. Cellular and mitochondrial effects of alcohol consumption. *Int J Environ Res Public Health* 2010; **7**: 4281-4304 [PMID: 21318009 DOI: 10.3390/ijerph7124281]
39 **Harijith A**, Ebenezer DL, Natarajan V. Reactive oxygen species at the crossroads of inflammasome and inflammation. *Front Physiol* 2014; **5**: 352 [PMID: 25324778 DOI: 10.3389/fphys.2014.00352]

40 **Seki E**, Brenner DA, Karin M. A liver full of JNK: signaling in regulation of cell function and disease pathogenesis, and clinical approaches. *Gastroenterology* 2012; **143**: 307-320 [PMID: 22705006 DOI: 10.1053/j.gastro.2012.06.004]

41 **Gunawan BK**, Liu ZX, Han D, Hanawa N, Gaarde WA, Kaplowitz N. c-Jun N-terminal kinase plays a major role in murine acetaminophen hepatotoxicity. *Gastroenterology* 2006; **131**: 165-178 [PMID: 16831600 DOI: 10.1053/j.gastro.2006.03.045]

42 **Pacher P**, Beckman JS, Liaudet L. Nitric oxide and peroxynitrite in health and disease. *Physiol Rev* 2007; **87**: 315-424 [PMID: 17237348 DOI: 10.1152/physrev.00029.2006]

43 **Mailloux RJ**, Harper ME. Uncoupling proteins and the control of mitochondrial reactive oxygen species production. *Free Radic Biol Med* 2011; **51**: 1106-1115 [PMID: 21762777 DOI: 10.1016/j.freeradbiomed.2011.06.022]

44 **Chavin KD**, Yang S, Lin HZ, Chatham J, Chacko VP, Hoek JB, Walajtys-Rode E, Rashid A, Chen CH, Huang CC, Wu TC, Lane MD, Diehl AM. Obesity induces expression of uncoupling protein-2 in hepatocytes and promotes liver ATP depletion. *J Biol Chem* 1999; **274**: 5692-5700 [PMID: 10026188 DOI: 10.1074/jbc.274.9.5692]

45 **Cederbaum AI**. Cytochrome P450 2E1-dependent oxidant stress and upregulation of anti-oxidant defense in liver cells. *J Gastroenterol Hepatol* 2006; **21** Suppl 3: S22-S25 [PMID: 16958665 DOI: 10.1111/j.1440-1746.2006.04595.x]

46 **Marí M**, Cederbaum AI. CYP2E1 overexpression in HepG2 cells induces glutathione synthesis by transcriptional activation of gamma-glutamylcysteine synthetase. *J Biol Chem* 2000; **275**: 15563-15571 [PMID: 10748080 DOI: 10.1074/jbc.M907022199]

47 **Candas D**, Li JJ. MnSOD in oxidative stress response-potential regulation via mitochondrial protein influx. *Antioxid Redox Signal* 2014; **20**: 1599-1617 [PMID: 23581847 DOI: 10.1089/ars.2013.5305]

48 **Degoul F**, Sutton A, Mansouri A, Cepanec C, Degott C, Fromenty B, Beaugrand M, Valla D, Pessayre D. Homozygosity for alanine in the mitochondrial targeting sequence of superoxide dismutase and risk for severe alcoholic liver disease. *Gastroenterology* 2001; **120**: 1468-1474 [PMID: 11313317 DOI: 10.1053/gast.2001.24051]

49 **Andringa KK**, King AL, Eccleston HB, Mantena SK, Landar A, Jhala NC, Dickinson DA, Squadrito GL, Bailey SM. Analysis of the liver mitochondrial proteome in response to ethanol and S-adenosylmethionine treatments: novel molecular targets of disease and hepatoprotection. *Am J Physiol Gastrointest Liver Physiol* 2010; **298**: G732-G745 [PMID: 20150243 DOI: 10.1152/ajpgi.00332.2009]

50 **Cederbaum AI**. Hepatoprotective effects of S-adenosyl-L-methionine against alcohol- and cytochrome P450 2E1-induced liver injury. *World J Gastroenterol* 2010; **16**: 1366-1376 [PMID: 20238404 DOI: 10.3748/wjg.v16.i11.1366]

51 **Fernández-Checa JC**, Colell A, García-Ruiz C. S-Adenosyl-L-methionine and mitochondrial reduced glutathione depletion in alcoholic liver disease. *Alcohol* 2002; **27**: 179-183 [PMID: 12163147 DOI: 10.1016/S0741-8329(02)00229-X]

52 **Mudd SH**, Wagner C, Luka Z, Stabler SP, Allen RH, Schroer R, Wood T, Wang J, Wong LJ. Two patients with hepatic mtDNA depletion syndromes and marked elevations of S-adenosylmethionine and methionine. *Mol Genet Metab* 2012; **105**: 228-236 [PMID: 22137549 DOI: 10.1016/j.ymgme.2011.11.006]

53 **Lee TD**, Sadda MR, Mendler MH, Bottiglieri T, Kanel G, Mato JM, Lu SC. Abnormal hepatic methionine and glutathione metabolism in patients with alcoholic hepatitis. *Alcohol Clin Exp Res* 2004; **28**: 173-181 [PMID: 14745316 DOI: 10.1097/01.ALC.0000108654.77178.03]

54 **Tkachenko P**, Maevskaya M, Pavlov A, Komkova I, Pavlov C, Ivashkin V. Prednisolone plus S-adenosil-L-methionine in severe alcoholic hepatitis. *Hepatol Int* 2016; **10**: 983-987 [PMID: 27337960 DOI: 10.1007/s12072-016-9751-4]

55 **Lucey MR**, Mathurin P, Morgan TR. Alcoholic hepatitis. *N Engl J Med* 2009; **360**: 2758-2769 [PMID: 19553649 DOI: 10.1056/NEJMra0805786]

56 **Fujimoto M**, Uemura M, Nakatani Y, Tsujita S, Hoppo K, Tamagawa T, Kitano H, Kikukawa M, Ann T, Ishii Y, Kojima H, Sakurai S, Tanaka R, Namisaki T, Noguchi R, Higashino T, Kikuchi E, Nishimura K, Takaya A, Fukui H. Plasma endotoxin and serum cytokine levels in patients with alcoholic hepatitis: relation to severity of liver disturbance. *Alcohol Clin Exp Res* 2000; **24**: 48S-54S [PMID: 10803780 DOI: 10.1111/j.1530-0277.2000.tb00012.x]

57 **Michelena J**, Altamirano J, Abraldes JG, Affò S, Morales-Ibanez O, Sancho-Bru P, Dominguez M, García-Pagán JC, Fernández J, Arroyo V, Ginès P, Louvet A, Mathurin P, Mehal WZ, Caballería J, Bataller R. Systemic inflammatory response and serum lipopolysaccharide levels predict multiple organ failure and death in alcoholic hepatitis. *Hepatology* 2015; **62**: 762-772 [PMID: 25761863 DOI: 10.1002/hep.27779]

58 **Vergis N**, Atkinson SR, Knapp S, Maurice J, Allison M, Austin A, Forrest EH, Masson S, McCune A, Patch D, Richardson P, Gleeson D, Ryder SD, Wright M, Thursz MR. In Patients With Severe Alcoholic Hepatitis, Prednisolone Increases Susceptibility to Infection and Infection-Related Mortality, and Is Associated With High Circulating Levels of Bacterial DNA. *Gastroenterology* 2017; **152**: 1068-1077.e4 [PMID: 28043903 DOI: 10.1053/j.gastro.2016.12.019]

59 **Mandrekar P**, Szabo G. Signalling pathways in alcohol-induced liver inflammation. *J Hepatol* 2009; **50**: 1258-1266 [PMID: 19398236 DOI: 10.1016/j.jhep.2009.03.007]

60 **Seki E**, Brenner DA. Toll-like receptors and adaptor molecules in liver disease: update. *Hepatology* 2008; **48**: 322-335 [PMID: 18506843 DOI: 10.1002/hep.22306]

61 **Li P**, He K, Li J, Liu Z, Gong J. The role of Kupffer cells in hepatic diseases. *Mol Immunol* 2017; **85**: 222-229 [PMID: 28314211 DOI: 10.1016/j.molimm.2017.02.018]

62 **Park HS**, Jung HY, Park EY, Kim J, Lee WJ, Bae YS. Cutting edge: direct interaction of TLR4 with NAD(P)H oxidase 4 isozyme is essential for lipopolysaccharide-induced production of reactive oxygen species and activation of NF-kappa B. *J Immunol* 2004; **173**: 3589-3593 [PMID: 15356101 DOI: 10.4049/jimmunol.173.6.3589]

63 **Petrasek J**, Mandrekar P, Szabo G. Toll-like receptors in the pathogenesis of alcoholic liver disease. *Gastroenterol Res Pract* 2010; **2010**: 710381 [PMID: 20827314 DOI: 10.1155/2010/710381]

64 **Shasthry SM**, Sarin SK. New treatment options for alcoholic hepatitis. *World J Gastroenterol* 2016;

22: 3892-3906 [PMID: 27099434 DOI: 10.3748/wjg.v22.i15.3892]
65 **Zhang J**, Wang X, Vikash V, Ye Q, Wu D, Liu Y, Dong W. ROS and ROS-Mediated Cellular Signaling. *Oxid Med Cell Longev* 2016; **2016**: 4350965 [PMID: 26998193 DOI: 10.1155/2016/4350965]
66 **Mu W**, Liu LZ. Reactive Oxygen Species Signaling in Cancer Development. *React Oxyg Species* 2017; **4**: 251-265 [DOI: 10.20455/ros.2017.843]
67 **Thakur V**, Pritchard MT, McMullen MR, Wang Q, Nagy LE. Chronic ethanol feeding increases activation of NADPH oxidase by lipopolysaccharide in rat Kupffer cells: role of increased reactive oxygen in LPS-stimulated ERK1/2 activation and TNF-alpha production. *J Leukoc Biol* 2006; **79**: 1348-1356 [PMID: 16554353 DOI: 10.1189/jlb.1005613]
68 **Cao Q**, Mak KM, Lieber CS. Dilinoleoylphosphatidylcholine decreases acetaldehyde-induced TNF-alpha generation in Kupffer cells of ethanol-fed rats. *Biochem Biophys Res Commun* 2002; **299**: 459-464 [PMID: 12445823 DOI: 10.1016/S0006-291X(02)02672-4]
69 **McMullen MR**, Pritchard MT, Wang Q, Millward CA, Croniger CM, Nagy LE. Early growth response-1 transcription factor is essential for ethanol-induced fatty liver injury in mice. *Gastroenterology* 2005; **128**: 2066-2076 [PMID: 15940638 DOI: 10.1053/j.gastro.2005.02.065]
70 **Yao J**, Mackman N, Edgington TS, Fan ST. Lipopolysaccharide induction of the tumor necrosis factor-alpha promoter in human monocytic cells. Regulation by Egr-1, c-Jun, and NF-kappaB transcription factors. *J Biol Chem* 1997; **272**: 17795-17801 [PMID: 9211933 DOI: 10.1074/jbc.272.28.17795]
71 **Kishore R**, McMullen MR, Nagy LE. Stabilization of tumor necrosis factor alpha mRNA by chronic ethanol: role of A + U-rich elements and p38 mitogen-activated protein kinase signaling pathway. *J Biol Chem* 2001; **276**: 41930-41937 [PMID: 11551956 DOI: 10.1074/jbc.M107181200]
72 **Win S**, Than TA, Zhang J, Oo C, Min RWM, Kaplowitz N. New insights into the role and mechanism of c-Jun-N-terminal kinase signaling in the pathobiology of liver diseases. *Hepatology* 2018; **67**: 2013-2024 [PMID: 29194686 DOI: 10.1002/hep.29689]
73 **Oak S**, Mandrekar P, Catalano D, Kodys K, Szabo G. TLR2- and TLR4-mediated signals determine attenuation or augmentation of inflammation by acute alcohol in monocytes. *J Immunol* 2006; **176**: 7628-7635 [PMID: 16751410 DOI: 10.4049/jimmunol.176.12.7628]
74 **Chang Q**, Zhang Y, Beezhold KJ, Bhatia D, Zhao H, Chen J, Castranova V, Shi X, Chen F. Sustained JNK1 activation is associated with altered histone H3 methylations in human liver cancer. *J Hepatol* 2009; **50**: 323-333 [PMID: 19041150 DOI: 10.1016/j.jhep.2008.07.037]
75 **Loomba R**, Lawitz E, Mantry PS, Jayakumar S, Caldwell SH, Arnold H, Diehl AM, Djedjos CS, Han L, Myers RP, Subramanian GM, McHutchison JG, Goodman ZD, Afdhal NH, Charlton MR; GS-US-384-1497 Investigators. The ASK1 inhibitor selonsertib in patients with nonalcoholic steatohepatitis: A randomized, phase 2 trial. *Hepatology* 2018; **67**: 549-559 [PMID: 28892558 DOI: 10.1002/hep.29514]
76 **Mathurin P**, Dufour J-F, Bzowej NH, Shiffman ML, Arterburn S, Nguyen T, Billin A, Chung C, Subramanian M, Myers RP, Szabo G, Thevenot T, Cramp ME, Ryder SD, Tilg H, Moreno C, Thursz MR, Agarwal K. Selonsertib in combination with prednisolone for the treatment of severe alcoholic hepatitis: a phase 2 randomized controlled trial. *Hepatology* 2018; 68; 8A-9A [DOI: 10.1002/hep.30256]
77 **Nogueira V**, Hay N. Molecular pathways: reactive oxygen species homeostasis in cancer cells and implications for cancer therapy. *Clin Cancer Res* 2013; **19**: 4309-4314 [PMID: 23719265 DOI: 10.1158/1078-0432.CCR-12-1424]
78 **Zhang Y**, Du Y, Le W, Wang K, Kieffer N, Zhang J. Redox control of the survival of healthy and diseased cells. *Antioxid Redox Signal* 2011; **15**: 2867-2908 [PMID: 21457107 DOI: 10.1089/ars.2010.3685]
79 **Zhao Y**, Hu X, Liu Y, Dong S, Wen Z, He W, Zhang S, Huang Q, Shi M. ROS signaling under metabolic stress: cross-talk between AMPK and AKT pathway. *Mol Cancer* 2017; **16**: 79 [PMID: 28407774 DOI: 10.1186/s12943-017-0648-1]
80 **Latvala J**, Hietala J, Koivisto H, Järvi K, Anttila P, Niemelä O. Immune Responses to Ethanol Metabolites and Cytokine Profiles Differentiate Alcoholics with or without Liver Disease. *Am J Gastroenterol* 2005; **100**: 1303-1310 [PMID: 15929761 DOI: 10.1111/j.1572-0241.2005.41509.x]
81 **Bird GL**, Sheron N, Goka AK, Alexander GJ, Williams RS. Increased plasma tumor necrosis factor in severe alcoholic hepatitis. *Ann Intern Med* 1990; **112**: 917-920 [PMID: 2339855 DOI: 10.7326/0003-4819-112-12-917]
82 **Szabo G**, Bala S. Alcoholic liver disease and the gut-liver axis. *World J Gastroenterol* 2010; **16**: 1321-1329 [PMID: 20238398 DOI: 10.3748/wjg.v16.i11.1321]
83 **Colmenero J**, Bataller R, Sancho-Bru P, Bellot P, Miquel R, Moreno M, Jares P, Bosch J, Arroyo V, Caballería J, Ginès P. Hepatic expression of candidate genes in patients with alcoholic hepatitis: correlation with disease severity. *Gastroenterology* 2007; **132**: 687-697 [PMID: 17258719 DOI: 10.1053/j.gastro.2006.12.036]
84 **McVicker BL**, Tuma DJ, Casey CA. Effect of ethanol on pro-apoptotic mechanisms in polarized hepatic cells. *World J Gastroenterol* 2007; **13**: 4960-4966 [PMID: 17854138 DOI: 10.3748/wjg.v13.i37.4960]
85 **Neuman MG**, Shear NH, Bellentani S, Tiribelli C. Role of cytokines in ethanol-induced cytotoxicity in vitro in Hep G2 cells. *Gastroenterology* 1998; **115**: 157-166 [PMID: 9649471 DOI: 10.1016/S0016-5085(98)70377-4]
86 **Micheau O**, Tschopp J. Induction of TNF receptor I-mediated apoptosis via two sequential signaling complexes. *Cell* 2003; **114**: 181-190 [PMID: 12887920 DOI: 10.1016/S0092-8674(03)00521-X]
87 **Kim YS**, Morgan MJ, Choksi S, Liu ZG. TNF-induced activation of the Nox1 NADPH oxidase and its role in the induction of necrotic cell death. *Mol Cell* 2007; **26**: 675-687 [PMID: 17560373 DOI: 10.1016/j.molcel.2007.04.021]
88 **Vanden Berghe T**, Declercq W, Vandenabeele P. NADPH oxidases: new players in TNF-induced necrotic cell death. *Mol Cell* 2007; **26**: 769-771 [PMID: 17588511 DOI: 10.1016/j.molcel.2007.06.002]
89 **Minayoshi Y**, Maeda H, Yanagisawa H, Hamasaki K, Mizuta Y, Nishida K, Kinoshita R, Enoki Y, Imafuku T, Chuang VTG, Koga T, Fujiwara Y, Takeya M, Sonoda K, Wakayama T, Taguchi K, Ishima Y, Ishida T, Iwakiri Y, Tanaka M, Sasaki Y, Watanabe H, Otagiri M, Maruyama T. Development of Kupffer cell targeting type-I interferon for the treatment of hepatitis via inducing anti-inflammatory and

immunomodulatory actions. *Drug Deliv* 2018; **25**: 1067-1077 [PMID: 29688069 DOI: 10.1080/10717544.2018.1464083]
90 **Sun B**, Karin M. NF-kappaB signaling, liver disease and hepatoprotective agents. *Oncogene* 2008; **27**: 6228-6244 [PMID: 18931690 DOI: 10.1038/onc.2008.300]
91 **Dhanda AD**, Yates E, Schewitz-Bowers LP, Lait PJ, Lee RWJ, Cramp ME. *Ex Vivo* T Cell Cytokine Expression Predicts Survival in Patients with Severe Alcoholic Hepatitis. *Gut Liver* 2020; **14**: 265-268 [PMID: 31158952 DOI: 10.5009/gnl19035]
92 **Lemmers A**, Moreno C, Gustot T, Maréchal R, Degré D, Demetter P, de Nadai P, Geerts A, Quertinmont E, Vercruysse V, Le Moine O, Devière J. The interleukin-17 pathway is involved in human alcoholic liver disease. *Hepatology* 2009; **49**: 646-657 [PMID: 19177575 DOI: 10.1002/hep.22680]
93 **Orman ES**, Odena G, Bataller R. Alcoholic liver disease: pathogenesis, management, and novel targets for therapy. *J Gastroenterol Hepatol* 2013; **28** Suppl 1: 77-84 [PMID: 23855300 DOI: 10.1111/jgh.12030]
94 **Kerr IM**, Costa-Pereira AP, Lillemeier BF, Strobl B. Of JAKs, STATs, blind watchmakers, jeeps and trains. *FEBS Lett* 2003; **546**: 1-5 [PMID: 12829228 DOI: 10.1016/S0014-5793(03)00411-3]
95 **Norkina O**, Dolganiuc A, Catalano D, Kodys K, Mandrekar P, Syed A, Efros M, Szabo G. Acute alcohol intake induces SOCS1 and SOCS3 and inhibits cytokine-induced STAT1 and STAT3 signaling in human monocytes. *Alcohol Clin Exp Res* 2008; **32**: 1565-1573 [PMID: 18616672 DOI: 10.1111/j.1530-0277.2008.00726.x]
96 **Tilg H**, Moschen AR, Szabo G. Interleukin-1 and inflammasomes in alcoholic liver disease/acute alcoholic hepatitis and nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. *Hepatology* 2016; **64**: 955-965 [PMID: 26773297 DOI: 10.1002/hep.28456]
97 **Tschopp J**, Schroder K. NLRP3 inflammasome activation: The convergence of multiple signalling pathways on ROS production? *Nat Rev Immunol* 2010; **10**: 210-215 [PMID: 20168318 DOI: 10.1038/nri2725]
98 **Revu S**, Wu J, Henkel M, Rittenhouse N, Menk A, Delgoffe GM, Poholek AC, McGeachy MJ. IL-23 and IL-1β Drive Human Th17 Cell Differentiation and Metabolic Reprogramming in Absence of CD28 Costimulation. *Cell Rep* 2018; **22**: 2642-2653 [PMID: 29514093 DOI: 10.1016/j.celrep.2018.02.044]
99 **Szabo G**, Csak T. Inflammasomes in liver diseases. *J Hepatol* 2012; **57**: 642-654 [PMID: 22634126 DOI: 10.1016/j.jhep.2012.03.035]
100 **Takeuchi D**, Yoshidome H, Kato A, Ito H, Kimura F, Shimizu H, Ohtsuka M, Morita Y, Miyazaki M. Interleukin 18 causes hepatic ischemia/reperfusion injury by suppressing anti-inflammatory cytokine expression in mice. *Hepatology* 2004; **39**: 699-710 [PMID: 14999688 DOI: 10.1002/hep.20117]
101 **Sakai A**, Han J, Cato AC, Akira S, Li JD. Glucocorticoids synergize with IL-1beta to induce TLR2 expression via MAP Kinase Phosphatase-1-dependent dual Inhibition of MAPK JNK and p38 in epithelial cells. *BMC Mol Biol* 2004; **5**: 2 [PMID: 15125785 DOI: 10.1186/1471-2199-5-2]
102 **Wintergerst ES**, Maggini S, Hornig DH. Contribution of selected vitamins and trace elements to immune function. *Ann Nutr Metab* 2007; **51**: 301-323 [PMID: 17726308 DOI: 10.1159/000107673]
103 **Zhu H**, Jia Z, Misra H, Li YR. Oxidative stress and redox signaling mechanisms of alcoholic liver disease: updated experimental and clinical evidence. *J Dig Dis* 2012; **13**: 133-142 [PMID: 22356308 DOI: 10.1111/j.1751-2980.2011.00569.x]
104 **Shah D**, Mahajan N, Sah S, Nath SK, Paudyal B. Oxidative stress and its biomarkers in systemic lupus erythematosus. *J Biomed Sci* 2014; **21**: 23 [PMID: 24636579 DOI: 10.1186/1423-0127-21-23]
105 **Wołonciej M**, Milewska E, Roszkowska-Jakimiec W. Trace elements as an activator of antioxidant enzymes. *Postepy Hig Med Dosw (Online)* 2016; **70**: 1483-1498 [PMID: 28100855 DOI: 10.5604/17322693.1229074]
106 **Czuczejko J**, Zachara BA, Staubach-Topczewska E, Halota W, Kedziora J. Selenium, glutathione and glutathione peroxidases in blood of patients with chronic liver diseases. *Acta Biochim Pol* 2003; **50**: 1147-1154 [PMID: 14740001 DOI: 10.18388/abp.2003_3638]
107 **Arakawa Y**, Moriyama M, Arakawa Y. Liver cirrhosis and metabolism (sugar, protein, fat and trace elements). *Hepatol Res* 2004; **30S**: 46-58 [PMID: 15607139 DOI: 10.1016/j.hepres.2004.10.009]
108 **Nangliya V**, Sharma A, Yadav D, Sunder S, Nijhawan S, Mishra S. Study of trace elements in liver cirrhosis patients and their role in prognosis of disease. *Biol Trace Elem Res* 2015; **165**: 35-40 [PMID: 25613584 DOI: 10.1007/s12011-015-0237-3]
109 **Burk RF**, Hill KE, Motley AK, Byrne DW, Norsworthy BK. Selenium deficiency occurs in some patients with moderate-to-severe cirrhosis and can be corrected by administration of selenate but not selenomethionine: a randomized controlled trial. *Am J Clin Nutr* 2015; **102**: 1126-1133 [PMID: 26468123 DOI: 10.3945/ajcn.115.110932]
110 **Prystupa A**, Błażewicz A, Kiciński P, Sak JJ, Niedziałek J, Załuska W. Serum Concentrations of Selected Heavy Metals in Patients with Alcoholic Liver Cirrhosis from the Lublin Region in Eastern Poland. *Int J Environ Res Public Health* 2016; **13**: 582 [PMID: 27304961 DOI: 10.3390/ijerph13060582]
111 **Huang MC**, Chen CH, Peng FC, Tang SH, Chen CC. Alterations in oxidative stress status during early alcohol withdrawal in alcoholic patients. *J Formos Med Assoc* 2009; **108**: 560-569 [PMID: 19586830 DOI: 10.1016/S0929-6646(09)60374-0]
112 **Saribal D**, Hocaoglu-Emre FS, Karaman F, Mırsal H, Akyolcu MC. Trace Element Levels and Oxidant/Antioxidant Status in Patients with Alcohol Abuse. *Biol Trace Elem Res* 2020; **193**: 7-13 [PMID: 30805875 DOI: 10.1007/s12011-019-01681-y]
113 **Grüngreiff K**, Reinhold D, Wedemeyer H. The role of zinc in liver cirrhosis. *Ann Hepatol* 2016; **15**: 7-16 [PMID: 26626635 DOI: 10.5604/16652681.1184191]
114 **Bode JC**, Hanisch P, Henning H, Koenig W, Richter FW, Bode C. Hepatic zinc content in patients with various stages of alcoholic liver disease and in patients with chronic active and chronic persistent hepatitis. *Hepatology* 1988; **8**: 1605-1609 [PMID: 3192174 DOI: 10.1002/hep.1840080622]
115 **Tapiero H**, Tew KD. Trace elements in human physiology and pathology: zinc and metallothioneins. *Biomed Pharmacother* 2003; **57**: 399-411 [PMID: 14652165 DOI: 10.1016/S0753-3322(03)00081-7]
116 **Wong CP**, Rinaldi NA, Ho E. Zinc deficiency enhanced inflammatory response by increasing immune cell

activation and inducing IL6 promoter demethylation. *Mol Nutr Food Res* 2015; **59**: 991-999 [PMID: 25656040 DOI: 10.1002/mnfr.201400761]

117 **Hennig B**, Meerarani P, Toborek M, McClain CJ. Antioxidant-like properties of zinc in activated endothelial cells. *J Am Coll Nutr* 1999; **18**: 152-158 [PMID: 10204831 DOI: 10.1080/07315724.1999.10718843]

118 **Shen H**, Arzuaga X, Toborek M, Hennig B. Zinc nutritional status modulates expression of ahr-responsive p450 enzymes in vascular endothelial cells. *Environ Toxicol Pharmacol* 2008; **25**: 197-201 [PMID: 19255596 DOI: 10.1016/j.etap.2007.10.016]

119 **Zhong W**, Zhao Y, Sun X, Song Z, McClain CJ, Zhou Z. Dietary zinc deficiency exaggerates ethanol-induced liver injury in mice: involvement of intrahepatic and extrahepatic factors. *PLoS One* 2013; **8**: e76522 [PMID: 24155903 DOI: 10.1371/journal.pone.0076522]

120 **Zhong W**, McClain CJ, Cave M, Kang YJ, Zhou Z. The role of zinc deficiency in alcohol-induced intestinal barrier dysfunction. *Am J Physiol Gastrointest Liver Physiol* 2010; **298**: G625-G633 [PMID: 20167873 DOI: 10.1152/ajpgi.00350.2009]

121 **Mohammad MK**, Song M, Falkner K, McClain C, Cave M. Zinc Sulfate for Alcoholic Cirrhosis (ZAC) Clinical Trial - Interim Analysis of Fiver Injury/Inflammation Biomarkers. *Hepatology* 2014; **60**: 794A [DOI: 10.1002/hep.28229]

122 **Wenzel G**, Kuklinski B, Rühlmann C, Ehrhardt D. [Alcohol-induced toxic hepatitis--a "free radical" associated disease. Lowering fatality by adjuvant antioxidant therapy]. *Z Gesamte Inn Med* 1993; **48**: 490-496 [PMID: 8256468]

123 **Pár A**, Pár G. [Alcoholic liver disease: the roles of genetic-epigenetic factors and the effect of abstinence]. *Orv Hetil* 2019; **160**: 524-532 [PMID: 30931596 DOI: 10.1556/650.2019.31352]

124 **Wu Q**, Ni X. ROS-mediated DNA methylation pattern alterations in carcinogenesis. *Curr Drug Targets* 2015; **16**: 13-19 [PMID: 25585126 DOI: 10.2174/1389450116666150113121054]

125 **Afanas'ev I**. New nucleophilic mechanisms of ros-dependent epigenetic modifications: comparison of aging and cancer. *Aging Dis* 2014; **5**: 52-62 [PMID: 24490117 DOI: 10.14336/AD.2014.050052]

126 **Boccuto L**, Abenavoli L. Genetic and Epigenetic Profile of Patients With Alcoholic Liver Disease. *Ann Hepatol* 2017; **16**: 490-500 [PMID: 28612750 DOI: 10.5604/01.3001.0010.0274]

127 **Meroni M**, Longo M, Rametta R, Dongiovanni P. Genetic and Epigenetic Modifiers of Alcoholic Liver Disease. *Int J Mol Sci* 2018; **19**: 3857 [PMID: 30513996 DOI: 10.3390/ijms19123857]

128 **Zahs A**, Curtis BJ, Waldschmidt TJ, Brown LA, Gauthier TW, Choudhry MA, Kovacs EJ, Bird MD. Alcohol and epigenetic changes: summary of the 2011 Alcohol and Immunology Research Interest Group (AIRIG) meeting. *Alcohol* 2012; **46**: 783-787 [PMID: 22738858 DOI: 10.1016/j.alcohol.2012.05.005]

129 **Kendrick SF**, O'Boyle G, Mann J, Zeybel M, Palmer J, Jones DE, Day CP. Acetate, the key modulator of inflammatory responses in acute alcoholic hepatitis. *Hepatology* 2010; **51**: 1988-1997 [PMID: 20232292 DOI: 10.1002/hep.23572]

130 **To Y**, Ito K, Kizawa Y, Failla M, Ito M, Kusama T, Elliott WM, Hogg JC, Adcock IM, Barnes PJ. Targeting phosphoinositide-3-kinase-delta with theophylline reverses corticosteroid insensitivity in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2010; **182**: 897-904 [PMID: 20224070 DOI: 10.1164/rccm.200906-0937OC]

131 **Ito K**, Caramori G, Adcock IM. Therapeutic potential of phosphatidylinositol 3-kinase inhibitors in inflammatory respiratory disease. *J Pharmacol Exp Ther* 2007; **321**: 1-8 [PMID: 17021257 DOI: 10.1124/jpet.106.111674]

132 **Barnes PJ**. Corticosteroid resistance in patients with asthma and chronic obstructive pulmonary disease. *J Allergy Clin Immunol* 2013; **131**: 636-645 [PMID: 23360759 DOI: 10.1016/j.jaci.2012.12.1564]

133 **Kendrick SF**, Henderson E, Palmer J, Jones DE, Day CP. Theophylline improves steroid sensitivity in acute alcoholic hepatitis. *Hepatology* 2010; **52**: 126-131 [PMID: 20578267 DOI: 10.1002/hep.23666]

134 **Hassanein T**, Stein LL, Flamm SL, Martin P, Cave MC, Blevins C, Scott, D, Krebs W, Lin W. Safety and efficacy of DUR-928: a potential new therapy for acute alcoholic hepatitis. *Hepatology* 2019; **70**: 1477A-1501A [DOI: 10.1002/hep.31033]

135 **Yu S**, Khor TO, Cheung KL, Li W, Wu TY, Huang Y, Foster BA, Kan YW, Kong AN. Nrf2 expression is regulated by epigenetic mechanisms in prostate cancer of TRAMP mice. *PLoS One* 2010; **5**: e8579 [PMID: 20062804 DOI: 10.1371/journal.pone.0008579]

136 **Khor TO**, Fuentes F, Shu L, Paredes-Gonzalez X, Yang AY, Liu Y, Smiraglia DJ, Yegnasubramanian S, Nelson WG, Kong AN. Epigenetic DNA methylation of antioxidative stress regulator NRF2 in human prostate cancer. *Cancer Prev Res (Phila)* 2014; **7**: 1186-1197 [PMID: 25266896 DOI: 10.1158/1940-6207.CAPR-14-0127]

137 **Wang D**, Ma Y, Yang X, Xu X, Zhao Y, Zhu Z, Wang X, Deng H, Li C, Gao F, Tong J, Yamanaka K, An Y. Hypermethylation of the Keap1 gene inactivates its function, promotes Nrf2 nuclear accumulation, and is involved in arsenite-induced human keratinocyte transformation. *Free Radic Biol Med* 2015; **89**: 209-219 [PMID: 26409248 DOI: 10.1016/j.freeradbiomed.2015.07.153]

138 **Correa F**, Mallard C, Nilsson M, Sandberg M. Activated microglia decrease histone acetylation and Nrf2-inducible anti-oxidant defence in astrocytes: restoring effects of inhibitors of HDACs, p38 MAPK and GSK3β. *Neurobiol Dis* 2011; **44**: 142-151 [PMID: 21757005 DOI: 10.1016/j.nbd.2011.06.016]

139 **Guo T**, Chang L, Xiao Y, Liu Q. S-adenosyl-L-methionine for the treatment of chronic liver disease: a systematic review and meta-analysis. *PLoS One* 2015; **10**: e0122124 [PMID: 25774783 DOI: 10.1371/journal.pone.0122124]

140 **Medici V**, Virata MC, Peerson JM, Stabler SP, French SW, Gregory JF 3rd, Albanese A, Bowlus CL, Devaraj S, Panacek EA, Richards JR, Halsted CH. S-adenosyl-L-methionine treatment for alcoholic liver disease: a double-blinded, randomized, placebo-controlled trial. *Alcohol Clin Exp Res* 2011; **35**: 1960-1965 [PMID: 22044287 DOI: 10.1111/j.1530-0277.2011.01547.x]

141 **Mato JM**, Cámara J, Fernández de Paz J, Caballería L, Coll S, Caballero A, García-Buey L, Beltrán J, Benita V, Caballería J, Solà R, Moreno-Otero R, Barrao F, Martín-Duce A, Correa JA, Parés A, Barrao E, García-Magaz I, Puerta JL, Moreno J, Boissard G, Ortiz P, Rodés J. S-adenosylmethionine in alcoholic liver

cirrhosis: a randomized, placebo-controlled, double-blind, multicenter clinical trial. *J Hepatol* 1999; **30**: 1081-1089 [PMID: 10406187 DOI: 10.1016/S0168-8278(99)80263-3]

142 **Collins P**, Jones C, Choudhury S, Damelin L, Hodgson H. Increased expression of uncoupling protein 2 in HepG2 cells attenuates oxidative damage and apoptosis. *Liver Int* 2005; **25**: 880-887 [PMID: 15998440 DOI: 10.1111/j.1478-3231.2005.01104.x]

143 **Jin X**, Yang YD, Chen K, Lv ZY, Zheng L, Liu YP, Chen SH, Yu CH, Jiang XY, Zhang CY, Li YM. HDMCP uncouples yeast mitochondrial respiration and alleviates steatosis in L02 and hepG2 cells by decreasing ATP and H2O2 levels: a novel mechanism for NAFLD. *J Hepatol* 2009; **50**: 1019-1028 [PMID: 19303656 DOI: 10.1016/j.jhep.2008.10.034]

144 **Cortez-Pinto H**, Machado MV. Uncoupling proteins and non-alcoholic fatty liver disease. *J Hepatol* 2009; **50**: 857-860 [PMID: 19328582 DOI: 10.1016/j.jhep.2009.02.019]

145 **Phillips M**, Curtis H, Portmann B, Donaldson N, Bomford A, O'Grady J. Antioxidants versus corticosteroids in the treatment of severe alcoholic hepatitis--a randomised clinical trial. *J Hepatol* 2006; **44**: 784-790 [PMID: 16469404 DOI: 10.1016/j.jhep.2005.11.039]

146 **Stewart S**, Prince M, Bassendine M, Hudson M, James O, Jones D, Record C, Day CP. A randomized trial of antioxidant therapy alone or with corticosteroids in acute alcoholic hepatitis. *J Hepatol* 2007; **47**: 277-283 [PMID: 17532088 DOI: 10.1016/j.jhep.2007.03.027]

147 **Nguyen-Khac E**, Thevenot T, Piquet MA, Benferhat S, Goria O, Chatelain D, Tramier B, Dewaele F, Ghrib S, Rudler M, Carbonell N, Tossou H, Bental A, Bernard-Chabert B, Dupas JL; AAH-NAC Study Group. Glucocorticoids plus N-acetylcysteine in severe alcoholic hepatitis. *N Engl J Med* 2011; **365**: 1781-1789 [PMID: 22070475 DOI: 10.1056/NEJMoa1101214]

148 **European Association for the Study of the Liver**; Thursz M, Gual A, Lackner C, Mathurin P, Moreno C, Spahr L, Sterneck M, Cortez-Pinto H. European Association for the Study of the Liver. EASL Clinical Practice Guidelines: Management of alcohol-related liver disease. *J Hepatol* 2018; **69**: 154-181 [PMID: 29628280 DOI: 10.1016/j.jhep.2018.03.018]

149 **McClain C**, Vatsalya V, Cave M. Role of Zinc in the Development/Progression of Alcoholic Liver Disease. *Curr Treat Options Gastroenterol* 2017; **15**: 285-295 [PMID: 28447197 DOI: 10.1007/s11938-017-0132-4]

150 **Singh AK**, Bishayee A, Pandey AK. Targeting Histone Deacetylases with Natural and Synthetic Agents: An Emerging Anticancer Strategy. *Nutrients* 2018; **10**: 731 [PMID: 29882797 DOI: 10.3390/nu10060731]

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ORIGINAL ARTICLE

Basic Study

Ipragliflozin-induced improvement of liver steatosis in obese mice may involve sirtuin signaling

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Abstract

BACKGROUND

Sodium glucose cotransporter 2 (SGLT2) inhibitors are newly developed oral antidiabetic drugs. SGLT2 is primarily expressed in the kidneys and reabsorbs approximately 90% of the glucose filtered by the renal glomeruli. SGLT2 inhibitors lower glucose levels independently of insulin action by facilitating urinary glucose excretion. The SGLT2 inhibitor ipragliflozin has reportedly improved liver steatosis in animal models and clinical studies. However, the mechanisms by which SGLT2 inhibitors improve liver steatosis are not fully understood.

AIM

To investigate the ameliorative effects of ipragliflozin on liver steatosis and the mechanisms of these effects in obese mice.

METHODS

We analyzed 8-wk-old male obese (*ob/ob*) mice that were randomly divided into a group receiving a normal chow diet and a group receiving a normal chow diet supplemented with ipragliflozin (3 mg/kg or 10 mg/kg) for 4 wk. We also analyzed their lean sex-matched littermates receiving a normal chow diet as

another control group. Body weight and liver weight were evaluated, and liver histology, immunoblotting, and reverse transcription-polymerase chain reaction analyses were performed.

RESULTS

Hepatic lipid accumulation was significantly ameliorated in *ob/ob* mice treated with 10 mg/kg ipragliflozin compared to untreated *ob/ob* mice irrespective of body weight changes. Ipragliflozin had no appreciable effects on hepatic oxidative stress-related gene expression levels or macrophage infiltration, but significantly reduced hepatic interleukin-1β (IL-1β) mRNA expression levels. Ipragliflozin increased both the mRNA and protein expression levels of sirtuin 1 (SIRT1) in the liver. The hepatic mRNA levels of peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α), peroxisome proliferator-activated receptor α (PPARα), and fibroblast growth factor-21 (FGF21) were also significantly higher in ipragliflozin-treated *ob/ob* mice than in untreated *ob/ob* mice.

CONCLUSION

Our study suggests that the liver steatosis-ameliorating effects of ipragliflozin in *ob/ob* mice may be mediated partly by hepatic SIRT1 signaling, possibly through the PGC-1α/PPARα-FGF21 pathway.

Key words: Selective sodium glucose cotransporter 2; Nonalcoholic fatty liver disease; Sirtuin 1; Peroxisome proliferator-activated receptor γ coactivator 1α; Peroxisome proliferator-activated receptor α; Fibroblast growth factor-21

Core tip: The selective sodium glucose cotransporter 2 inhibitor ipragliflozin significantly ameliorated hepatic lipid accumulation in genetically obese (*ob/ob)* mice and increased both the mRNA and protein expression levels of sirtuin 1 (SIRT1), a NAD+-dependent protein deacetylase with numerous substrates, in the liver. Ipragliflozin also significantly increased the hepatic mRNA levels of peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α), peroxisome proliferator-activated receptor α (PPARα), and fibroblast growth factor-21 (FGF21). The liver steatosis-attenuating effects of ipragliflozin in *ob/ob* mice may have been mediated partly by hepatic SIRT1 signaling, possibly through the PGC-1α/PPARα-FGF21 pathway.

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INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD), a hepatic manifestation of metabolic syndrome, is a common chronic liver disease. It includes isolated fatty liver and nonalcoholic steatohepatitis (NASH), the latter of which can progress to cirrhosis and liver cancer in some individuals[1]. This disease is associated with obesity, insulin resistance, and type 2 diabetes mellitus (T2DM). As lifestyles have become increasingly sedentary and dietary patterns have changed, the worldwide prevalence of NAFLD has dramatically increased[2]. The most challenging problem is that no pharmacological therapies have been established for NAFLD so far[3].

Sodium glucose cotransporter 2 (SGLT2) inhibitors are newly developed oral antidiabetic drugs. SGLT2 is primarily expressed in the kidneys and reabsorbs approximately 90% of the glucose filtered by the renal glomeruli. SGLT2 inhibitors, which lower glucose levels independently of insulin action by facilitating the excretion of glucose in urine, are expected to become candidate therapeutic agents not only for T2DM but also for NASH/NAFLD[4,5]. Ipragliflozin is a selective SGLT2 inhibitor that

University Animal Care and Experimentation Committee and the Gunma University Safety Committee for Recombinant DNA Experiments prior to the experiments under approval numbers 15-016 and 15-030, respectively.

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is orally administered. Previous reports have shown that ipragliflozin improves liver steatosis in animal models[6-8] and clinical settings[9,10]. However, the mechanisms by which SGLT2 inhibitors improve liver steatosis are not fully understood.

Recently, chronic administration of an SGLT2 inhibitor was reported to drive a fuel shift, decreasing tissue glucose disposal and increasing lipid use[11]. Therefore, we hypothesized that sirtuin 1 (SIRT1), a NAD^+-dependent protein deacetylase with numerous substrates, might be associated with the amelioration of liver steatosis by SGLT2 inhibitors. SIRT1 plays important roles in controlling energy homeostasis and longevity in mammals[12,13]. For example, SIRT1 improves sensitivity to both leptin and insulin, which act on proopiomelanocortin neurons to increase sympathetic activity toward adipose tissues and to promote the browning of white fat, and is involved in energy and glucose homeostasis[14]. Pharmacological activation of SIRT1 signaling reportedly ameliorates fatty liver[15,16]. In contrast, hepatocyte-specific deletion of SIRT1 impairs peroxisome proliferator-activated receptor α (PPARα) signaling, decreases fatty acid β-oxidation, and results in liver steatosis and inflammation[17]. Peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α), a key coactivator for PPARα signaling[18], is known to be a direct substrate of SIRT1[19]. PGC-1α interacts with multiple transcription factors to enhance mitochondrial metabolic capacity[20]. Moreover, hepatic SIRT1 attenuates liver steatosis and controls energy balance by inducing the activation of fibroblast growth factor-21 (FGF21)[21]. Hepatic FGF21 is regulated by PPARα and is a key mediator of hepatic metabolism[22]. All of the above findings suggest that the SIRT1-PGC-1α/PPARα-FGF21 pathway is important in lipid homeostasis in the liver.

It has not been fully elucidated whether the amelioration of liver steatosis mediated by the SGLT2 inhibitor ipragliflozin is associated with SIRT1 signaling. The objectives of our study were thus to evaluate the *in vivo* effects of the selective SGLT2 inhibitor ipragliflozin on liver steatosis and to investigate the mechanisms by which this SGLT2 inhibitor improves liver steatosis in obese (*ob/ob*) mice. In particular, the primary experimental aim was to clarify the role of SIRT1 signaling in ipragliflozin-mediated attenuation of liver steatosis in *ob/ob* mice.

MATERIALS AND METHODS

Animals and animal treatment protocol

We purchased 6-wk-old male *ob/ob* mice and their lean sex-matched littermates from Charles River Co., Ltd. (Yokohama, Japan). All mice were kept under a 12:12 h light-dark cycle with free access to food and water. After the mice had acclimated to the rearing environment for 2 wk, they were fed a normal chow diet (CLEA Rodent Diet CE-2) from CLEA Japan, Inc. (Tokyo, Japan). The diet was changed to a normal chow diet (D12450B) from Research Diets (Tokyo, Japan) or an ipragliflozin-supplemented D12450B chow diet when the mice were 8 wk old. The treatment groups were composed of *ob/ob* mice that were fed a normal chow diet only or a normal chow diet supplemented with one of two different doses of ipragliflozin (3 mg/kg or 10 mg/kg, Astellas Pharma Inc., Tokyo, Japan), and the control group was composed of lean littermates fed a normal chow diet. The *ob/ob* mice were randomly assigned to the 3 treatment groups, each of which comprised 8 mice. After 4 weeks of feeding, all mice were sacrificed, total liver resection was performed, and the specimens were analyzed. For verification of SGLT2 mRNA expression, liver and kidney specimens were obtained from C57BL/6 mice purchased from Charles River Laboratories Japan, Inc.

Histological analysis

We used Oil Red O staining to evaluate liver fat deposition in paraffin-embedded liver tissue specimens. The ImageJ software (NIH) image software program was used to quantify the Oil Red O-stained areas in 8 microscopic fields at 400-fold magnification.

Immunoblot analyses

Proteins extracted from liver tissue were resolved *via* polyacrylamide gel electrophoresis, and the separated proteins in the gels were transferred to nitrocellulose membranes. The membranes were then probed with primary antibodies against SIRT1 (Merck, Tokyo, Japan), α-tubulin (Santa Cruz Biotechnology, Inc., TX, United States), phospho-AMP-activated protein kinase (AMPK), and total AMPK (Cell Signaling Technology Japan, K. K., Tokyo, Japan). The membranes were then incubated with corresponding horseradish peroxidase-conjugated secondary antibodies. The immunoreactive proteins were assessed with an LAS-4000 Image

analyzer (FUJIFILM Holdings Corporation, Tokyo, Japan), and densitometry was performed using NIH.

Quantitative reverse transcription-polymerase chain reaction analysis

An RNAiso Plus kit (Takara Bio Inc., Shiga, Japan) was used for total RNA isolation. An Improm-II Reverse Transcription System (Promega Japan, Tokyo, Japan) was used for reverse transcription of isolated RNA into cDNA. cDNA samples (1 μg) were subjected to reverse transcription-polymerase chain reaction (RT-PCR) with a PCR Kit (TaKaRa) or to quantitative PCR with an Applied Biosystems ViiA™ 7 Real-Time PCR System (Life Technologies Japan, Ltd., Tokyo, Japan) and PowerUp™ SYBR™ Green Master Mix (Fisher Scientific International, Inc., Pittsburgh, PA, United States). The specific primer sequences are listed in Table 1. The target mRNA expression levels were assessed relative to mouse β-actin mRNA (control gene) levels.

Statistical analysis

All data are presented as the mean ± SD. Multiple comparisons were performed with analysis of variance followed by post hoc tests, as appropriate. *P* values of less than 0.05 were considered to indicate statistical significance.

RESULTS

Ipragliflozin reduced hepatic lipid accumulation regardless of body weight changes in ob/ob mice

All mice showed sensitive reactions, normal movement, normal appetite, normal stool, and stable breathing at the start of the experiment. The mice did not show any adverse events during the experiment, and no modifications of the experimental protocols were necessary.

We first tested whether ipragliflozin improved liver steatosis in *ob/ob* mice. There were no significant changes in body weight in either the 3 mg/kg or the 10 mg/kg ipragliflozin-treated *ob/ob* mice compared with the untreated *ob/ob* mice after 4 wk of treatment (Figure 1A). In addition, ipragliflozin did not significantly change the ratio of liver weight to body weight at the end of the experimental period (Figure 1B). On the other hand, we found that the livers of the 10 mg/kg ipragliflozin-treated *ob/ob* mice had significantly lower Oil Red O-stained areas than those of the untreated *ob/ob* mice (Figure 2). These results indicated that ipragliflozin improved liver steatosis irrespective of body weight changes.

Ipragliflozin increased hepatic SIRT1 protein expression levels in ob/ob mice

To elucidate the mechanism by which ipragliflozin improved liver steatosis in *ob/ob* mice, we examined the protein expression levels of hepatic SIRT1. Interestingly, compared with no treatment, ipragliflozin treatment significantly increased hepatic SIRT1 protein expression levels by approximately 2-fold in *ob/ob* mice (Figure 3). These results suggested that ipragliflozin upregulated the protein expression of SIRT1 in the livers of *ob/ob* mice.

Ipragliflozin-mediated attenuation of liver steatosis in ob/ob mice was associated with SIRT1 signaling

Based on the abovementioned effect of ipragliflozin on the hepatic expression of SIRT1 protein, we analyzed the role of the SIRT1-PGC-1α/PPARα-FGF21 pathway in our mouse model. Specifically, we examined the mRNA expression levels of SIRT1, PGC-1α, PPARα, and FGF21 in the liver. Consistent with the findings regarding hepatic SIRT1 protein expression, we found that hepatic SIRT1 mRNA expression was significantly higher in 10 mg/kg ipragliflozin-treated *ob/ob* mice than in untreated *ob/ob* control mice (Figure 4A). Moreover, we found that liver PGC-1α, PPARα, and FGF21 mRNA expression was significantly higher in ipragliflozin-treated *ob/ob* mice than in untreated *ob/ob* control mice (Figure 4B-D). On the other hand, the hepatic mRNA levels of fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC), key regulators of *de novo* hepatic lipogenesis, did not significantly differ between the ipragliflozin-treated *ob/ob* mice and the untreated *ob/ob* control mice (Figure 4E and F). These results indicated that the ameliorative effects of ipragliflozin on liver steatosis were possibly mediated by the SIRT1-PGC-1α/PPARα-FGF21 pathway.

Table 1 Sequences of the primers used for reverse transcription-polymerase chain reaction

	Forward primer sequence	Reverse primer sequence
SIRT1	5′-GTA AGC GGC TTG AGG G-3′	5′-TTC GGG CCT CTC CGT A-3′
PGC-1α	5′-TTG ACT GGC GTC ATT CGG GAG-3′	5′-ATC TGG GCA AAG AGG CTG GTC-3′
PPARα	5′-AGG AAG CCG TTC TGT GAC AT-3′	5′-TTG AAG GAG CTT TGG GAA GA-3′
FGF21	5′-AGA TCA GGG AGG ATG GAA CA-3′	5′-TCA AAG TGA GGC GAT CCA TA-3′
FAS	5′-ACC ACT GCA TTG ACG GCC GG-3′	5′-GGG TCA GGC GGG AGA CCG AT-3′
ACC	5′-GGG CAC AGA CCG TGG TAG TT-3′	5′-CAG GAT CAG CTG GGA TAC TGA-3′
ACOX1	5′-TGG TAT GGT GTC GTA CTT GAA TGA C-3′	5′-AAT TTC TAC CAA TCT GGC TGC AC-3′
ACS	5′-AAA GAT GGC TGG TTA CAC ACG-3′	5′-CGA TAA TCT TCA AGG TGC CAT T-3′
CPT1	5′-CCC TGG GCA TGA TTG CAA-3′	5′-AAG AGG ACG CCA CTC ACG AT-3′
CPT2	5′-CAG ACA GTG GCT ACC TAT GAA TCC T-3′	5′-TGG TCA GCT GGC CAT GGT ATT TGG A-3′
Nox2	5′-GAA AAC TCC TTG GGT CAG CAC T-3′	5′-ATT TCG ACA CAC TGG CAG CA-3′
GPx-1	5′-TTA CAT TGT TTG AGA AGT GCG A-3′	5′-CAA AGT TCC AGG CAA TGT C-3′
SOD-1	5′-CAT TCC ATC ATT GGC CGT-3′	5′-TCA GAC CAC ACA GGG AAT GTT TA-3′
SOD-2	5′-TGT ATA TCT CTG GAG AAC TGG AC-3′	5′-GGC CCT CTT GTG ACT GTA A-3′
IL-1β	5′-AAA CGG TTT GTC TTC AAC-3′	5′-ATG GTG AAG TCA ATT ATG TC-3′
F4/80	5′-CAT CTT GCT GGA GAC TGT-3′	5′-CTG CCA AGT TAA TGG ACT CA-3′
β-actin	5′-AGC CTT CCT TCT TGG GTA-3′	5′-GAG CAA TGA TCT TGA TCT TC-3′

ACC: Acetyl-CoA carboxylase; ACOX1: Acyl-CoA oxidase 1; ACS: Acyl-CoA synthetase; CPT1: Carnitine palmitoyltransferase 1; CPT2: Carnitine palmitoyltransferase 2; FAS: Fatty acid synthase; FGF21: Fibroblast growth factor-21; GPx-1: Glutathione peroxidase 1; IL-1β: Interleukin-1β; Nox2: NADPH oxidase 2; PGC-1α: Peroxisome proliferator-activated receptor γ coactivator 1α; PPARα: Peroxisome proliferator-activated receptor α; SIRT1: Sirtuin 1; SOD-1: Superoxide dismutase 1; SOD-2: Superoxide dismutase 2.

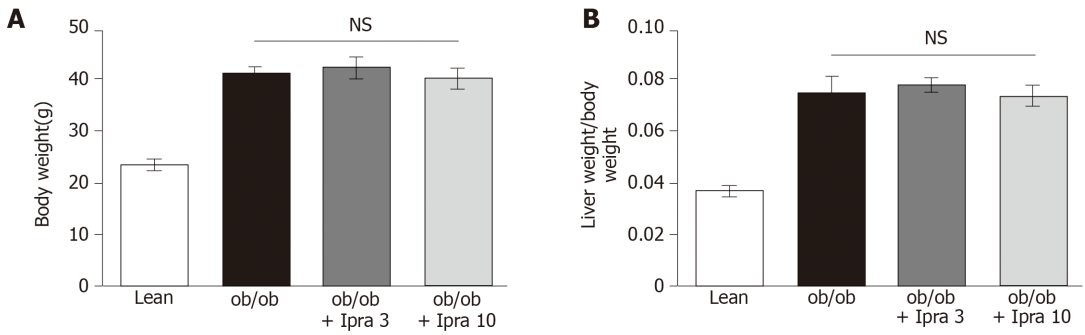

Figure 1 Body weights and liver-to-body weight ratios of obese mice treated with or without ipragliflozin and their lean littermates. A: Body weights of mice in the lean, obese (*ob/ob*), *ob/ob* + ipragliflozin 3 mg/kg, and *ob/ob* + ipragliflozin 10 mg/kg groups at the end of therapy; B: Liver-to-body weight ratios of mice in the lean, *ob/ob*, *ob/ob* + ipragliflozin 3 mg/kg, and *ob/ob* + ipragliflozin 10 mg/kg groups at the end of therapy. NS: Not significant; *ob/ob*: Obese; Ipra: Ipragliflozin. *n* = 8.

Ipragliflozin increased the mRNA expression levels of β-oxidation-related enzymes in ob/ob mice

Furthermore, we analyzed the mRNA expression levels of β-oxidation-related enzymes in our mouse model. We found that the hepatic mRNA expression levels of acyl-CoA oxidase 1 (ACOX1), acyl-CoA synthetase (ACS), carnitine palmitoyltransferase (CPT) 1, and CPT2 were significantly higher in 10 mg/kg ipragliflozin-treated *ob/ob* mice than in untreated *ob/ob* control mice (Figure 5A-D). These results suggested that ipragliflozin increased both peroxisomal and mitochondrial β-oxidation in *ob/ob* mice.

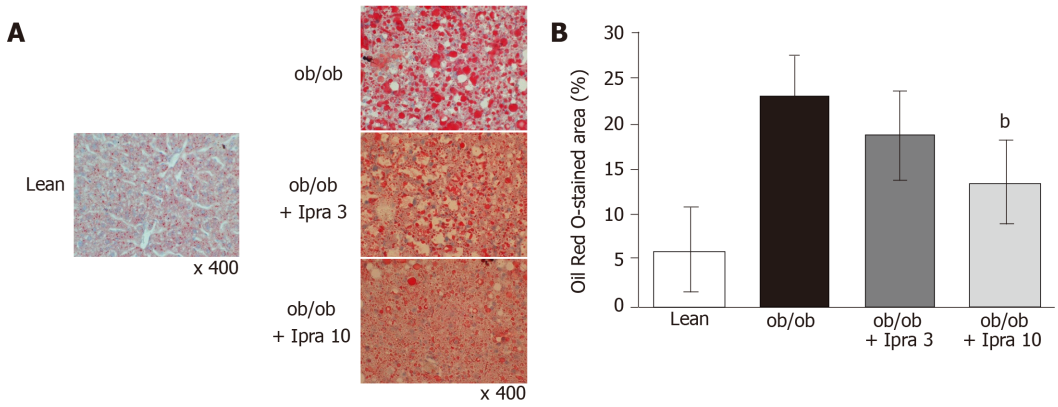

Figure 2 Evaluation of liver histology in obese mice treated with or without ipragliflozin and their lean littermates. A: Representative hepatic histology of mice in the lean, obese (*ob/ob*), *ob/ob* + ipragliflozin 3 mg/kg, and *ob/ob* + ipragliflozin 10 mg/kg groups at the end of therapy. The liver sections were stained with Oil Red O; B: Results of quantitative histomorphometric analysis of the total hepatic lipid content for each experimental group. The Oil Red O-stained areas were quantified in 8 microscopic fields at 400-fold magnification. $^{b}P < 0.01$ *vs* the *ob/ob* group. *ob/ob*: Obese; Ipra: Ipragliflozin.

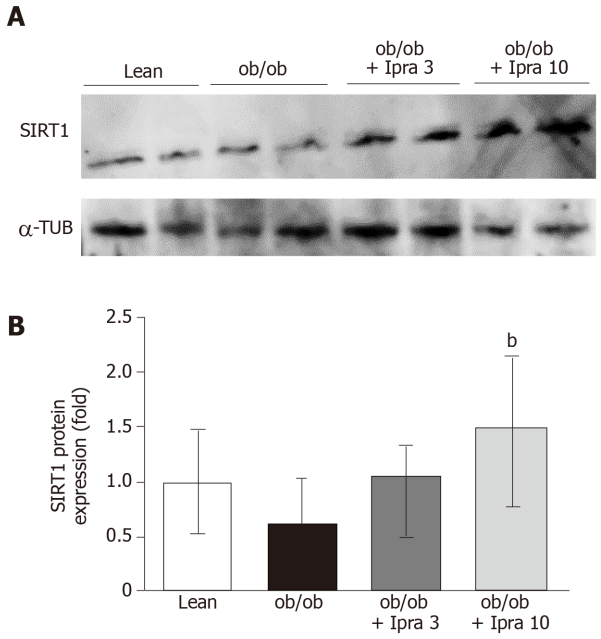

Figure 3 Hepatic sirtuin 1 protein expression in obese mice treated with or without ipragliflozin and their lean littermates. A: Representative western blot showing the expression of hepatic sirtuin 1 (SIRT1) protein at the end of the treatment period; B: The bar graph below shows the expression of SIRT1 normalized to α-tubulin. $^{b}P < 0.01$ *vs* the *ob/ob* group. Ipra: Ipragliflozin; *ob/ob*: Obese; SIRT1: Sirtuin 1; α-TUB: α-tubulin. $n = 8$.

Ipragliflozin decreased the mRNA expression levels of interleukin-1β but had no appreciable effects on those of oxidative stress-related genes or macrophage marker in ob/ob mice

We also analyzed oxidative stress, inflammatory cytokine levels, and macrophage infiltration in our mouse model. Treatment with 3 mg/kg ipragliflozin significantly increased the hepatic mRNA levels of NADPH oxidase 2 (Nox2), but treatment with 10 mg/kg ipragliflozin did not (Figure 5E). The hepatic mRNA levels of glutathione peroxidase 1, superoxide dismutase (SOD)-1, or SOD-2, key regulators of oxidative stress, did not significantly differ between ipragliflozin-treated *ob/ob* mice and untreated *ob/ob* control mice (Figure 5F-H). Ipragliflozin decreased the mRNA expression levels of interleukin-1β (IL-1β) in *ob/ob* mice (Figure 5I). However, the hepatic mRNA expression levels of F4/80 were unchanged by ipragliflozin treatment (Figure 5J).

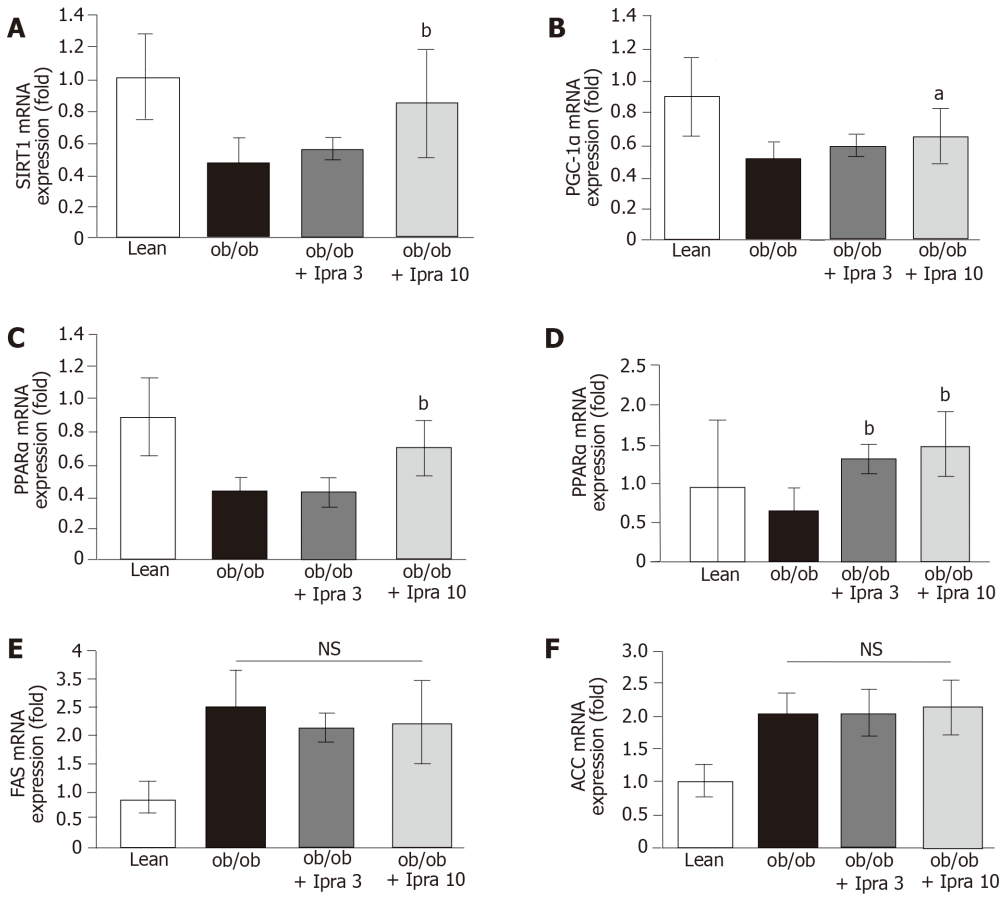

Figure 4 Hepatic mRNA expression of genes related to sirtuin 1 signaling in obese mice treated with or without ipragliflozin and their lean littermates. A: Hepatic mRNA expression of sirtuin 1 in obese (*ob/ob*) mice treated with or without ipragliflozin and their lean littermates; B: Hepatic mRNA expression of peroxisome proliferator-activated receptor γ coactivator 1α in *ob/ob* mice treated with or without ipragliflozin and their lean littermates; C: Hepatic mRNA expression of peroxisome proliferator-activated receptor α in *ob/ob* mice treated with or without ipragliflozin and their lean littermates; D: Hepatic mRNA expression of fibroblast growth factor-21 in *ob/ob* mice treated with or without ipragliflozin and their lean littermates; E: Hepatic mRNA expression of fatty acid synthase in *ob/ob* mice treated with or without ipragliflozin and their lean littermates; F: Hepatic mRNA expression of acetyl-CoA carboxylase in *ob/ob* mice treated with or without ipragliflozin and their lean littermates. $^{a}P < 0.05$, $^{b}P < 0.01$ *vs* the *ob/ob* group. ACC: Acetyl-CoA carboxylase; FAS: Fatty acid synthase; FGF21: Fibroblast growth factor-21; Ipra: Ipragliflozin; NS: Not significant; PPARα: Peroxisome proliferator-activated receptor α; PGC-1α: Peroxisome proliferator-activated receptor γ coactivator 1α; *ob/ob*: Obese; SIRT1: Sirtuin 1. $n = 8$.

SGLT2 was expressed in the kidneys but not in the liver

We next investigated why hepatic SIRT1 signaling was increased in ipragliflozin-treated mice. We hypothesized that ipragliflozin increased hepatic SIRT1 signaling by directly inhibiting SGLT2 in the mouse liver. Therefore, we tested whether SGLT2 was expressed in the livers of mice. RT-PCR revealed that SGLT2 was expressed in mouse kidneys but not in mouse livers (Figure 6A).

Ipragliflozin increased AMPK activation in the whole liver

We further assessed the effects of ipragliflozin treatment on the activation of AMPK, a major metabolic energy sensor and master regulator of metabolic homeostatic processes, including SIRT1 signaling[23]. Interestingly, ipragliflozin significantly increased the activation of AMPK in whole livers obtained from mice in the treated groups (Figure 6B and C).

DISCUSSION

In our study, the SGLT2 inhibitor ipragliflozin ameliorated hepatic lipid accumulation in a manner associated with hepatic SIRT1 signaling in an experimental obese mouse model. It was unlikely that the inhibitory effect of ipragliflozin on liver steatosis was

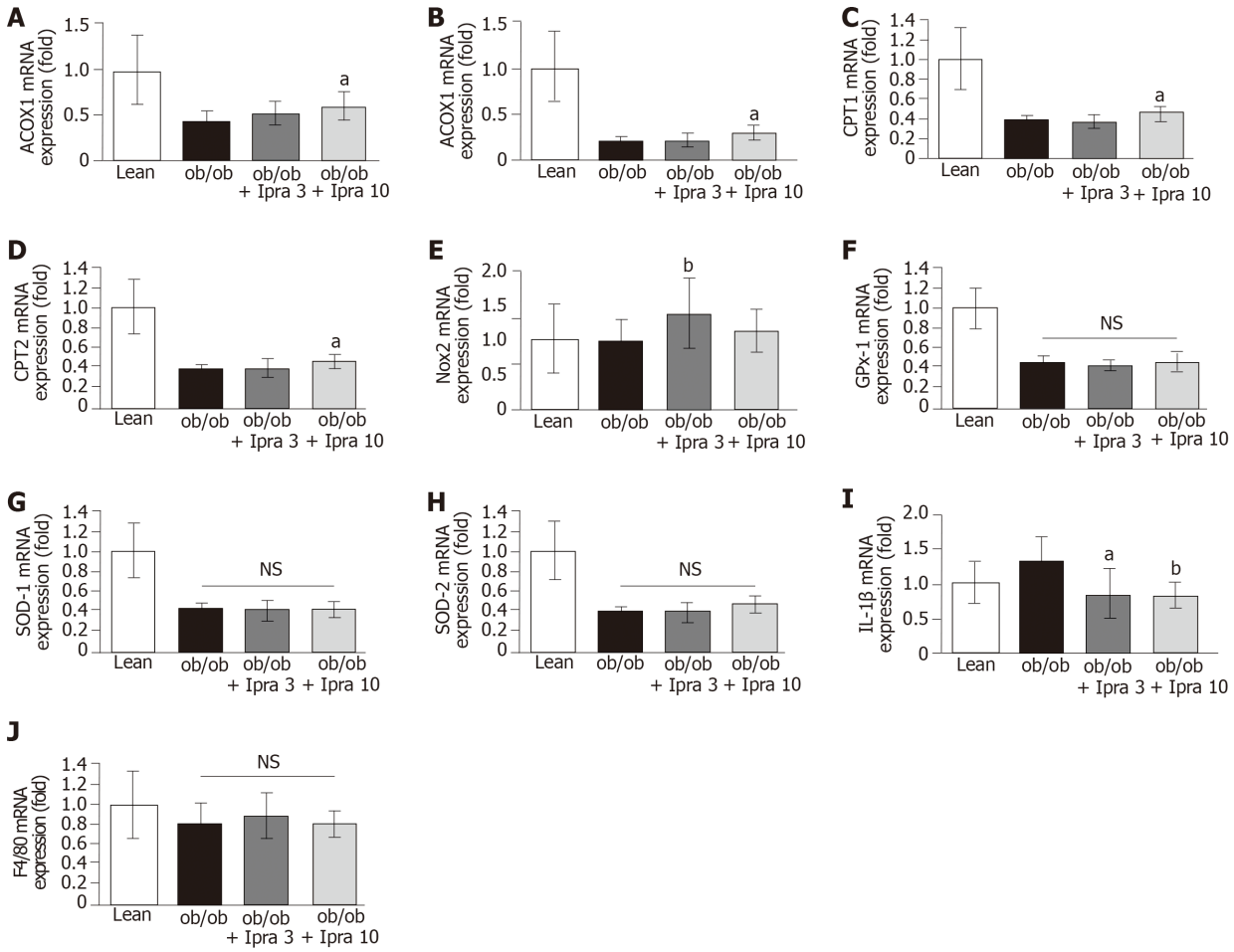

Figure 5 Hepatic mRNA expression of genes related to β-oxidation, oxidative stress, inflammatory cytokine, and macrophage marker in obese mice treated with or without ipragliflozin and their lean littermates. A: Hepatic mRNA expression of acyl-CoA oxidase 1 in obese (*ob/ob*) mice treated with or without ipragliflozin and their lean littermates; B: Hepatic mRNA expression of acyl-CoA synthetase in *ob/ob* mice treated with or without ipragliflozin and their lean littermates; C: Hepatic mRNA expression of carnitine palmitoyltransferase 1 in *ob/ob* mice treated with or without ipragliflozin and their lean littermates; D: Hepatic mRNA expression of carnitine palmitoyltransferase 2 in *ob/ob* mice treated with or without ipragliflozin and their lean littermates; E: Hepatic mRNA expression of NADPH oxidase 2 in *ob/ob* mice treated with or without ipragliflozin and their lean littermates; F: Hepatic mRNA expression of glutathione peroxidase 1 in *ob/ob* mice treated with or without ipragliflozin and their lean littermates; G: Hepatic mRNA expression of superoxide dismutase 1 in *ob/ob* mice treated with or without ipragliflozin and their lean littermates; H: Hepatic mRNA expression of superoxide dismutase 2 in *ob/ob* mice treated with or without ipragliflozin and their lean littermates; I: Hepatic mRNA expression of interleukin-1β in *ob/ob* mice treated with or without ipragliflozin and their lean littermates; J: Hepatic mRNA expression of F4/80 in *ob/ob* mice treated with or without ipragliflozin and their lean littermates. $^{a}P < 0.05$, $^{b}P < 0.01$ *vs* the *ob/ob* group. NS: Not significant; *ob/ob*: Obese; Ipra: Ipragliflozin; ACOX1: Acyl-CoA oxidase 1; ACS: Acyl-CoA synthetase; CPT-1: Carnitine palmitoyltransferase 1; CPT-2: Carnitine palmitoyltransferase 2; GPx-1: Glutathione peroxidase 1; IL-1β: Interleukin-1β; Ipra: Ipragliflozin; Nox2: NADPH oxidase 2; SOD-1: Superoxide dismutase 1; SOD-2: Superoxide dismutase 2. $n = 8$.

mediated by a decrease in body weight because ipragliflozin did not significantly affect body weight in the mouse model. Some SGLT2 inhibitors (empagliflozin, dapagliflozin, and canagliflozin) are generally reported to cause weight loss in obese mice and rats[24-26]. However, consistent with previous reports[7,8], our results showed that ipragliflozin did not significantly alter body weight in obese mice (Figure 1A). It is possible that there are pharmacologic differences among SGLT2 inhibitors that cause them to have different effects on body weight. Further studies are required to confirm this hypothesis.

The ameliorative effect of ipragliflozin on fatty liver in our experimental obese mouse model may have involved upregulation of the SIRT1 protein and subsequent enhancement of hepatic SIRT1 signaling. This hypothetical mechanism is supported by the following evidence: 1) hepatic SIRT1 protein expression levels in *ob/ob* mice were significantly increased by ipragliflozin treatment, and 2) activation of the SIRT1-PGC-1α/PPARα-FGF21 pathway was observed by mRNA expression analysis after ipragliflozin treatment. Ipragliflozin-mediated activation of the SIRT1-PGC-1α/PPARα-FGF21 pathway might result in promotion of mitochondrial fatty acid β-

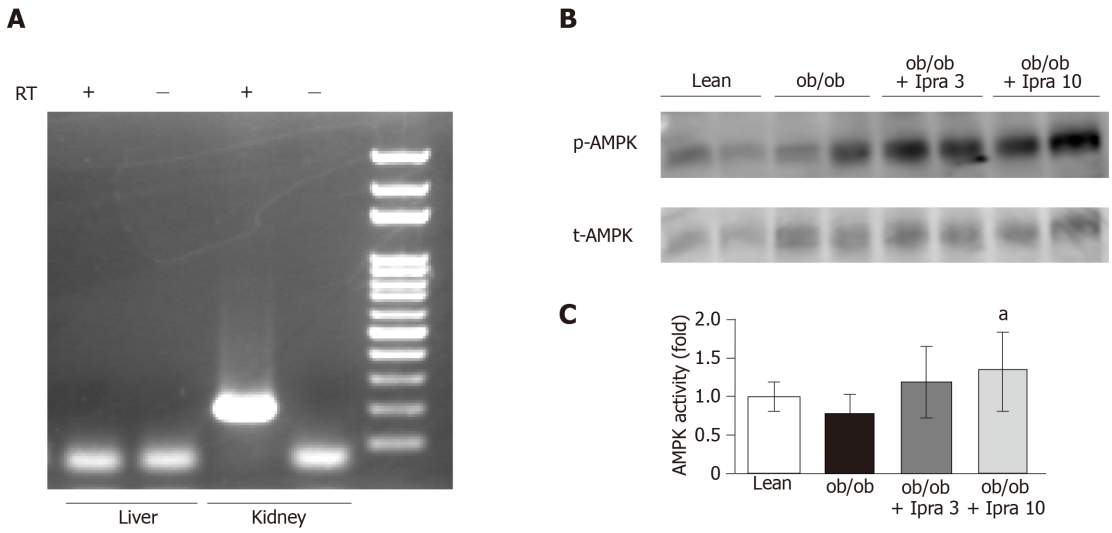

Figure 6 Hepatic and renal mRNA expression of sodium glucose cotransporter 2 and phosphorylation of AMP-activated protein kinase in whole livers of obese mice treated with or without ipragliflozin and their lean littermates. A: Reverse transcription-polymerase chain reaction analysis of SGLT2 expression in mouse livers and kidneys; B: Representative western blot showing the levels of phosphorylated phospho-AMP-activated protein kinase (p-AMPK) at the end of the 4-wk period of ipragliflozin treatment; C: The expression of p-AMPK protein was normalized to that of t-AMPK protein. $^{a}P < 0.05$ *vs* the *ob/ob* group. Ipra: Ipragliflozin; p-AMPK: Phospho AMP-activated protein kinase; SGLT2: Sodium glucose cotransporter 2; t-AMPK: Total AMP-activated protein kinase. $n = 8$.

oxidation and could account for the attenuation of liver steatosis in *ob/ob* mice. This mechanism is supported by a prior study demonstrating that ipragliflozin increases the hepatic mRNA expression levels of PPARα, a marker of lipid outflow, in Amylin liver NASH model mice[7]. Moreover, the SGLT2 inhibitor empagliflozin has been reported to increase the hepatic mRNA levels of PGC-1α and FGF21 in mice with high-fat-diet-induced obesity[24]. However, a recent clinical study on NAFLD patients with T2DM reported that treatment with the SGLT2 inhibitor dapagliflozin decreased plasma FGF21 levels, while treatment with a combination of dapagliflozin and omega-3 carboxylic acids did not[27]. FGF21 contributes to the regulation of mitochondrial activity and lipolysis in white adipose tissue[23,28] and increases fatty acid oxidation in the liver[22]. Therefore, an increase in FGF21 in the liver following therapy with ipragliflozin may promote fat utilization. On the other hand, ipragliflozin did not change the hepatic mRNA expression levels of FAS and ACC, markers of lipid inflow, probably because it promoted hepatic fatty acid oxidation without suppressing *de novo* hepatic lipogenesis in *ob/ob* mice. However, a previous report showed that the expression levels of FAS and ACC, which are upregulated in C57BL/6J wild-type mice fed a high-fat diet, are significantly suppressed by ipragliflozin[8]. The exact reason for the inconsistency among these findings is unknown but might be related to the differences between genetically engineered and wild-type mice or the differences between the ipragliflozin administration methods used (dietary supplementation *vs* drinking water supplementation).

Ipragliflozin did not significantly change the expression levels of oxidative stress-related genes, with the exception of Nox2, the mRNA expression levels of which were altered in 3 mg/d ipragliflozin-treated livers; these findings are contradictory to the findings of a previous study[29]. In addition, ipragliflozin did not significantly change macrophage infiltration based on the F4/80 mRNA expression data. In contrast, ipragliflozin significantly decreased IL-1β mRNA expression levels in the liver, consistent with the findings of a previous study[29]. However, the inhibitory effect of ipragliflozin on IL-1β mRNA expression was relatively small; thus, ipragliflozin might have no appreciable effects on oxidative stress. The exact causes of the discrepancy between our results and previous results regarding hepatic oxidative stress are unknown; however, differences in the mice and/or experimental protocols used might have affected the results.

To our knowledge, this is the first report to show that the therapeutic effects of the SGLT2 inhibitor ipragliflozin are associated with the hepatic expression of the SIRT1 protein. Our findings are supported by a recent report to show that a decrease in renal

SIRT1 protein expression was rescued by treatment with the SGLT2 inhibitor canagliflozin in *db/db* mice[30]. However, because SGLT2 is expressed in the kidneys but has not been reported to be expressed in the liver, further studies are needed to elucidate whether the effects of ipragliflozin on the liver are direct or indirect. Because SIRT1 is an energy-sensing molecule responsible for the promotion of healthy longevity mediated by caloric restriction[14], it is possible that temporary calorie loss due to the urinary glucose excretion caused by ipragliflozin may stimulate hepatic SIRT1. Similar conclusions were reached by Kim *et al*[31]. In addition, AMPK enhances SIRT1 activity by increasing cellular NAD+ levels[32], and the interplay between SIRT1 and AMPK is suggested to be reciprocal[33]. Several SGLT2 inhibitors, including canagliflozin, dapagliflozin, and empagliflozin, activate AMPK in HEK-293 cells, and canagliflozin activates AMPK in mouse livers *in vivo*[34]. Such findings are consistent with our finding that ipragliflozin significantly enhanced AMPK activation in our mouse model. The activation of hepatic SIRT1 might have been partly due to the activation of AMPK in our mouse model. Further investigation is needed to elucidate the mechanism by which hepatic SIRT1 signaling is activated after treatment with the SGLT2 inhibitor ipragliflozin.

In a previous study, the hepatic mRNA and protein expression levels of not only SIRT1 but also SIRT3, SIRT5, and SIRT6 were found to be lower in a human NAFLD group than in a control group[35]. Our findings from the comparison between the lean mouse group and the untreated *ob/ob* control mouse group regarding SIRT1 mRNA and protein expression are consistent with these results[35]. The previous finding that SIRT1 activators inhibit the expression of lipogenic genes such as FAS and ACC[36,37] and similar findings that FAS and ACC expressions are increased while hepatic SIRT1 expression is repressed in the human NAFLD group[35] are also consistent with our data. Interestingly, the expression of SIRT4 has been found to be upregulated in humans with NAFLD compared with controls[35]. SIRT4 mediates fatty acid oxidation in liver cells[38] and inhibits the interaction of SIRT1 and PPARα to decrease fatty acid oxidation[38]. In our study, the mRNA expression levels of genes related to fatty acid oxidation, ACOX1, CPT1, ACS, and CPT2, were lower in untreated *ob/ob* control mice than in lean mice. These results suggest that the expression of SIRT4 might have been higher in untreated *ob/ob* control mice than in lean mice in our study.

The limitations of our study are that only one mouse model and only one SGLT2 inhibitor were used. The mechanisms of the effects of SGLT2 inhibitors on NAFLD should be verified in the future using several animal models of NAFLD and additional SGLT2 inhibitors.

In conclusion, this study suggests that the liver steatosis-attenuating effects of ipragliflozin in *ob/ob* mice may be mediated partly by hepatic SIRT1 signaling, possibly through the PGC-1α/PPARα-FGF21 pathway. Because SGLT2 inhibitors are widely used in clinical practice and are characterized by good safety and tolerability profiles, treatment with these inhibitors may be an effective therapeutic strategy for patients with liver steatosis induced by T2DM.

ARTICLE HIGHLIGHTS

Research background

The sodium glucose cotransporter 2 (SGLT2) inhibitor ipragliflozin has been reported to improve liver steatosis in animal models and clinical studies. However, the mechanisms by which SGLT2 inhibitors improve liver steatosis are not fully understood. To our knowledge, this is the first report to show that the therapeutic effects of the SGLT2 inhibitor ipragliflozin are associated with activation of sirtuin 1 (SIRT1) signaling in the liver.

Research motivation

SGLT2 inhibitors are reportedly effective in fatty liver model mice as well as human nonalcoholic fatty liver disease patients. The mechanisms still need to be elucidated. Evaluating the mechanisms further may help identify molecules related to ameliorating fatty liver, allowing us to develop novel therapeutic strategies for fatty liver in the future.

Research objectives

The main objectives were to investigate the ameliorative effects of ipragliflozin on liver steatosis and the mechanisms of these effects in obese mice. Another objective was to

evaluate the effect of ipragliflozin on β-oxidation, oxidative stress, inflammatory cytokine, and macrophage infiltration in the liver. Our study confirms the ameliorative effects of SGLT2 inhibitors on liver steatosis and the previously proposed mechanisms, and proposes a new mechanism, which can promote further research.

Research methods

Obese (*ob/ob*) mice and their littermates received a normal chow diet or a normal chow diet plus 2 doses of ipragliflozin for 4 weeks. We examined lipid accumulation, β-oxidation, oxidative stress, inflammatory cytokine, and macrophage infiltration in the liver. *Ob/ob* mice were suitable for this experiment as they developed fatty liver even when they received normal chow. In addition, we used two control mouse groups, *ob/ob* control mice that received ipragliflozin and *ob/ob* littermates. In particular, SIRT1 signaling in the liver as a new candidate mechanism by which SGLT2 inhibitors improve liver steatosis was also assessed.

Research results

Amelioration of hepatic lipid accumulation by SGLT2 inhibitors was confirmed in our obese mouse model with ipragliflozin. Ipragliflozin-induced SIRT1 upregulation and SIRT1 signaling, which we propose might be involved in the mechanism by which ipragliflozin induces improvement of liver steatosis. The hypothesis should be further verified with different SGLT2 inhibitors in additional models and human samples. The observed effects of ipragliflozin on oxidative stress and macrophage infiltration, which were inconsistent with previous studies in the liver, need to be further evaluated.

Research conclusions

The new findings in our study are that SIRT1 signaling may be involved in the mechanism of ipragliflozin-induced improvement of liver steatosis in *ob/ob* mice. Thus, our study offers a new mechanism of ipragliflozin-induced improvement of liver steatosis. To be more specific, our proposed theory (hypothesis) is that activation of SIRT1 signaling due to ipragliflozin may ameliorate liver steatosis in *ob/ob* mice. This hypothesis and new phenomena were confirmed in our obese mouse model. In summary, the liver steatosis-attenuating effects of ipragliflozin in *ob/ob* mice may be mediated partly by hepatic SIRT1 signaling, possibly through the PGC-1α/PPARα-FGF21 pathway. The original insights into our results are that temporary calorie loss due to urinary glucose excretion caused by ipragliflozin may stimulate hepatic SIRT1, which might also be partly due to the activation of phospho-AMP-activated protein kinase in our mouse model. Our study provides additional evidence of SGLT2 inhibitor-induced improvement of liver steatosis; thus, we think that SGLT2 inhibitors are likely to be beneficial in diabetes mellitus patients with fatty liver in clinical practice.

Research perspectives

We take particular note of the role of SIRT1 as it plays important roles in controlling energy homeostasis and longevity in mammals and because the regulation of SIRT1 expression affects fatty liver. Thus, new target molecules that may be involved in amelioration of liver steatosis by SGLT2 inhibitors may be associated with energy homeostasis and longevity. We propose that future research should confirm our hypothesis using different animal models and human samples with different SGLT2 inhibitors or by confirming that upregulation or downregulation of SIRT1 signaling by the different methods used in our model or previous studies alters hepatic lipid accumulation. Genetically engineered mice, such as SIRT1 knockout mice, may be one of the best ways to confirm our results, and could be used to evaluate ipragliflozin-induced improvement of liver steatosis.

REFERENCES

1 **Cohen JC**, Horton JD, Hobbs HH. Human fatty liver disease: old questions and new insights. *Science* 2011; **332**: 1519-1523 [PMID: 21700865 DOI: 10.1126/science.1204265]

2 **Anstee QM**, Targher G, Day CP. Progression of NAFLD to diabetes mellitus, cardiovascular disease or cirrhosis. *Nat Rev Gastroenterol Hepatol* 2013; **10**: 330-344 [PMID: 23507799 DOI: 10.1038/nrgastro.2013.41]

3 **Torres DM**, Williams CD, Harrison SA. Features, diagnosis, and treatment of nonalcoholic fatty liver disease. *Clin Gastroenterol Hepatol* 2012; **10**: 837-858 [PMID: 22446927 DOI: 10.1016/j.cgh.2012.03.011]

4 **Sumida Y**, Seko Y, Yoneda M; Japan Study Group of NAFLD (JSG-NAFLD). Novel antidiabetic medications for non-alcoholic fatty liver disease with type 2 diabetes mellitus. *Hepatol Res* 2017;

47: 266-280 [PMID: 28019064 DOI: 10.1111/hepr.12856]
5 **Cusi K**. Treatment of patients with type 2 diabetes and non-alcoholic fatty liver disease: current approaches and future directions. *Diabetologia* 2016; **59**: 1112-1120 [PMID: 27101131 DOI: 10.1007/s00125-016-3952-1]
6 **Hayashizaki-Someya Y**, Kurosaki E, Takasu T, Mitori H, Yamazaki S, Koide K, Takakura S. Ipragliflozin, an SGLT2 inhibitor, exhibits a prophylactic effect on hepatic steatosis and fibrosis induced by choline-deficient l-amino acid-defined diet in rats. *Eur J Pharmacol* 2015; **754**: 19-24 [PMID: 25701721 DOI: 10.1016/j.ejphar.2015.02.009]
7 **Honda Y**, Imajo K, Kato T, Kessoku T, Ogawa Y, Tomeno W, Kato S, Mawatari H, Fujita K, Yoneda M, Saito S, Nakajima A. The Selective SGLT2 Inhibitor Ipragliflozin Has a Therapeutic Effect on Nonalcoholic Steatohepatitis in Mice. *PLoS One* 2016; **11**: e0146337 [PMID: 26731267 DOI: 10.1371/journal.pone.0146337]
8 **Komiya C**, Tsuchiya K, Shiba K, Miyachi Y, Furuke S, Shimazu N, Yamaguchi S, Kanno K, Ogawa Y. Ipragliflozin Improves Hepatic Steatosis in Obese Mice and Liver Dysfunction in Type 2 Diabetic Patients Irrespective of Body Weight Reduction. *PLoS One* 2016; **11**: e0151511 [PMID: 26977813 DOI: 10.1371/journal.pone.0151511]
9 **Takase T**, Nakamura A, Miyoshi H, Yamamoto C, Atsumi T. Amelioration of fatty liver index in patients with type 2 diabetes on ipragliflozin: an association with glucose-lowering effects. *Endocr J* 2017; **64**: 363-367 [PMID: 27980239 DOI: 10.1507/endocrj.EJ16-0295]
10 **Ito D**, Shimizu S, Inoue K, Saito D, Yanagisawa M, Inukai K, Akiyama Y, Morimoto Y, Noda M, Shimada A. Comparison of Ipragliflozin and Pioglitazone Effects on Nonalcoholic Fatty Liver Disease in Patients With Type 2 Diabetes: A Randomized, 24-Week, Open-Label, Active-Controlled Trial. *Diabetes Care* 2017; **40**: 1364-1372 [PMID: 28751548 DOI: 10.2337/dc17-0518]
11 **Ferrannini E**, Baldi S, Frascerra S, Astiarraga B, Heise T, Bizzotto R, Mari A, Pieber TR, Muscelli E. Shift to Fatty Substrate Utilization in Response to Sodium-Glucose Cotransporter 2 Inhibition in Subjects Without Diabetes and Patients With Type 2 Diabetes. *Diabetes* 2016; **65**: 1190-1195 [PMID: 26861783 DOI: 10.2337/db15-1356]
12 **Bordone L**, Guarente L. Calorie restriction, SIRT1 and metabolism: understanding longevity. *Nat Rev Mol Cell Biol* 2005; **6**: 298-305 [PMID: 15768047 DOI: 10.1038/nrm1616]
13 **Rodgers JT**, Lerin C, Gerhart-Hines Z, Puigserver P. Metabolic adaptations through the PGC-1 alpha and SIRT1 pathways. *FEBS Lett* 2008; **582**: 46-53 [PMID: 18036349 DOI: 10.1016/j.febslet.2007.11.034]
14 **Sasaki T**. Age-Associated Weight Gain, Leptin, and SIRT1: A Possible Role for Hypothalamic SIRT1 in the Prevention of Weight Gain and Aging through Modulation of Leptin Sensitivity. *Front Endocrinol (Lausanne)* 2015; **6**: 109 [PMID: 26236282 DOI: 10.3389/fendo.2015.00109]
15 **Ajmo JM**, Liang X, Rogers CQ, Pennock B, You M. Resveratrol alleviates alcoholic fatty liver in mice. *Am J Physiol Gastrointest Liver Physiol* 2008; **295**: G833-G842 [PMID: 18755807 DOI: 10.1152/ajpgi.90358.2008]
16 **Yamazaki Y**, Usui I, Kanatani Y, Matsuya Y, Tsuneyama K, Fujisaka S, Bukhari A, Suzuki H, Senda S, Imanishi S, Hirata K, Ishiki M, Hayashi R, Urakaze M, Nemoto H, Kobayashi M, Tobe K. Treatment with SRT1720, a SIRT1 activator, ameliorates fatty liver with reduced expression of lipogenic enzymes in MSG mice. *Am J Physiol Endocrinol Metab* 2009; **297**: E1179-E1186 [PMID: 19724016 DOI: 10.1152/ajpendo.90997.2008]
17 **Purushotham A**, Schug TT, Xu Q, Surapureddi S, Guo X, Li X. Hepatocyte-specific deletion of SIRT1 alters fatty acid metabolism and results in hepatic steatosis and inflammation. *Cell Metab* 2009; **9**: 327-338 [PMID: 19356714 DOI: 10.1016/j.cmet.2009.02.006]
18 **Vega RB**, Huss JM, Kelly DP. The coactivator PGC-1 cooperates with peroxisome proliferator-activated receptor alpha in transcriptional control of nuclear genes encoding mitochondrial fatty acid oxidation enzymes. *Mol Cell Biol* 2000; **20**: 1868-1876 [PMID: 10669761 DOI: 10.1128/mcb.20.5.1868-1876.2000]
19 **Rodgers JT**, Lerin C, Haas W, Gygi SP, Spiegelman BM, Puigserver P. Nutrient control of glucose homeostasis through a complex of PGC-1alpha and SIRT1. *Nature* 2005; **434**: 113-118 [PMID: 15744310 DOI: 10.1038/nature03354]
20 **Wu Z**, Puigserver P, Andersson U, Zhang C, Adelmant G, Mootha V, Troy A, Cinti S, Lowell B, Scarpulla RC, Spiegelman BM. Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. *Cell* 1999; **98**: 115-124 [PMID: 10412986 DOI: 10.1016/S0092-8674(00)80611-X]
21 **Li Y**, Wong K, Giles A, Jiang J, Lee JW, Adams AC, Kharitonenkov A, Yang Q, Gao B, Guarente L, Zang M. Hepatic SIRT1 attenuates hepatic steatosis and controls energy balance in mice by inducing fibroblast growth factor 21. *Gastroenterology* 2014; **146**: 539-49.e7 [PMID: 24184811 DOI: 10.1053/j.gastro.2013.10.059]
22 **Badman MK**, Pissios P, Kennedy AR, Koukos G, Flier JS, Maratos-Flier E. Hepatic fibroblast growth factor 21 is regulated by PPARalpha and is a key mediator of hepatic lipid metabolism in ketotic states. *Cell Metab* 2007; **5**: 426-437 [PMID: 17550778 DOI: 10.1016/j.cmet.2007.05.002]
23 **Chau MD**, Gao J, Yang Q, Wu Z, Gromada J. Fibroblast growth factor 21 regulates energy metabolism by activating the AMPK-SIRT1-PGC-1alpha pathway. *Proc Natl Acad Sci USA* 2010; **107**: 12553-12558 [PMID: 20616029 DOI: 10.1073/pnas.1006962107]
24 **Xu L**, Nagata N, Nagashimada M, Zhuge F, Ni Y, Chen G, Mayoux E, Kaneko S, Ota T. SGLT2 Inhibition by Empagliflozin Promotes Fat Utilization and Browning and Attenuates Inflammation and Insulin Resistance by Polarizing M2 Macrophages in Diet-induced Obese Mice. *EBioMedicine* 2017; **20**: 137-149 [PMID: 28579299 DOI: 10.1016/j.ebiom.2017.05.028]
25 **Devenny JJ**, Godonis HE, Harvey SJ, Rooney S, Cullen MJ, Pelleymounter MA. Weight loss induced by chronic dapagliflozin treatment is attenuated by compensatory hyperphagia in diet-induced obese (DIO) rats. *Obesity (Silver Spring)* 2012; **20**: 1645-1652 [PMID: 22402735 DOI: 10.1038/oby.2012.59]
26 **Liang Y**, Arakawa K, Ueta K, Matsushita Y, Kuriyama C, Martin T, Du F, Liu Y, Xu J, Conway B, Conway J, Polidori D, Ways K, Demarest K. Effect of canagliflozin on renal threshold for glucose, glycemia, and body weight in normal and diabetic animal models. *PLoS One* 2012;

7: e30555 [PMID: 22355316 DOI: 10.1371/journal.pone.0030555]
27 **Eriksson JW**, Lundkvist P, Jansson PA, Johansson L, Kvarnström M, Moris L, Miliotis T, Forsberg GB, Risérus U, Lind L, Oscarsson J. Effects of dapagliflozin and n-3 carboxylic acids on non-alcoholic fatty liver disease in people with type 2 diabetes: a double-blind randomised placebo-controlled study. *Diabetologia* 2018; **61**: 1923-1934 [PMID: 29971527 DOI: 10.1007/s00125-018-4675-2]
28 **Inagaki T**, Dutchak P, Zhao G, Ding X, Gautron L, Parameswara V, Li Y, Goetz R, Mohammadi M, Esser V, Elmquist JK, Gerard RD, Burgess SC, Hammer RE, Mangelsdorf DJ, Kliewer SA. Endocrine regulation of the fasting response by PPARalpha-mediated induction of fibroblast growth factor 21. *Cell Metab* 2007; **5**: 415-425 [PMID: 17550777 DOI: 10.1016/j.cmet.2007.05.003]
29 **Tahara A**, Kurosaki E, Yokono M, Yamajuku D, Kihara R, Hayashizaki Y, Takasu T, Imamura M, Li Q, Tomiyama H, Kobayashi Y, Noda A, Sasamata M, Shibasaki M. Effects of SGLT2 selective inhibitor ipragliflozin on hyperglycemia, hyperlipidemia, hepatic steatosis, oxidative stress, inflammation, and obesity in type 2 diabetic mice. *Eur J Pharmacol* 2013; **715**: 246-255 [PMID: 23707905 DOI: 10.1016/j.ejphar.2013.05.014]
30 **Umino H**, Hasegawa K, Minakuchi H, Muraoka H, Kawaguchi T, Kanda T, Tokuyama H, Wakino S, Itoh H. High Basolateral Glucose Increases Sodium-Glucose Cotransporter 2 and Reduces Sirtuin-1 in Renal Tubules through Glucose Transporter-2 Detection. *Sci Rep* 2018; **8**: 6791 [PMID: 29717156 DOI: 10.1038/s41598-018-25054-y]
31 **Kim JW**, Lee YJ, You YH, Moon MK, Yoon KH, Ahn YB, Ko SH. Effect of sodium-glucose cotransporter 2 inhibitor, empagliflozin, and α-glucosidase inhibitor, voglibose, on hepatic steatosis in an animal model of type 2 diabetes. *J Cell Biochem* 2019; **120**: 8534-8546 [PMID: 30474134 DOI: 10.1002/jcb.28141]
32 **Cantó C**, Gerhart-Hines Z, Feige JN, Lagouge M, Noriega L, Milne JC, Elliott PJ, Puigserver P, Auwerx J. AMPK regulates energy expenditure by modulating NAD+ metabolism and SIRT1 activity. *Nature* 2009; **458**: 1056-1060 [PMID: 19262508 DOI: 10.1038/nature07813]
33 **Feige JN**, Lagouge M, Canto C, Strehle A, Houten SM, Milne JC, Lambert PD, Mataki C, Elliott PJ, Auwerx J. Specific SIRT1 activation mimics low energy levels and protects against diet-induced metabolic disorders by enhancing fat oxidation. *Cell Metab* 2008; **8**: 347-358 [PMID: 19046567 DOI: 10.1016/j.cmet.2008.08.017]
34 **Hawley SA**, Ford RJ, Smith BK, Gowans GJ, Mancini SJ, Pitt RD, Day EA, Salt IP, Steinberg GR, Hardie DG. The Na+/Glucose Cotransporter Inhibitor Canagliflozin Activates AMPK by Inhibiting Mitochondrial Function and Increasing Cellular AMP Levels. *Diabetes* 2016; **65**: 2784-2794 [PMID: 27381369 DOI: 10.2337/db16-0058]
35 **Wu T**, Liu YH, Fu YC, Liu XM, Zhou XH. Direct evidence of sirtuin downregulation in the liver of non-alcoholic fatty liver disease patients. *Ann Clin Lab Sci* 2014; **44**: 410-418 [PMID: 25361925]
36 **Walker AK**, Yang F, Jiang K, Ji JY, Watts JL, Purushotham A, Boss O, Hirsch ML, Ribich S, Smith JJ, Israelian K, Westphal CH, Rodgers JT, Shioda T, Elson SL, Mulligan P, Najafi-Shoushtari H, Black JC, Thakur JK, Kadyk LC, Whetstine JR, Mostoslavsky R, Puigserver P, Li X, Dyson NJ, Hart AC, Näär AM. Conserved role of SIRT1 orthologs in fasting-dependent inhibition of the lipid/cholesterol regulator SREBP. *Genes Dev* 2010; **24**: 1403-1417 [PMID: 20595232 DOI: 10.1101/gad.1901210]
37 **Ponugoti B**, Kim DH, Xiao Z, Smith Z, Miao J, Zang M, Wu SY, Chiang CM, Veenstra TD, Kemper JK. SIRT1 deacetylates and inhibits SREBP-1C activity in regulation of hepatic lipid metabolism. *J Biol Chem* 2010; **285**: 33959-33970 [PMID: 20817729 DOI: 10.1074/jbc.M110.122978]
38 **Min Z**, Gao J, Yu Y. The Roles of Mitochondrial SIRT4 in Cellular Metabolism. *Front Endocrinol (Lausanne)* 2018; **9**: 783 [PMID: 30666234 DOI: 10.3389/fendo.2018.00783]

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ORIGINAL ARTICLE

Basic Study

Anti-inflammatory and anti-oxidant effects of aloe vera in rats with non-alcoholic steatohepatitis

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Abstract

BACKGROUND

Aloe vera exerts several biological activities, such as, anti-inflammatory, antioxidant, and antimicrobial effects. It was recently shown to reduce insulin resistance and triglyceride level. We hypothesized that aloe vera would have beneficial effects in alleviating non-alcoholic steatohepatitis (NASH) in rats.

AIM

To examine the therapeutic effects of aloe vera in NASH rats.

METHODS

All rats were randomly divided into 3 groups (n = 6 in each group). Rats in the control group were fed ad libitum with a standard diet for 8 wk. Rats in the NASH group were fed ad libitum with a high-fat high-fructose diet (HFHFD) for 8 wk. Rats in the aloe vera group were fed ad libitum with a HFHFD and aloe vera in dimethylsulfoxide (50 mg/kg) by gavage daily for 8 wk. Liver samples were collected at the end of the treatment period.

RESULTS

Hepatic malondialdehyde (MDA) levels increased significantly in the NASH group as compared with the control group (377 ± 77 nmol/mg *vs* 129 ± 51 nmol/mg protein, respectively, $P < 0.001$). Glutathione (GSH) levels were significantly lower in the NASH group than the control group (9 ± 2 nmol/mg *vs* 24 ± 8 nmol/mg protein, respectively, $P = 0.001$). The expression of interleukin-18

Institutional animal care and use committee statement: That *in vivo* experiments were performed only after receiving formal authorization by the Institutional "Animal Care and Use Committee" of the Chulalongkorn University, Bangkok, Thailand. The procedures for the care and handling of the animals used in the study were in accordance with the European Directive 2010/63/EU on the protection of animals used for scientific purposes.

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(IL-18), nuclear factor-kappa β, and caspase-3 increased, while peroxisome proliferator-activated receptor gamma decreased in the NASH group compared with the controls. Following aloe vera administration, MDA levels decreased (199 ± 35 nmol/mg protein) and GSH increased (18 ± 4 nmol/mg protein) markedly. Steatosis, hepatocyte ballooning, lobular inflammation and increased hepatocyte apoptosis were observed in the NASH group. Aloe vera treatment attenuated these changes in liver histology.

CONCLUSION
Aloe vera attenuated oxidative stress, hepatic inflammation and hepatocyte apoptosis, thus improving liver pathology in rats with NASH.

Key words: Non-alcoholic steatohepatitis; Aloe vera; Oxidative stress; Hepatic inflammation; Hepatocyte apoptosis; Peroxisome proliferator-activated receptor

Core tip: To the best of our knowledge, this is the first study to evaluate the therapeutic effects of aloe vera in non-alcoholic steatohepatitis (NASH). In this animal model of NASH, we found that aloe vera decreased oxidative stress markers, replenished natural antioxidants, and reduced hepatic inflammation and hepatocyte apoptosis. Thus, aloe vera can alleviate the pathologic changes seen in NASH.

Citation: Klaikeaw N, Wongphoom J, Werawatganon D, Chayanupatkul M, Siriviriyakul P. Anti-inflammatory and anti-oxidant effects of aloe vera in rats with non-alcoholic steatohepatitis. *World J Hepatol* 2020; 12(7): 363-377
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INTRODUCTION

Due to the obesity epidemic, non-alcoholic fatty liver disease (NAFLD) has become the most common liver disease worldwide with an estimated prevalence of 24%[1]. In the United States, NAFLD has now surpassed alcoholic liver disease as the leading indication for liver transplantation in women[2]. A subset of patients with NAFLD develop non-alcoholic steatohepatitis (NASH) which can lead to fibrosis progression and cirrhosis[3,4]. Currently, there are no Food and Drug Administration approved medications for the treatment of NASH. Weight loss, the mainstay of treatment for NASH, is difficult to achieve and hardly sustainable. Alternative therapies that are safe, effective and inexpensive are attractive options for the management of lifelong diseases such as NASH.

Indigenous to Africa, Asia and Mediterranean regions, aloe vera has long been used as a medicinal plant for various conditions[5,6]. Aloe vera contains at least 75 potentially active constituents such as vitamins, enzymes, minerals, sugars, plant steroids, hormones and amino acids[7]. Aloe vera and its constituents exert several biological activities, for instance, anti-inflammatory (salicylic acid, campesterol, β-sitosterol and C-glucosyl chromone), antioxidant (vitamin A, C and E), antitumor (anthraquinones and phorbol myristic acetate), and antimicrobial effects (aloin and emodin)[5,7,8]. Aloe vera has never been directly studied in NASH but it has shown potential benefits in other liver conditions such as amelioration of acetaminophen-induced liver damage[9]. Moreover, aloe vera has been shown to reduce insulin resistance and hepatic triglyceride levels which are major components of NASH[10,11]. With the aforementioned evidence, we hypothesized that aloe vera could alleviate NASH *via* its anti-inflammatory and antioxidant properties. To the best of our knowledge, this is the first study to evaluate the effects of aloe vera on NASH development in an animal model.

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MATERIALS AND METHODS

Animal preparation

The study protocol was approved by the Institutional Review Board for Animal Research Studies, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand. Male Sprague-Dawley® rats weighing 220-260 g were obtained from the National Laboratory Animal Center, Mahidol University, Nakorn Pathom, Thailand. The animals were kept in a controlled temperature room at 25 ± 1°C under standard conditions with a normal 12 h light-12 h dark cycle. All rats had free access to drinking water. The animals were allowed to acclimate to the new environment for 1 wk prior to initiation of the experiment.

Aloe vera preparation

Leaves of 1-year-old aloe vera plants were cut and washed thoroughly with water to cleanse the aloin-containing juice. The spiked edges were sliced off to extract the pulp. The pulp was then mixed in a blender and sieved through fine gauze. Aloe vera gel was turned into powder by freeze drying using a lyophilizer. Before use, the aloe vera powder was reconstituted into gel form and dispensed in distilled water (DW).

Experimental protocol

A total of 18 rats were randomly divided into 3 groups as follows: (1) Group 1 (control group, n = 6): Rats were fed ad libitum with standard laboratory chow (National Laboratory Animal Center, Mahidol University, Nakorn Pathom, Thailand) which contained 35% of total energy from fat, 47% from carbohydrate, and 18% from protein for 8 wk; (2) Group 2 (NASH group, n = 6): Rats were fed ad libitum with a made-in-house high-fat high-fructose diet (HFHFD) which contained 55% of total energy from fat, 35% from carbohydrate (20% from fructose and 15% from starch), and 10% from protein for 8 wk; and (3) Group 3 (aloe vera group, n = 6): Rats were fed ad libitum with the HFHFD plus daily administration of aloe vera (50 mg/kg) dissolved in DW by gavage for 8 wk. Aloe vera powder was supplied by Lipo Chemical Co., United States.

Animals were weighed weekly during the experimental period. At the end of 8 wk, all rats were euthanized with sodium thiopental overdose after a 12-h fast. The liver was surgically removed and cut into several pieces. Three small pieces of liver were immediately frozen in liquid nitrogen and stored at -80°C until malondialdehyde (MDA) and glutathione (GSH) analysis. The remaining liver specimen was fixed in 10% formaldehyde for histopathological examination and the expression of IL-18, PPARγ, caspase-3, cytochrome-C and NF-kβ was analyzed using an immunohistochemistry technique.

Hepatic MDA determination

MDA level was measured from homogenized tissue using a commercial assay kit (Cayman Chemical Company, Ann Arbor, MI, United States). The test involved measuring the rate of production of thiobarbituric acid-reactive substances under high-temperature and acidic conditions. The process is described as follows: One gram of liver tissue was homogenized in radioimmunoprecipitation assay buffer (RIPA buffer) containing protease inhibitor and sonicated on ice for 15 s. Supernatants were obtained after centrifugation at 1600 × g for 10 min at 4°C. The absorbance of the supernatant fraction was read at a wavelength of 532 nm. MDA levels were calculated from a standard curve and expressed as nmol/mg protein.

Hepatic GSH measurement

GSH level was quantified using a commercial assay kit (Cayman Chemical Company, Ann Arbor, MI, United States). Liver tissues were washed with phosphate buffered saline (PBS) solution. Tissues were then homogenized with cold MES buffer before being centrifuged at 10000 × g for 15 min at 4°C. The supernatants were collected and deproteinated. The absorbance of the supernatant fraction was read at a wavelength of 405 nm and GSH values were calculated from a standard curve and expressed as nmol/mg protein.

Immunohistochemistry for hepatic IL-18, PPAR-γ, NF-kβ, caspase-3, and cytochrome-C expression

After being fixed in formaldehyde, liver samples were embedded in paraffin and sliced at a thickness of 3 μm. The tissue sections were then deparaffinized with xylene

and ethanol for 10 min. Antigen retrieval was achieved by treating the slides with citrate buffer at pH 6.0 and heating in a microwave for 13 min. The slides were incubated with 3% hydrogen peroxide to block endogenous peroxidase activity for 5 min and with 3% normal horse serum to block nonspecific binding for 20 min. Tissues were then washed with PBS solution. The sections were subsequently incubated with primary antibodies for IL-18 (Gene Tex, CA, United States), PPAR-γ (Santa Cruz Biotechnology, CA, United States), NF-kβ (Abcam, MA, United States), caspase-3, and cytochrome-C (R and D, United States) for 30 min at room temperature and washed again with PBS solution. The slides were then incubated with specific secondary antibodies for 30 min at room temperature. When color development with diaminobenzidine was detected, the sections were counterstained with hematoxylin.

Under light microscopy, IL-18-positive cells were defined as Kupffer cells with dark brown-stained nuclei. Hepatocytes with PPAR-γ, NF-kβ, caspase-3 and cytochrome-C expression were characterized as liver cells with brownish nuclei. Images of each sample were taken at high-magnification (40 ×). The numbers of positive stained cells were counted using Aperio ImageScope software (Leica Biosystems Imaging, Inc., MD, United States) and expressed as the percentage of immunoreactive cells or average intensity (pixel).

Histopathological examination

Liver samples were processed using a standard technique. Collected liver tissue was fixed in 10% formalin at room temperature for 24-48 h, embedded in paraffin and sectioned at 3 μm using a microtome. Each tissue section was stained with hematoxylin and eosin and placed on glass slides for light microscopic examination. An experienced pathologist blinded to the experiment evaluated all samples. All fields in each section were examined and graded for steatosis (0-3), hepatocyte ballooning (0-3) and lobular inflammation (0-3) according to the criteria described by Brunt *et al*[12]. The percentage of apoptotic hepatocytes was determined by terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) method using the ApopTag® Peroxidase In Situ Apoptosis Detection kit (Millipore, CA, United States). The procedure was performed according to the manufacturer's instructions.

Statistical analysis

Continuous data are presented as mean ± SD. One-way ANOVA and the post-hoc Tukey HSD were used to compare results between the groups. A P value of less than 0.05 was considered statistically significant. All statistical analyses were performed using SPSS Statistics for Windows version 17 (SPSS, Inc., Chicago, IL, United States).

RESULTS

Body weight changes in each group

There were no differences in body weight among the groups at the beginning of the experiment. After eight weeks, rats fed with the HFHFD (NASH group) had lower body weight than those in the control group (223 ± 14.0 g *vs* 417 ± 11.2 g, respectively, $P < 0.001$). Following aloe vera administration, rats in the treatment arm gained more weight than those in the NASH group (276 ± 3.6 g *vs* 223 ± 14.0 g, respectively, $P < 0.001$) (Figure 1).

Liver histopathology

The histologic scores in each group are summarized in Table 1. Liver histology was normal in the control group. In contrast, liver pathology in the NASH group revealed significant macrovesicular and microvesicular steatosis, hepatocyte ballooning and lobular inflammation. Following aloe vera treatment, liver pathology significantly improved with only mild steatosis, minimal hepatocyte ballooning and lobular inflammation present (Figure 2A).

Hepatic MDA and GSH levels

As shown in Figure 3A, MDA levels in the NASH group were significantly higher than those in the control group (377 ± 77 nmol/mg *vs* 129 ± 51 nmol/mg protein, $P < 0.001$). MDA levels declined significantly in rats receiving aloe vera along with HFHFD compared to those receiving HFHFD alone (199 ± 35 nmol/mg *vs* 377 ± 77 nmol/mg protein, $P < 0.001$). As demonstrated in Figure 3B, GSH levels in the NASH group were significantly lower than those in the control group (9 ± 2 nmol/mg *vs* 24 ± 8

Table 1 Summary of steatohepatitis and necroinflammation scores in all experimental groups

Group	Steatosis				Inflammation				Ballooning		
	0	1	2	3	0	1	2	3	0	1	2
Control	-	-	-	-	-	-	-	-	-	-	-
Non-alcoholic steatohepatitis	-	-	3	3	0	5	1	-	-	4	2
Aloe vera	3	3	-	-	2	4	-	-	3	3	-

The scoring system was based on the study by Brunt *et al*[11].

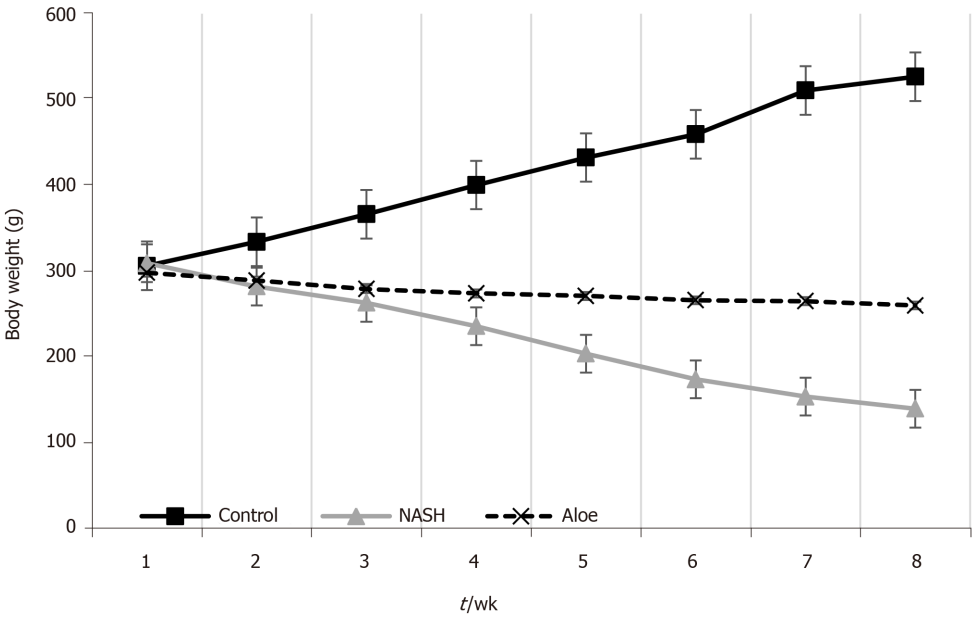


Figure 1 Mean body weight changes in rats in each group. Data at each time point are expressed as mean ± SD. NASH: Non-alcoholic steatohepatitis.

nmol/mg protein, $P < 0.001$). Aloe vera treatment led to a notable rise in hepatic GSH levels (18 ± 4 nmol/mg *vs* 9 ± 2 nmol/mg protein in the aloe vera and NASH groups, respectively, $P = 0.04$).

Hepatic expression of IL-18, PPAR-γ, and NF-kβ by immunohistochemistry

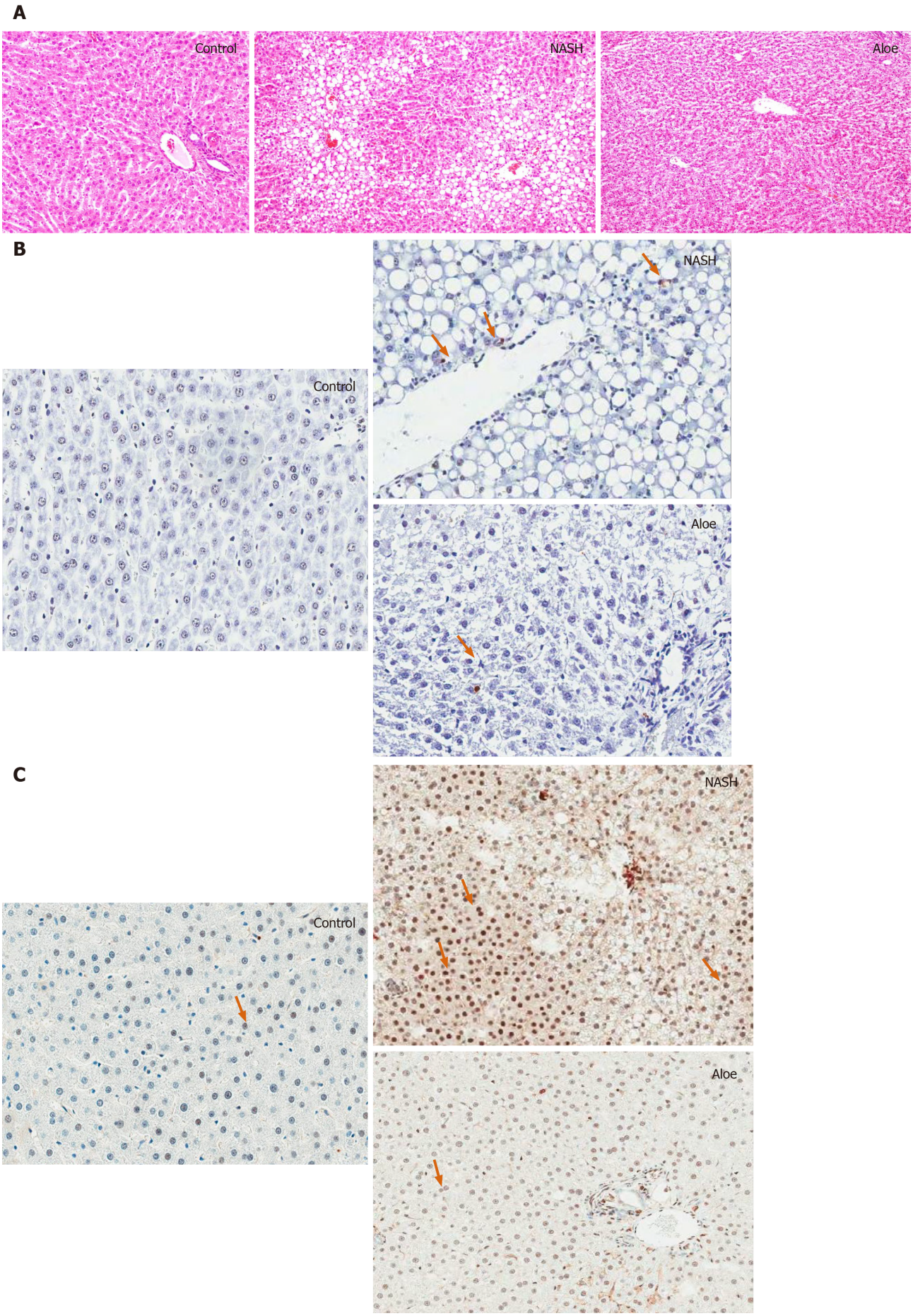
As illustrated in Figures 2, 4, and 5, the expression of IL-18 and NF-kβ increased, while the percentage of PPAR-γ positive cells decreased in the NASH group as compared to controls. In contrast, aloe vera treatment restored the changes in hepatic IL-18, PPAR-γ, and NF-kβ expression to the levels close to those observed in the control group.

Hepatic expression of caspase-3 and cytochrome-C, and the degree of hepatocyte apoptosis by the TUNEL method

Using the TUNEL method, we found that the degree of hepatocyte apoptosis was significantly higher in the NASH group as compared with the control and aloe vera groups. Similarly, markers of apoptosis such as caspase-3 and cytochrome-C were also higher in the NASH group, while the expression of these 2 markers was similar in the control and aloe vera groups (Figures 2 and 6).

DISCUSSION

The pathogenesis of NAFLD is a complex process involving insulin resistance and lipid accumulation in the liver followed by lipid peroxidation, oxidative stress, and inflammatory responses[13]. Insulin resistance facilitates adipose tissue lipolysis followed by the release of free fatty acids (FFA) in the serum, and promotes


A
Control
NASH
Aloe
B
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NASH
Aloe
C
Control
NASH
Aloe

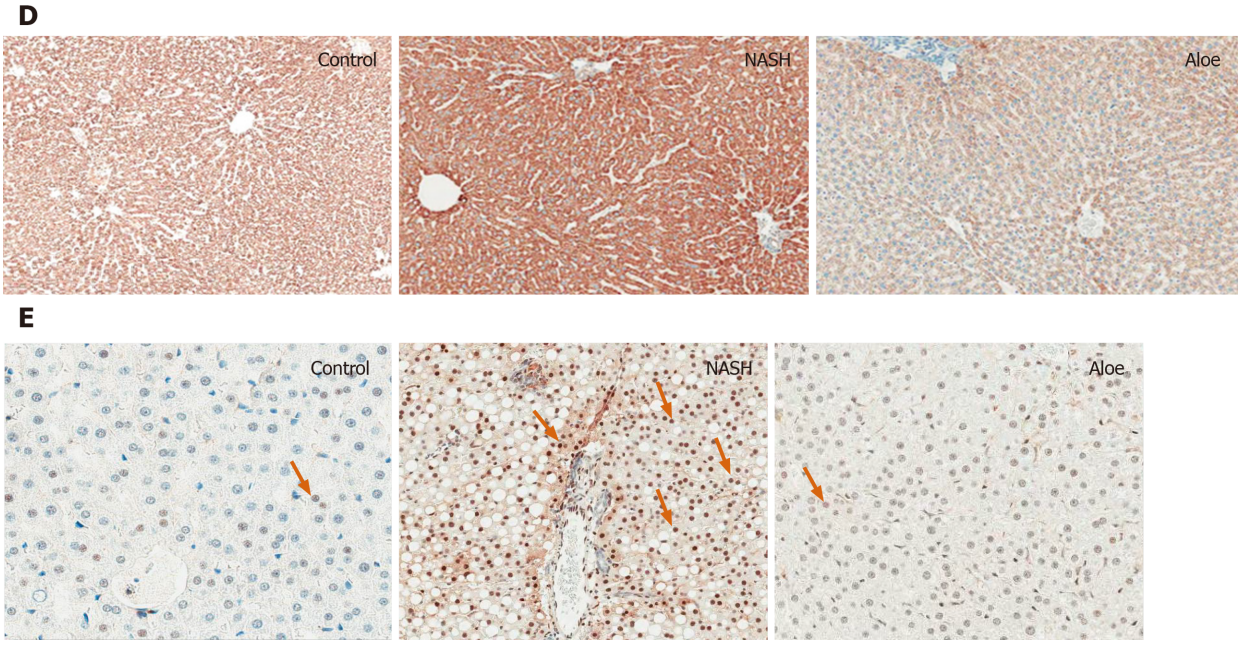


Figure 2 Representative images on light microscopy using hematoxylin and eosin stain, terminal deoxynucleotidyl transferase dUTP nick end labeling stain for apoptosis, or immunohistochemistry stains for NF-kβ, cytochrome-C and caspase-3. A: Hematoxylin and eosin stain; B: Terminal deoxynucleotidyl transferase dUTP nick end labeling stain; C: Immunohistochemistry stains; D: Cytochrome-C; E: Caspase-3. Control groups are presented on the left column; non-alcoholic steatohepatitis group in the middle and aloe vera group on the right column. Arrowheads point to positive cells. NASH: Non-alcoholic steatohepatitis.

lipogenesis in the liver, thus increasing hepatic fat accumulation. These lipids, especially saturated fatty acids (SFA), lead to lipotoxic stress in the endoplasmic reticulum and mitochondria and subsequently hepatocyte apoptosis. Moreover, SFA can activate toll-like receptor-4 leading to NF-kβ activation and TNF-α and IL-6 production, the important cytokines associated with inflammatory responses in the liver[14].

Our results showed that aloe vera improved liver histopathological changes associated with HFHFD. In this experiment, we used aloe vera crude extract; therefore, we could not pinpoint the actual active ingredient of aloe vera that might have therapeutic effects against NASH. Previous studies suggested that phytosterols were the potential substances of interest. Misawa and colleagues evaluated the effects of lophenol and cycloartanol extracted from aloe vera gel on glucose and lipid metabolism in diabetic, obese rats. The authors found that lophenol and cycloartanol reduced the expression of both gluconeogenic and lipogenic genes in the liver along with the reduction in hepatic fat contents. This study, however, did not evaluate liver histology[15]. Similarly, Nomaguchi *et al*[16] used five phytosterols isolated from aloe vera gel in mice fed with high fat diet and found that aloe vera phytosterols could reduce body fat and liver triglyceride.

Accumulating evidence supports the implication of lipid peroxidation and oxidative stress in the development of NAFLD[17-19]. In accordance with other research, we found that MDA levels, a marker of oxidative stress, increased in rats with NASH as compared with the control group. Moreover, natural antioxidants, such as GSH, significantly declined in animals receiving HFHFD further perpetuating oxidative stress in the liver. The administration of aloe vera attenuated the increment in MDA levels and restored GSH levels in rats with NASH. Despite not being studied directly in animal models of NASH, aloe vera has been shown to reduce oxidative stress markers such as thiobarbituric acid reactive substances and increase natural antioxidants such as GSH and superoxide dismutase in streptozotocin-induced diabetic rats[20].

PPAR-γ, a member of the nuclear hormone receptor superfamily, is involved in the regulation of adipocyte differentiation, lipid metabolism, and liver inflammation[21-23]. *In vitro* and *in vivo* studies suggested that PPAR-γ provided protection against NASH by inhibiting hepatic stellate cell proliferation and migration[24], reducing pro-inflammatory cytokine production, and suppressing fatty acid synthesis[22]. Zhao *et al*[21] previously demonstrated that the mRNA levels of PPAR-γ were lower in rats fed with high fat diet and the levels were negatively correlated with the degree of hepatic

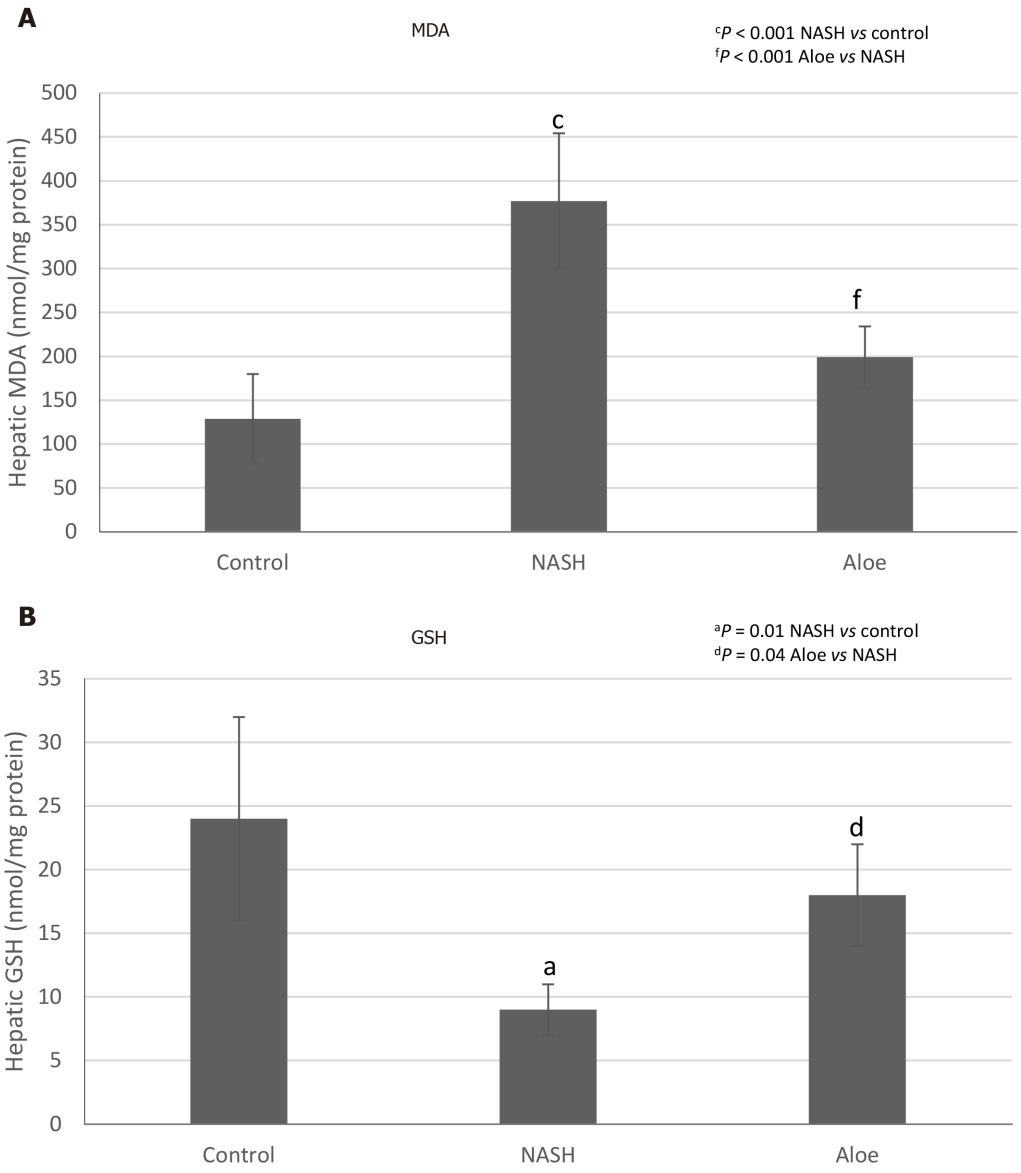
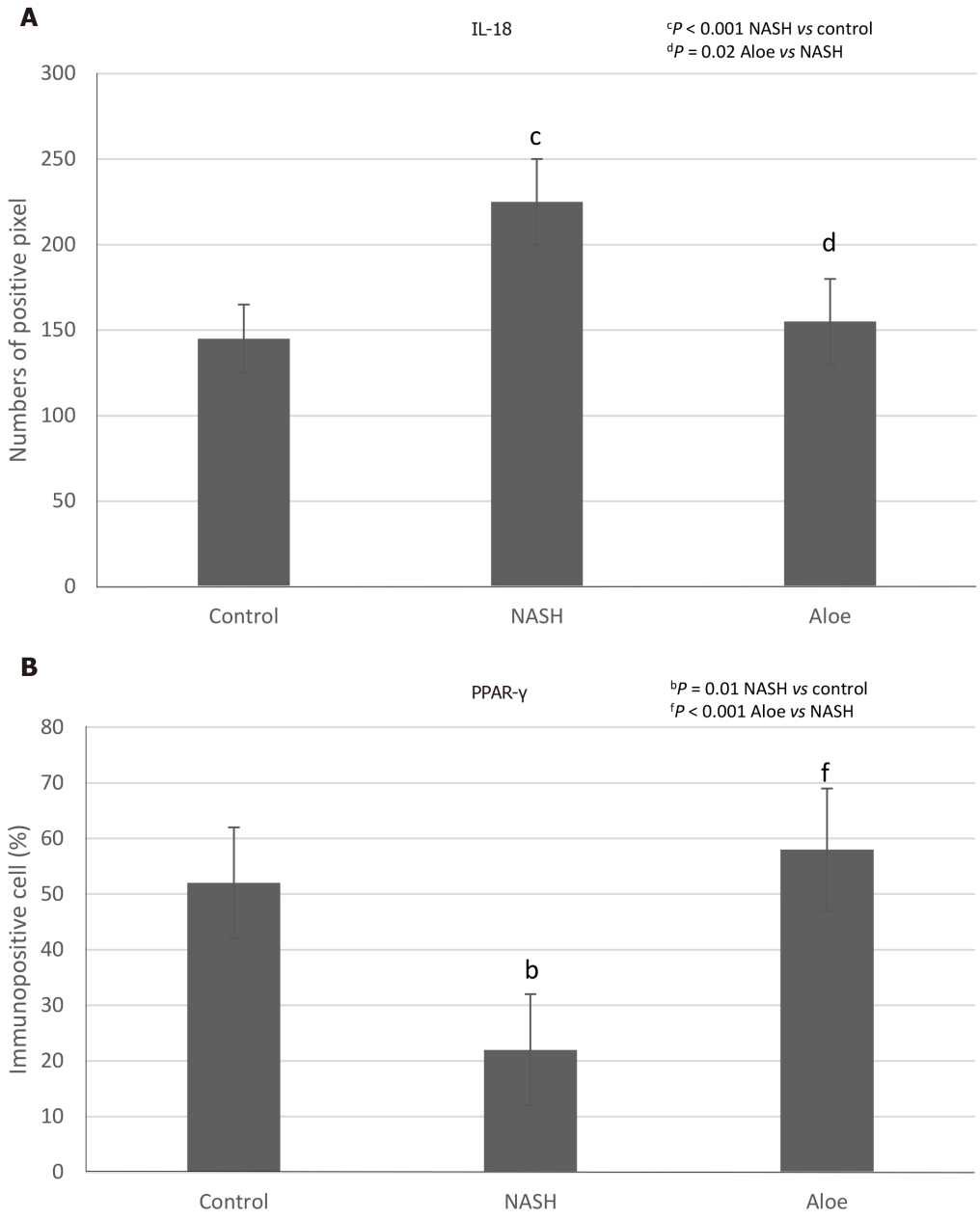


Figure 3 Hepatic malondialdehyde and glutathione levels in each group. A: Malondialdehyde; B: Glutathione. Data are expressed as mean ± SD. NASH: Non-alcoholic steatohepatitis; MDA: Malondialdehyde; GSH: Glutathione.

inflammation, necrosis and fibrosis, as well as serum TNF-α and hepatic MDA contents. Similarly, we found that PPAR-γ expression was significantly lower in rats with NASH and this was restored to the level of control rats with aloe vera treatment. Comparable with our results, Nomaguchi *et al*[16] found that aloe vera could stimulate PPAR-γ and α activities in a dose-dependent manner as well as decrease body fat, hepatic triglyceride levels and serum lipid panels in diet-induced obese mice[16].

Recent data suggested that hepatocyte apoptosis may play a pivotal role in the progression of NAFLD[25,26]. Lipid accumulation, especially saturated FFAs and free cholesterol, may sensitize Fas- and TNF-mediated hepatocyte apoptosis and induce mitochondrial dysfunction, thus activating both extrinsic and intrinsic pathways of apoptosis[25-27]. The activation of both pathways leads to the release of pro-apoptotic proteins such as cytochrome-C, which then triggers the downstream effector caspases 3, 6, and 7 to initiate the apoptotic processes[28]. In this study, we found increases in hepatocyte apoptosis on liver histology, and cytochrome-C and caspase-3 expression in rats with NASH. Conversely, the degree of apoptosis and its markers decreased significantly with aloe vera treatment. To the best of our knowledge, this is the first study to evaluate the effect of aloe vera on hepatocyte apoptosis.

IL-18 has previously been shown to be involved in both innate and acquired immune responses by inducing several cytokines such as interferon-γ, TNF-α and IL-1[29]. However, recent studies demonstrated that IL-18 also played an important role in the regulation of metabolic functions and the development of NAFLD and NASH.


A
IL-18
[c]P < 0.001 NASH vs control
[d]P = 0.02 Aloe vs NASH
Numbers of positive pixel
300
250
200
150
100
50
0
c
d
Control
NASH
Aloe
B
PPAR-γ
[b]P = 0.01 NASH vs control
[f]P < 0.001 Aloe vs NASH
Immunopositive cell (%)
80
70
60
50
40
30
20
10
0
b
f
Control
NASH
Aloe

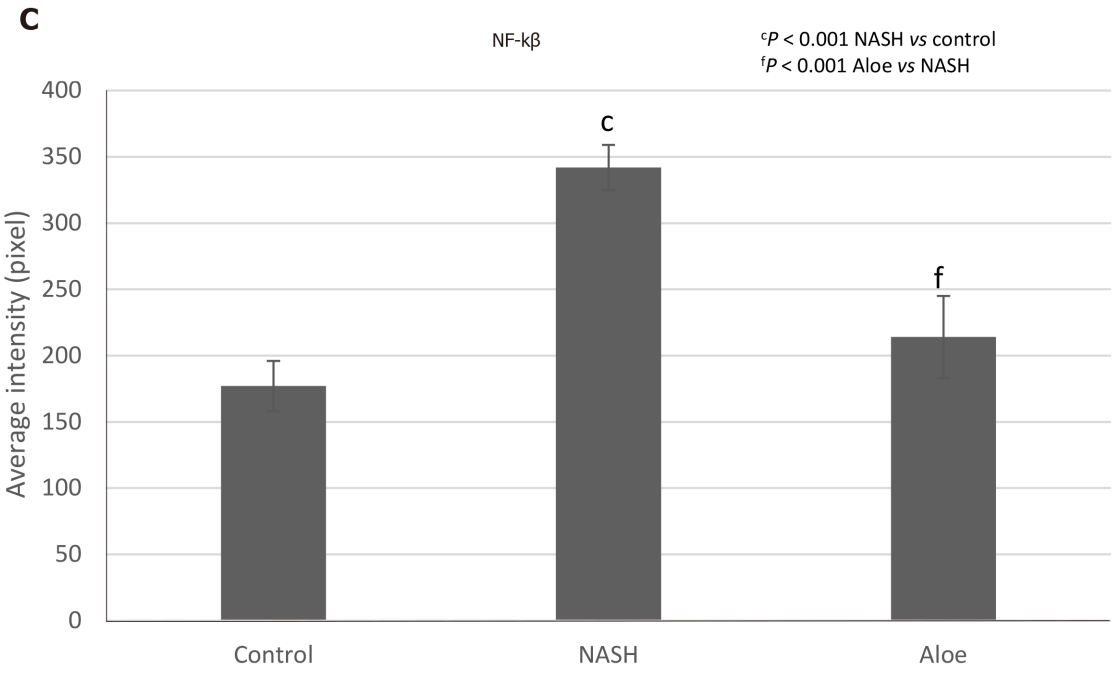


Figure 4 The expression of IL-18, PPAR-γ, and NF-kβ using immunohistochemistry methods. A: IL-18; B: PPAR-γ; C: NF-kβ. The positive cells were counted by Aperio ImageScope software. Data are expressed as mean ± SD. NASH: Non-alcoholic steatohepatitis.

Animal studies reported increases in food intake, body weight, insulin resistance, serum glucose and serum lipid levels, and eventually the development of NASH in IL-18 deficient mice[30,31]. The severity of NASH also appeared to be higher in IL-18 knockout mice as compared to wild-type mice[32]. In our study, we found increased expression of IL-18 in rats fed with HFHFD and this was normalized by the administration of aloe vera. These findings could be explained in 2 ways. The elevated IL-18 expression could be an attempt to offset the metabolic derangement due to HFHFD or simply the inflammatory responses from fat accumulation in the liver. Human studies showed similar results of elevated IL-18 levels in patients with NAFLD and these levels were positively correlated with the degree of liver injury[33,34]. Although the presence of IL-18 is crucial in maintaining energy homeostasis, the overexpression of IL-18 could accelerate hepatocyte apoptosis and perpetuate severe liver damage[35]. Aloe vera treatment decreased IL-18 expression through its anti-inflammatory and insulin sensitizing effects[10].

A unique finding in our study was the presence of weight loss in rats fed with HFHFD. This was unexpected given that several experimental models of NASH showed significant weight gain in rats receiving HFHFD compared with control diet[36-38]. This finding of weight loss was previously seen in a NASH model produced by a methionine and choline deficient diet[39,40]. We could not ascertain the amount of methionine and choline in our diet formula but it was possible that both nutrients were at low levels given a protein content of only 10% in our custom diet. Another hypothesis was the presence of high monounsaturated and polyunsaturated fatty acid contents in our diet (19% and 6% of total energy, respectively). Studies have shown that mono- and polyunsaturated fatty acids are associated with higher post-prandial fat oxidation, diet-induced thermogenesis, decreased appetite and less weight gain as compared with SFAs[41-44]. Lastly, we did not measure the total caloric intake of rats in each group; therefore, we could not say with absolute certainty that rats with HFHFD diet received an equal amount of calories compared to control rats. It is important to note, however, that liver histology in our model was consistent with NASH despite weight loss.

Our study, however, was not without limitations. First, rats with NASH in our model were slim, which differed from the usual NASH phenotype in humans. Our findings may be useful in the understanding of "lean" NASH in humans, but translating our results to "obese" NASH should be done with caution. Second, aloe vera and HFHFD were administered simultaneously resulting in our study representing more of a prevention model than treatment model. Further studies are warranted to confirm the therapeutic effects of aloe vera. Third, we only evaluated the gross effects of aloe vera on NASH development in this study. Additional *in vitro*

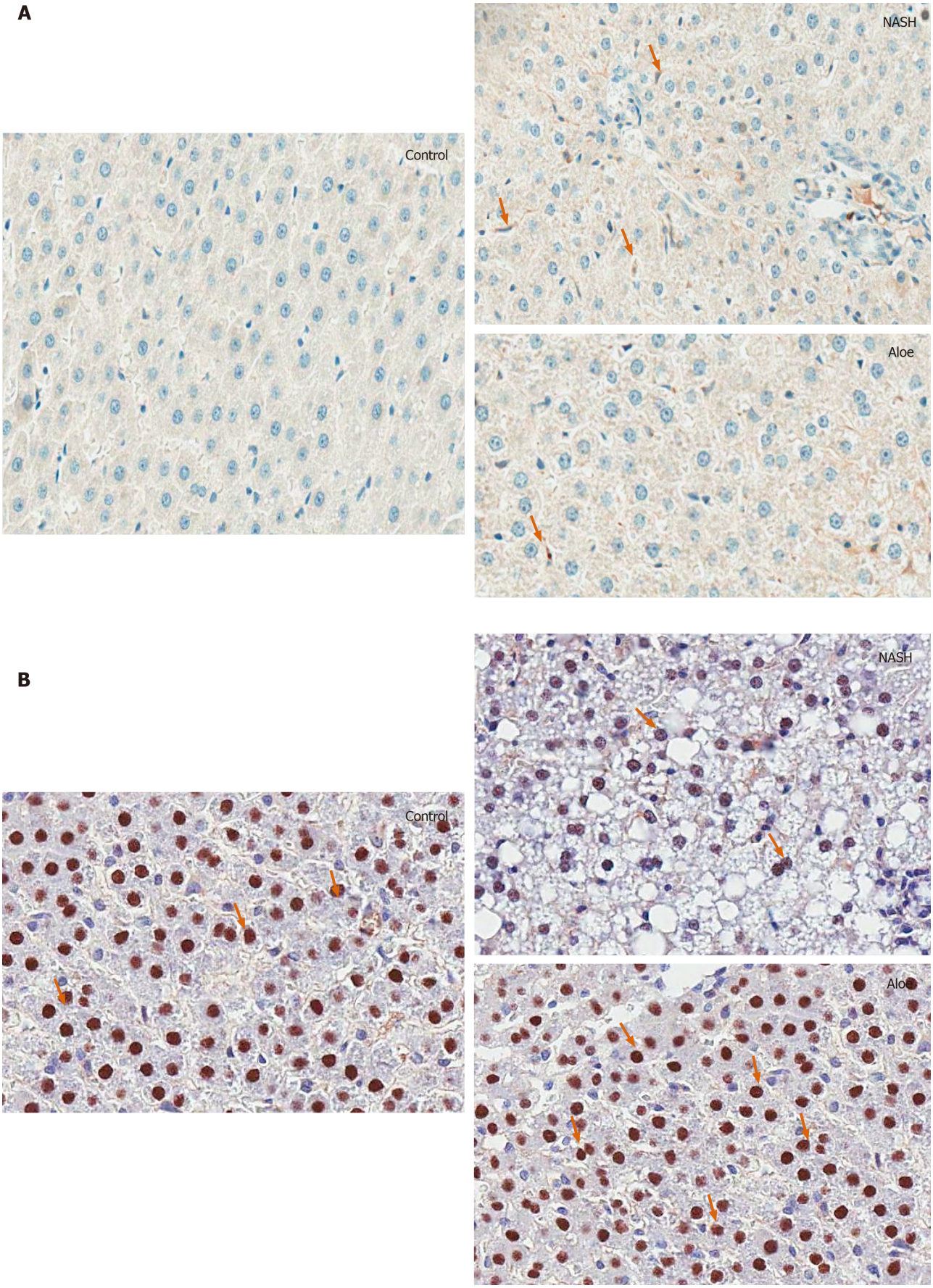


Figure 5 Representative images on light microscopy using immunohistochemistry stains for IL-18 and PPAR-γ. A: IL-18; B: PPAR-γ. Control groups are presented on the left column; non-alcoholic steatohepatitis group in the middle and aloe vera group on the right column. Arrowheads point to positive cells. NASH: Non-alcoholic steatohepatitis.

studies are needed to determine cellular and subcellular targets of aloe vera.

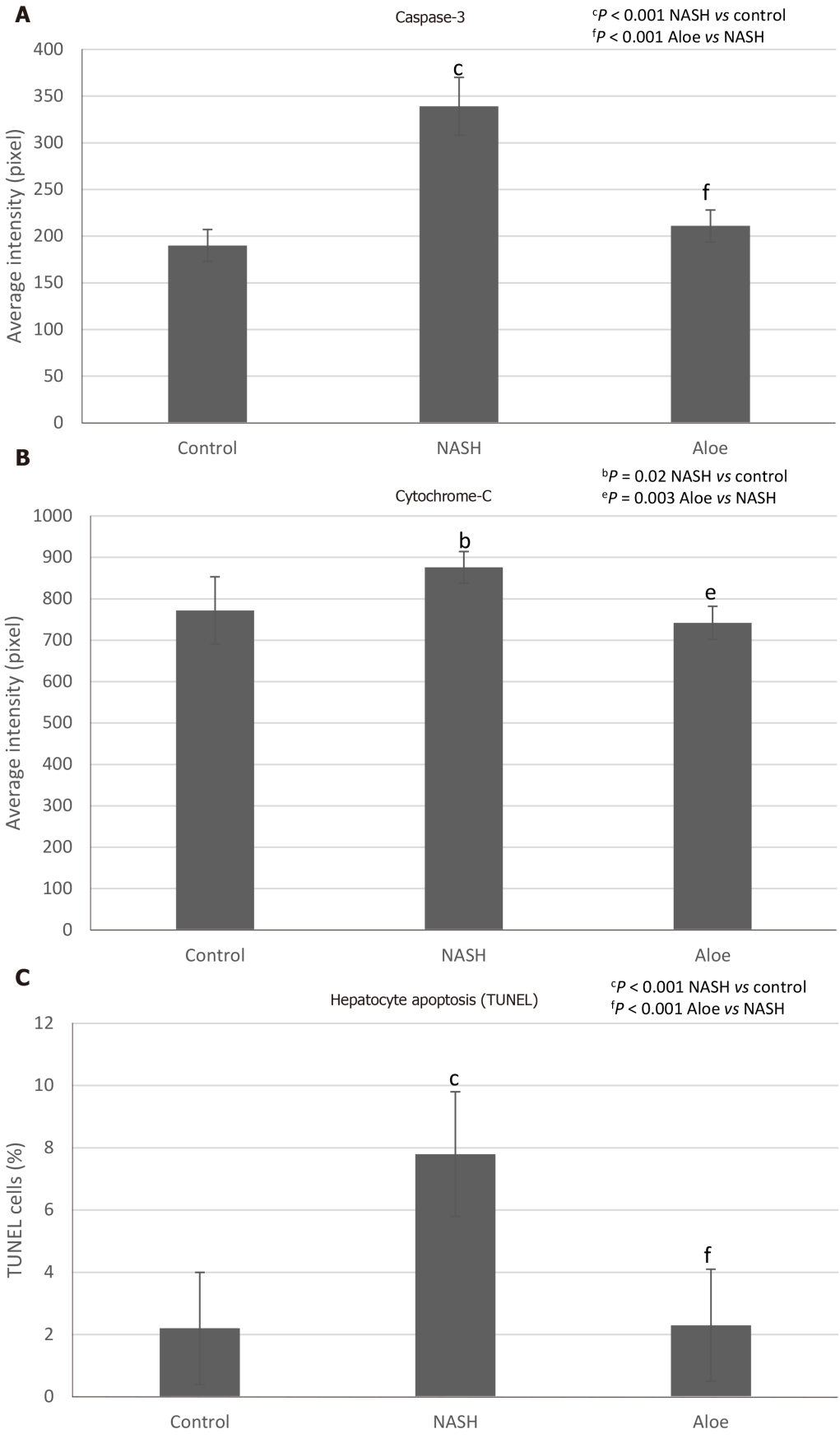


Figure 6 The expression of caspase-3 and cytochrome-C using immunohistochemistry methods, and the degree of hepatocyte apoptosis using terminal deoxynucleotidyl transferase dUTP nick end labeling methods. A: Caspase-3; B: Cytochrome-C; C: Degree of hepatocyte apoptosis. The positive cells were counted by Aperio ImageScope software. Data are expressed as mean ± SD. NASH: Non-alcoholic steatohepatitis; TUNEL: Terminal deoxynucleotidyl transferase dUTP nick end labeling.

In summary, aloe vera reduced lipid accumulation, oxidative stress, hepatic inflammation, hepatocyte apoptosis and histologic changes in this rat model of NASH.

ARTICLE HIGHLIGHTS

Research background

Non-alcoholic fatty liver disease (NAFLD) is one of the most common liver diseases worldwide. However, there is no Food and Drug Administration (FDA) approved medication for the treatment of NAFLD. Aloe vera has previously been shown to have anti-inflammatory and anti-oxidant properties, which might be beneficial in the treatment of NAFLD.

Research motivation

With the absence of FDA-approved treatment for NAFLD, we attempted to find a safe and effective treatment for NAFLD. Alternative medicines that are safe, effective and inexpensive are attractive options for the management of life-long diseases, such as non-alcoholic steatohepatitis (NASH).

Research objectives

The main objective of this study was to evaluate the effects of aloe vera on NASH development in an animal model.

Research methods

Rats were divided into 3 groups: Control, NASH [rats received high-fat high-fructose diet (HFHFD) to induce NASH pathology], and NASH + aloe vera. We compared liver histopathology, oxidative stress marker [malondialdehyde (MDA)], anti-oxidant level [glutathione (GSH)], inflammatory marker (IL-18), degree and markers of hepatocyte apoptosis [terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL), caspase-3, cytochrome-C], and PPAR-γ expression among the three groups.

Research results

We found that by administering aloe vera along with HFHFD, we were able to significantly reduce the severity of NASH pathology in this animal model. In this study, aloe vera treatment increased the level of natural anti-oxidant (GSH), reduced oxidative stress (MDA) and inflammatory markers (IL-18), and decreased the degree of hepatocyte apoptosis (TUNEL). At the subcellular level, we also found that aloe vera increased the expression of PPAR-γ and reduced the expression of NF-kβ, caspase-3 and cytochrome-C.

Research conclusions

This is the first study to evaluate the effects of aloe vera in rats with NASH. We found that aloe vera reduced the severity of NASH pathology in rats that received HFHFD. We hypothesized that aloe vera exerted its treatment effects by reducing oxidative stress and inflammation in the liver.

Research perspectives

The rats in our model were lean, so our results might not be entirely applicable to obese NASH that is seen more commonly in humans. Further studies with an obese rat model are warranted to confirm the effects of aloe vera in those conditions. Moreover, we used aloe vera crude extract in this study. Additional studies will be needed to identify the active ingredients in aloe vera that have anti-NASH effects.

REFERENCES

1 **Younossi Z**, Anstee QM, Marietti M, Hardy T, Henry L, Eslam M, George J, Bugianesi E. Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention. *Nat Rev Gastroenterol Hepatol* 2018; **15**: 11-20 [PMID: 28930295 DOI: 10.1038/nrgastro.2017.109]

2 **Noureddin M**, Vipani A, Bresee C, Todo T, Kim IK, Alkhouri N, Setiawan VW, Tran T, Ayoub WS, Lu SC, Klein AS, Sundaram V, Nissen NN. NASH Leading Cause of Liver Transplant in Women: Updated Analysis of Indications For Liver Transplant and Ethnic and Gender Variances. *Am J Gastroenterol* 2018; **113**: 1649-1659 [PMID: 29880964 DOI: 10.1038/s41395-018-0088-6]

3 **Ekstedt M**, Nasr P, Kechagias S. Natural History of NAFLD/NASH. *Curr Hepatol Rep* 2017;

16: 391-397 [PMID: 29984130 DOI: 10.1007/s11901-017-0378-2]
4 **Ong JP**, Younossi ZM. Epidemiology and natural history of NAFLD and NASH. *Clin Liver Dis* 2007; **11**: 1-16, vii [PMID: 17544968 DOI: 10.1016/j.cld.2007.02.009]
5 **Rahmani AH**, Aldebasi YH, Srikar S, Khan AA, Aly SM. Aloe vera: Potential candidate in health management via modulation of biological activities. *Pharmacogn Rev* 2015; **9**: 120-126 [PMID: 26392709 DOI: 10.4103/0973-7847.162118]
6 **Rajeswari R**, Umadevi M, Sharmila Rahale C, Pushpa R, Selvavenkadesh S, Sampath Kumar KP, Bhowmik D. Aloe vera: The Miracle Plant Its Medicinal and Traditional Uses in India. *J Pharm Phytochem* 2012; **1**: 118-124
7 **Surjushe A**, Vasani R, Saple DG. Aloe vera: a short review. *Indian J Dermatol* 2008; **53**: 163-166 [PMID: 19882025 DOI: 10.4103/0019-5154.44785]
8 **Foster M**, Hunter D, Samman S, Benzie IFF, Wachtel-Galor S. Evaluation of the Nutritional and Metabolic Effects of Aloe vera. In: Herbal Medicine: Biomolecular and Clinical Aspects. 2nd edition. Herbal Medicine: Biomolecular and Clinical Aspects. Boca Raton (FL): CRC Press/Taylor & Francis; 2011 [PMID: 22593933 DOI: 10.1201/b10787-4]
9 **Werawatganon D**, Linlawan S, Thanapirom K, Somanawat K, Klaikeaw N, Rerknimitr R, Siriviriyakul P. Aloe vera attenuated liver injury in mice with acetaminophen-induced hepatitis. *BMC Complement Altern Med* 2014; **14**: 229 [PMID: 25005608 DOI: 10.1186/1472-6882-14-229]
10 **Shin E**, Shim KS, Kong H, Lee S, Shin S, Kwon J, Jo TH, Park YI, Lee CK, Kim K. Dietary Aloe Improves Insulin Sensitivity via the Suppression of Obesity-induced Inflammation in Obese Mice. *Immune Netw* 2011; **11**: 59-67 [PMID: 21494375 DOI: 10.4110/in.2011.11.1.59]
11 **Rajasekaran S**, Ravi K, Sivagnanam K, Subramanian S. Beneficial effects of aloe vera leaf gel extract on lipid profile status in rats with streptozotocin diabetes. *Clin Exp Pharmacol Physiol* 2006; **33**: 232-237 [PMID: 16487267 DOI: 10.1111/j.1440-1681.2006.04351.x]
12 **Brunt EM**, Janney CG, Di Bisceglie AM, Neuschwander-Tetri BA, Bacon BR. Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. *Am J Gastroenterol* 1999; **94**: 2467-2474 [PMID: 10484010 DOI: 10.1111/j.1572-0241.1999.01377.x]
13 **Chen Z**, Yu R, Xiong Y, Du F, Zhu S. A vicious circle between insulin resistance and inflammation in nonalcoholic fatty liver disease. *Lipids Health Dis* 2017; **16**: 203 [PMID: 29037210 DOI: 10.1186/s12944-017-0572-9]
14 **Fuchs M**, Sanyal AJ. Lipotoxicity in NASH. *J Hepatol* 2012; **56**: 291-293 [PMID: 21741924 DOI: 10.1016/j.jhep.2011.05.019]
15 **Misawa E**, Tanaka M, Nomaguchi K, Nabeshima K, Yamada M, Toida T, Iwatsuki K. Oral ingestion of aloe vera phytosterols alters hepatic gene expression profiles and ameliorates obesity-associated metabolic disorders in zucker diabetic fatty rats. *J Agric Food Chem* 2012; **60**: 2799-2806 [PMID: 22352711 DOI: 10.1021/jf204465j]
16 **Nomaguchi K**, Tanaka M, Misawa E, Yamada M, Toida T, Iwatsuki K, Goto T, Kawada T. Aloe vera phytosterols act as ligands for PPAR and improve the expression levels of PPAR target genes in the livers of mice with diet-induced obesity. *Obes Res Clin Pract* 2011; **5**: e190–e201 [PMID: 24331101 DOI: 10.1016/j.orcp.2011.01.002]
17 **Sumida Y**, Niki E, Naito Y, Yoshikawa T. Involvement of free radicals and oxidative stress in NAFLD/NASH. *Free Radic Res* 2013; **47**: 869-880 [PMID: 24004441 DOI: 10.3109/10715762.2013.837577]
18 **Videla LA**, Rodrigo R, Orellana M, Fernandez V, Tapia G, Quiñones L, Varela N, Contreras J, Lazarte R, Csendes A, Rojas J, Maluenda F, Burdiles P, Diaz JC, Smok G, Thielemann L, Poniachik J. Oxidative stress-related parameters in the liver of non-alcoholic fatty liver disease patients. *Clin Sci (Lond)* 2004; **106**: 261-268 [PMID: 14556645 DOI: 10.1042/cs20030285]
19 **Li S**, Tan HY, Wang N, Zhang ZJ, Lao L, Wong CW, Feng Y. The Role of Oxidative Stress and Antioxidants in Liver Diseases. *Int J Mol Sci* 2015; **16**: 26087-26124 [PMID: 26540040 DOI: 10.3390/ijms161125942]
20 **Rajasekaran S**, Sivagnanam K, Subramanian S. Antioxidant effect of Aloe vera gel extract in streptozotocin-induced diabetes in rats. *Pharmacol Rep* 2005; **57**: 90-96 [PMID: 15849382]
21 **Zhao CY**, Jiang LL, Li L, Deng ZJ, Liang BL, Li JM. Peroxisome proliferator activated receptor-gamma in pathogenesis of experimental fatty liver disease. *World J Gastroenterol* 2004; **10**: 1329-1332 [PMID: 15112352 DOI: 10.3748/wjg.v10.i9.1329]
22 **Wu CW**, Chu ES, Lam CN, Cheng AS, Lee CW, Wong VW, Sung JJ, Yu J. PPARgamma is essential for protection against nonalcoholic steatohepatitis. *Gene Ther* 2010; **17**: 790-798 [PMID: 20376096 DOI: 10.1038/gt.2010.41]
23 **Everett L**, Galli A, Crabb D. The role of hepatic peroxisome proliferator-activated receptors (PPARs) in health and disease. *Liver* 2000; **20**: 191-199 [PMID: 10902968 DOI: 10.1034/j.1600-0676.2000.020003191.x]
24 **Marra F**, Efsen E, Romanelli RG, Caligiuri A, Pastacaldi S, Batignani G, Bonacchi A, Caporale R, Laffi G, Pinzani M, Gentilini P. Ligands of peroxisome proliferator-activated receptor gamma modulate profibrogenic and proinflammatory actions in hepatic stellate cells. *Gastroenterology* 2000; **119**: 466-478 [PMID: 10930382 DOI: 10.1053/gast.2000.9365]
25 **Feldstein AE**, Gores GJ. Apoptosis in alcoholic and nonalcoholic steatohepatitis. *Front Biosci* 2005; **10**: 3093-3099 [PMID: 15970563 DOI: 10.2741/1765]
26 **Alkhouri N**, Carter-Kent C, Feldstein AE. Apoptosis in nonalcoholic fatty liver disease: diagnostic and therapeutic implications. *Expert Rev Gastroenterol Hepatol* 2011; **5**: 201-212 [PMID: 21476915 DOI: 10.1586/egh.11.6]
27 **Marí M**, Caballero F, Colell A, Morales A, Caballeria J, Fernandez A, Enrich C, Fernandez-Checa JC, García-Ruiz C. Mitochondrial free cholesterol loading sensitizes to TNF- and Fas-mediated steatohepatitis. *Cell Metab* 2006; **4**: 185-198 [PMID: 16950136 DOI: 10.1016/j.cmet.2006.07.006]
28 **Thapaliya S**, Wree A, Povero D, Inzaugarat ME, Berk M, Dixon L, Papouchado BG, Feldstein AE. Caspase 3 inactivation protects against hepatic cell death and ameliorates fibrogenesis in a diet-induced NASH

model. *Dig Dis Sci* 2014; **59**: 1197-1206 [PMID: 24795036 DOI: 10.1007/s10620-014-3167-6]
29 **Dinarello CA**. Interleukin-18. *Methods* 1999; **19**: 121-132 [PMID: 10525448 DOI: 10.1006/meth.1999.0837]
30 **Netea MG**, Joosten LA, Lewis E, Jensen DR, Voshol PJ, Kullberg BJ, Tack CJ, van Krieken H, Kim SH, Stalenhoef AF, van de Loo FA, Verschueren I, Pulawa L, Akira S, Eckel RH, Dinarello CA, van den Berg W, van der Meer JW. Deficiency of interleukin-18 in mice leads to hyperphagia, obesity and insulin resistance. *Nat Med* 2006; **12**: 650-656 [PMID: 16732281 DOI: 10.1038/nm1415]
31 **Yamanishi K**, Maeda S, Kuwahara-Otani S, Watanabe Y, Yoshida M, Ikubo K, Okuzaki D, El-Darawish Y, Li W, Nakasho K, Nojima H, Yamanishi H, Hayakawa T, Okamura H, Matsunaga H. Interleukin-18-deficient mice develop dyslipidemia resulting in nonalcoholic fatty liver disease and steatohepatitis. *Transl Res* 2016; **173**: 101-114.e7 [PMID: 27063959 DOI: 10.1016/j.trsl.2016.03.010]
32 **Henao-Mejia J**, Elinav E, Jin C, Hao L, Mehal WZ, Strowig T, Thaiss CA, Kau AL, Eisenbarth SC, Jurczak MJ, Camporez JP, Shulman GI, Gordon JI, Hoffman HM, Flavell RA. Inflammasome-mediated dysbiosis regulates progression of NAFLD and obesity. *Nature* 2012; **482**: 179-185 [PMID: 22297845 DOI: 10.1038/nature10809]
33 **Vecchiet J**, Falasca K, Cacciatore P, Zingariello P, Dalessandro M, Marinopiccoli M, D'Amico E, Palazzi C, Petrarca C, Conti P, Pizzigallo E, Guagnano MT. Association between plasma interleukin-18 levels and liver injury in chronic hepatitis C virus infection and non-alcoholic fatty liver disease. *Ann Clin Lab Sci* 2005; **35**: 415-422 [PMID: 16254258 DOI: 10.1097/01.ftd.0000175910.68539.33]
34 **Flisiak-Jackiewicz M**, Bobrus-Chociej A, Tarasów E, Wojtkowska M, Białokoz-Kalinowska I, Lebensztejn DM. Predictive Role of Interleukin-18 in Liver Steatosis in Obese Children. *Can J Gastroenterol Hepatol* 2018; **2018**: 3870454 [PMID: 29854715 DOI: 10.1155/2018/3870454]
35 **Finotto S**, Siebler J, Hausding M, Schipp M, Wirtz S, Klein S, Protschka M, Doganci A, Lehr HA, Trautwein C, Khosravi-Far R, Strand D, Lohse A, Galle PR, Blessing M, Neurath MF. Severe hepatic injury in interleukin 18 (IL-18) transgenic mice: a key role for IL-18 in regulating hepatocyte apoptosis in vivo. *Gut* 2004; **53**: 392-400 [PMID: 14960523 DOI: 10.1136/gut.2003.018572]
36 **Lozano I**, Van der Werf R, Bietiger W, Seyfritz E, Peronet C, Pinget M, Jeandidier N, Maillard E, Marchioni E, Sigrist S, Dal S. High-fructose and high-fat diet-induced disorders in rats: impact on diabetes risk, hepatic and vascular complications. *Nutr Metab (Lond)* 2016; **13**: 15 [PMID: 26918024 DOI: 10.1186/s12986-016-0074-1]
37 **Jensen VS**, Hvid H, Damgaard J, Nygaard H, Ingvorsen C, Wulff EM, Lykkesfeldt J, Fledelius C. Dietary fat stimulates development of NAFLD more potently than dietary fructose in Sprague-Dawley rats. *Diabetol Metab Syndr* 2018; **10**: 4 [PMID: 29410708 DOI: 10.1186/s13098-018-0307-8]
38 **Kucera O**, Cervinkova Z. Experimental models of non-alcoholic fatty liver disease in rats. *World J Gastroenterol* 2014; **20**: 8364-8376 [PMID: 25024595 DOI: 10.3748/wjg.v20.i26.8364]
39 **Veteläinen R**, van Vliet A, van Gulik TM. Essential pathogenic and metabolic differences in steatosis induced by choline or methione-choline deficient diets in a rat model. *J Gastroenterol Hepatol* 2007; **22**: 1526-1533 [PMID: 17716355 DOI: 10.1111/j.1440-1746.2006.04701.x]
40 **George J**, Pera N, Phung N, Leclercq I, Yun Hou J, Farrell G. Lipid peroxidation, stellate cell activation and hepatic fibrogenesis in a rat model of chronic steatohepatitis. *J Hepatol* 2003; **39**: 756-764 [PMID: 14568258 DOI: 10.1016/S0168-8278(03)00376-3]
41 **Yang SC**, Lin SH, Chang JS, Chien YW. High Fat Diet with a High Monounsaturated Fatty Acid and Polyunsaturated/Saturated Fatty Acid Ratio Suppresses Body Fat Accumulation and Weight Gain in Obese Hamsters. *Nutrients* 2017; **9**: 1148 [PMID: 29048361 DOI: 10.3390/nu9101148]
42 **Piers LS**, Walker KZ, Stoney RM, Soares MJ, O'Dea K. The influence of the type of dietary fat on postprandial fat oxidation rates: monounsaturated (olive oil) vs saturated fat (cream). *Int J Obes Relat Metab Disord* 2002; **26**: 814-821 [PMID: 12037652 DOI: 10.1038/sj.ijo.0801993]
43 **Piers LS**, Walker KZ, Stoney RM, Soares MJ, O'Dea K. Substitution of saturated with monounsaturated fat in a 4-week diet affects body weight and composition of overweight and obese men. *Br J Nutr* 2003; **90**: 717-727 [PMID: 13129479 DOI: 10.1079/BJN2003948]
44 **Lawton CL**, Delargy HJ, Brockman J, Smith FC, Blundell JE. The degree of saturation of fatty acids influences post-ingestive satiety. *Br J Nutr* 2000; **83**: 473-482 [PMID: 10953671 DOI: 10.1017/S000711450000060X]

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ORIGINAL ARTICLE

Observational Study

Non-alcoholic steatohepatitis and the risk of myocardial infarction: A population-based national study

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Abstract

BACKGROUND

Non-alcoholic fatty liver disease (NAFLD) is a systemic disease with bidirectional relationships with cardiovascular disease (CVD). Non-alcoholic steatohepatitis (NASH) is a more severe subtype of NAFLD. Patients with NASH exhibit more intra and extrahepatic inflammation, procoagulant imbalances and proatherogenic lipid profiles. Whether NASH increases the risk of ischemic heart disease is currently unclear.

AIM

To investigate the relationship between acute myocardial infarction (MI) and NASH in a large cohort of subjects in the United States.

METHODS

We reviewed data from a large commercial database (Explorys IBM) that aggregates electronic health records from 26 large nationwide healthcare systems. Using systemized nomenclature of clinical medical terms (SNOMED CT), we identified adult with the diagnosis of NASH from 1999-2019. We included patients with the diagnosis of acute MI from 2018-2019. Comorbidities known to be associated with NASH and MI such as obesity, diabetes mellitus, hyperlipidemia, smoking, male gender, and hypertension were collected. Univariable and multivariable analyses were performed to investigate whether NASH is independently associated with the risk of MI.

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RESULTS

Out of 55099280 patients, 43170 were diagnosed with NASH (0.08%) and 107000 (0.194%) had a MI within 2018-2019. After adjusting for traditional risk factors, NASH conferred greater odds of MI odds ratio (OR) 1.5 [95% confidence interval (CI): 1.40-1.62]. Hyperlipidemia had the strongest association with MI OR 8.39 (95%CI: 8.21-8.58) followed by hypertension OR 3.11 (95%CI: 3.05-3.17) and smoking OR 2.83 (95%CI: 2.79-2.87). NASH had a similar association with MI as the following traditional risk factors like age above 65 years OR 1.47 (95%CI: 1.45-1.49), male gender OR 1.53 (95%CI: 1.51-1.55) diabetes mellitus OR 1.89 (95%CI: 1.86-1.91).

CONCLUSION

MI appears to be a prevalent disease in NASH. Patients with NASH may need early identification and aggressive cardiovascular risk modification.

Key words: Non-alcoholic steatohepatitis; Myocardial infarction; Non-alcoholic fatty liver disease; Ischemic cardiovascular disease; United States population; Atherosclerosis

Core tip: Non-alcoholic steatohepatitis (NASH) is a severe subtype of nonalcoholic fatty liver disease. The progression of non-alcoholic fatty liver disease is mirrored by activation of hepatic and systemic inflammatory cascades that maybe implicated in the pathogenesis of cardiovascular disease. Using a large electronic medical record database, we performed a national-based population study to investigate the association between NASH and myocardial infarction (MI). In this large cohort study, NASH was associated with increased risk of MI independent of traditional risk factors. Close follow up and aggressive risk modification maybe indicated to prevent major cardiovascular events in patients with NASH.

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is the hepatic manifestation of metabolic syndrome (MetS) defined by the presence of central obesity, insulin resistance and dyslipidemia[1-3]. The severity of NAFLD ranges from simple steatosis to non-alcoholic steatohepatitis (NASH), liver fibrosis progressing to cirrhosis and hepatocellular carcinoma[1,2]. Non-alcoholic liver disease is a world-wide chronic disease and the most common liver disorder in North America[2-5]. The prevalence of NAFLD in the United States is reported to be 10%-46% with approximately 7%-30% meeting the criteria of NASH[6].

NAFLD is not only associated with increased liver-related morbidity and mortality, it is also associated with increased risk of mortality due to cardiovascular disease (CVD)[7-9]. The distillation of NAFLD as an independent risk factor for CVD is impeded by overlap with established risk factors for CVD that are also risk factors for NAFLD. Even in the absence of cardiovascular mortality, CVD is highly prevalent in patients with NAFLD[7-9]. There is convincing data supporting the association of NAFLD with subclinical atherosclerosis, carotid arthrosclerosis and venous thromboembolic events[10-13]. The severity of coronary artery disease is also higher in patients with NAFLD diagnosed by coronary angiography[14]. Several mechanisms have been proposed by which NAFLD may give rise to CVD. Endothelial damage is considered the first step in atherosclerosis. Intrahepatic and systemic endothelial damage have been noted in NAFLD and are more pronounced in NASH[15]. Second, inflammation fuels CVD and promotes endothelial damage, by altering vascular tone and enhancing platelet dysfunction[16]. Circulating proinflammatory cytokines are increased in

NAFLD and are highly expressed in NASH[17]. Moreover, the liver plays a key role in whole body lipid metabolism. Serum lipid profiles mirror disease severity and are significantly higher in NASH[17]. Furthermore disturbances in haemostasis are seen in NAFLD and correlate with disease severity[18]. Nonetheless, the role of NAFLD as an independent cardiovascular risk is still debatable with research yielding contradictory results. In one meta-analysis that included 34043 subjects, NALFD was associated with increased overall mortality deriving from liver-related and CVD odds ratio (OR) 1.57 [95% confidence interval (CI): 1.18-2.10)[19]. In a prospective study, Baratta *et al*[20] reported NAFLD to have more than a 2-fold risk increase and those with fibrosis had a fourfold increase in cardiovascular events defined as fatal or nonfatal ischemic stroke or myocardial infarction (MI).

Other studies have failed to confirm this association[7,8]. This data however should be interpreted with caution because of methodological issues. To illustrate, the diagnosis of NAFLD relied on ultrasound imaging or biochemical testing both of which correlate poorly with histological findings. Furthermore, the degree of disease severity was not stratified in any of these studies. As we highlighted above, the progression of NAFLD is mirrored by increased activation of bidirectional pathways that maybe involved in ischemic CVD. Whether NASH is associated with an increased risk of MI in the United States population remains unclear. Given the lack of consensus on this important issue, and the ambiguity of data around this question, the aim of this study is to investigate the association between NASH and MI in United States population.

MATERIALS AND METHODS

Study design

This is a retrospective analysis of a large electronic health record (EHR)-based commercial database called Explorys (IBM). Explorys platform assimilates patient information from 26 major healthcare systems spread over 50 states in the United States. It stores over 60 million unique patient records[20]. Patient information is then de-identified, standardized and stored in a cloud database[20,21]. The Explorys platform uses SNOMED CT for medical diagnoses and procedures. For diagnoses, International Classification of Disease, Ninth Revision, Clinical Modification (ICD-9-CM) codes are mapped into the SNOMED-CT hierarchy[20]. Cohorts can further be refined demographically and comorbid diseases can be extracted[20,21]. The use of Explorys has been validated in multiple fields including Cardiology and Gastroenterology[20]. This platform is Health Insurance Portability and Accountability Act and Health Information Technology for Economic and Clinical Health Act compliant[20]. The Case Western Reserve University/Metrohealth Medical Center Institutional Review Board deemed studies using Explorys, as the dataset of record, as exempt from approval because all patient information is de-identified. Explorys protects patient confidentiality by approximating each population count to 10. All counts between 0 and 10 are treated equally. For the purpose of this study, counts between 0 and 10 were approximated to be five[21].

Patient selection

Subjects with active electronic health records since 1999 were identified using the search tool in Explorys. Using the SNOMED-CT diagnosis "Non-alcoholic steatohepatitis", we identified patients who were diagnosed with NASH between 1999-2019. Patients with the diagnosis of "fatty liver disease", "alcoholic hepatitis" and "alcoholic fatty liver disease" were excluded. Patients with MI were identified as those with the following SNOMED-CT diagnosis "Acute myocardial infarction". To establish a temporal relationship with NASH, only those with the diagnosis of acute MI within the last year of the study, 2018-2019, were included. Controls were identified as those patients without the diagnosis of NASH. For our analysis we identified cohorts of patients with NASH, with and without MI. It was not possible to perform propensity-score matching because this database only provides population-level data and not individual cases.

Covariates

The following characteristics were collected: demographic factors such as age, sex, and comorbidities, known to be associated with NASH and MI (hyperlipidemia, hypertension, obesity, diabetes mellitus, smoking age and gender), by searching the database for their respective SNOMED-CT terms.

Statistical analysis

To assess the association between MI with risk factors we divided the whole cohort of patients into MI and Non-MI patients. The prevalence of MI was calculated by dividing the number of patients with MI in each risk group (NASH, hypertension, diabetes mellitus, obesity, hyperlipidemia, age, gender and smoking). Frequency and percentages were used for statistical description of categorical variables and compared using the Pearson χ^2 test. ORs are presented with 95%CIs. Univariate binary logistic model was constructed using MI as dependent variable and other variables as independent variables. To adjust for possible confounding, a multivariable model adjusting for all covariates mentioned in univariate variables were added to test the main effect. Analyses were performed using R version 3.6.1 (The R Foundation, Vienna, Austria). Independence among covariate risk factors was assessed using the variance inflating factor (VIF) with cut-off of significant collinearity set at VIF > 1.5. "Goodness-of-fit" was assessed for all regression models using the Hosmer-Lemeshow test, with $P > 0.05$ indicating good fit. Two-sided P values were used and were considered statistically significant if $P < 0.05$.

RESULTS

Out of 55099280 patients, 43170 patients were diagnosed with NASH (0.08%) and 107000 (0.194%) had acute MI within 2018-2019. Baseline characteristics of subjects included in this study are displayed in Table 1.

The prevalence of MI in subjects with NASH was 10.24% and 0.18% in the non-NASH group. In overall unadjusted analysis, the unadjusted OR for MI in patients with NASH was 10.66 (95%CI: 9.58-10.94). Hyperlipidemia was strongly associated with increased risk of MI OR 40.13 (95%CI: 39.47-40.79), followed by hypertension OR 27.43 (95%CI: 27.00-7.86) and the male gender OR 17.61 (95%CI: 17.41-17.81). Diabetes, obesity, smoking and age above 65 years had slightly lower association with MI than NASH OR 12.57 (95%CI: 12.44–12.71), 8.19 (95%CI: 8.09-8.28), 6.71 (95%CI: 6.64-6.79) and 4.97 (95%CI: 4.92-5.03), respectively (Table 2).

Compared to the older NASH population, the younger NASH population had a higher relative risk of MI. The relative risk of MI decreased with older age [40-49 (10.1, $P < 0.0001$), 50-59 (7.7, $P < 0.0001$), 60-69 (5.5, $P < 0.0001$), 70-79 (5.2, $P < 0.0001$) and age above 80 years (5.1, $P < 0.0001$)] (Figure 1).

Gender differences in NASH contributed to the disparities observed in the relative risk of MI among each age group. Within the 40-49 years group, females had a higher risk of MI compared to their male counterparts (15.8 *vs* 6.3, $P < 0.0001$).

Compared to the younger NASH females, the older group had a lower relative risk of MI; while males with NASH maintained a relatively more stable relative risk of MI throughout their lifetime [age 50-59 (11.6 *vs* 5.4, $P < 0.0001$); age 60-69 (7.1 *vs* 4.8, $P < 0.0001$); age 70-79 (5.1 *vs* 5.7, $P < 0.0001$)] (Figure 2).

The absolute risk of MI was also higher in subjects with NASH than non-NASH and increased with age [40-49 (0.74 *vs* 0.07), 50-59 (1.4 *vs* 0.2), 60-69 (1.8 *vs* 0.3), age 70-79 (2.4 *vs* 0.5), and above 80 years (1.9 *vs* 0.4)] (Figure 1).

After adjusting for age, gender, smoking, hyperlipidemia, hypertension, diabetes and smoking, the diagnosis of NASH had a significant association with MI OR 1.5 (95%CI: 1.40-1.62) (Table 3).

Hyperlipidemia had the strongest association with MI OR 8.39 (95%CI: 8.21-8.58) followed by hypertension OR 3.11 [95%CI: 3.05-3.17), and smoking OR 2.83 [95%CI: 2.79-2.87) (Figure 3). NASH had comparable association with MI when compared to traditional risk factors such as age above 65 OR 1.47 (95%CI: 1.45-1.49), male gender OR 1.53 (95%CI: 1.51-1.55) and diabetes mellitus OR 1.89 (95%CI: 1.86-1.91) (Table 3).

DISCUSSION

There is increasing evidence suggesting the relationship between NAFLD and CVD extends beyond risk factors common to both chronic conditions[22,23]. A number of population studies have also confirmed this association and have suggested that individuals with NAFLD should be screened for CVD. Other studies have shown that CVD risk increases with the severity of NAFLD[24,25]. Our study used a large population-based database to evaluate the relationship between NASH and MI in the United States. We included over 55 million patients in our study, of which 43170 were

Table 1 Baseline characteristics of subjects with co-morbidities included in this study

	Total cohort	Proportion (%)	NASH cohort	Proportion (%)
Total cohort	55099280		43170	
Male gender	24569555	45	17380	40
Age above 65 yr	15333620	28	17020	39
Essential hypertension	11043970	20	32040	74
Hyperlipidemia	9539460	17	31030	72
Smoking	4993910	9	9920	23
Diabetes mellitus	4785840	9	24640	57
Obesity	4226640	8	25570	59

NASH: Non-alcoholic steatohepatitis.

Table 2 Unadjusted odds ratio of having an associated diagnosis of myocardial infarction based on subjects' co-morbidities

	Odds ratio	95%CI[b]
Hyperlipidemia	40.13	39.47-40.79
Essential hypertension	27.43	27.00-7.86
Male gender	17.61	17.41-17.81
Diabetes mellitus	12.57	12.44–12.71
NASH	10.66	9.58-10.94
Obesity	8.19	8.09-8.28
Smoking	6.71	6.64-6.79
Age above 65 yr	4.97	4.92-5.03

[b]$P < 0.01$ unless otherwise stated. NASH: Non-alcoholic steatohepatitis; CI: Confidence interval.

Table 3 Adjusted odds ratio for acute myocardial infarction based on subject co-morbidities

	Odds ratio	95%CI[b]
Hyperlipidemia	8.39	8.21-8.58
Hypertension	3.11	3.05-3.17
Smoking	2.83	2.79-2.87
Diabetes mellitus	1.89	1.86-1.91
Obesity	1.78	1.75-1.80
Male	1.53	1.51-1.55
NASH	1.5	1.40-1.62
Age above 65 yr	1.47	1.45-1.49

[b]$P < 0.01$ unless otherwise stated. NASH: Non-alcoholic steatohepatitis; CI: Confidence interval.

diagnosed with NASH and 107000 had an acute MI within 2018-2019.

The main findings of our study are: (1) NASH is associated with MI independent of traditional risk factors; (2) Compared to the older NASH population, younger patients were more likely to have a higher relative risk of MI; and (3) MI is a prevalent outcome among patients with NASH.

Although an association between ischemic heart disease and NAFLD has been

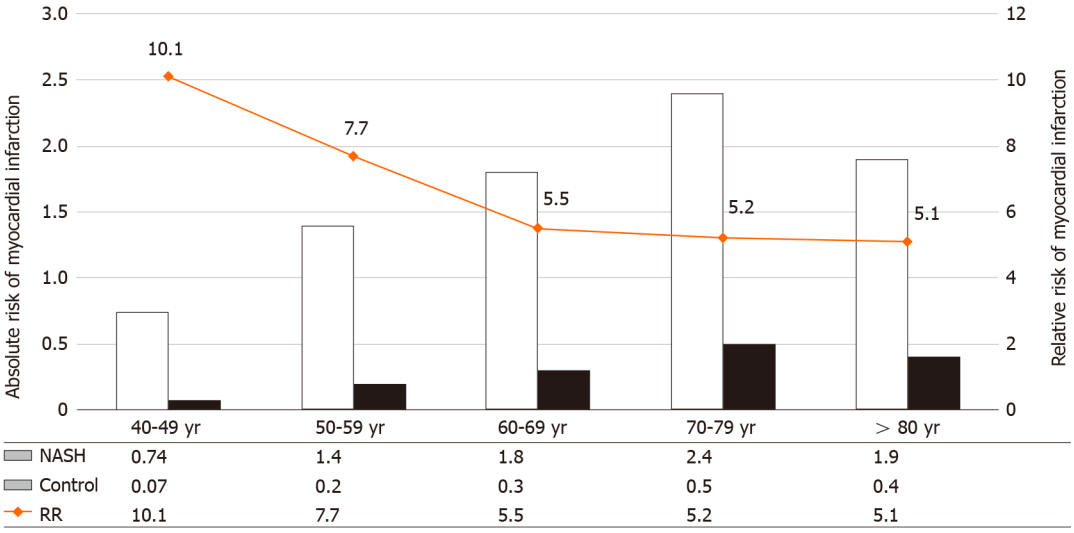

Figure 1 The relative and absolute risk of acute myocardial infarction in the study population. The relative risk of myocardial infarction is higher in the younger patient group compared to the older population. The absolute risk of myocardial infarction however increases with aging. RR: relative risk; NASH: Non-alcoholic steatohepatitis.

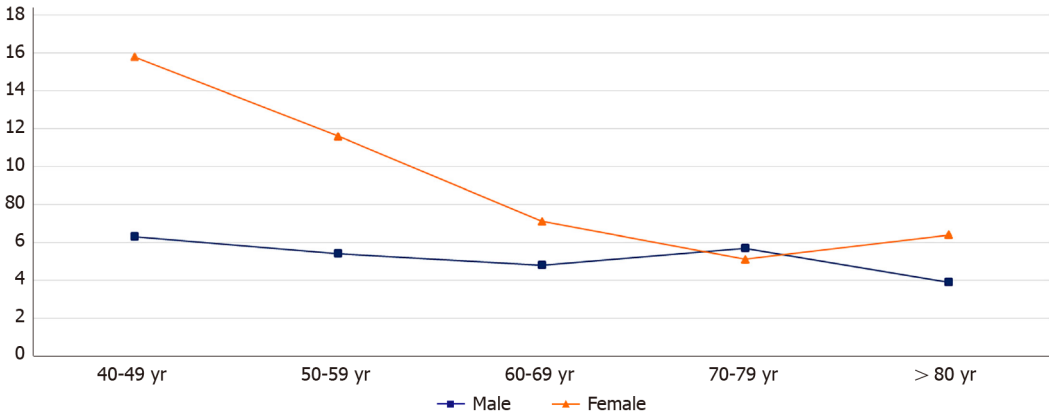

Figure 2 Gender based differences in relative risk of acute myocardial infarction in patients with non-alcoholic steatohepatitis. Female patients had higher relative risk of myocardial infarction compared to their male counterparts in almost all age groups.

suggested, the strength of this relationship remains controversial. In this study, NASH was independently associated with MI OR 1.5 (95%CI: 1.40-1.62) after adjusting for classically known risk factors for the latter. Our study is in line with other studies that have shown an association between NASH or NAFLD and ischemic heart disease[19,20].

At the pathophysiological level, this observation could be explained by several mechanisms. First, NASH is considered a more severe manifestation of NAFLD with augmented hepatic steatosis and aberrant intrahepatic inflammation[26]. Oxidative stress is thought to be the driving force of NASH propagating intra-hepatic proinflammatory cascades[13,27-30]. Free fatty acids themselves contribute to inflammatory mechanisms when they undergo esterification[31]. The liver is an important immune tissue containing the largest number of macrophages and other immune cells[32]. Hence, diseased liver will drain an increasing number of cytokines into systemic circulation and promote extrahepatic inflammation. Systemic cytokines and inflammatory markers are also associated with secondary cardiovascular effects[29,30]. Chronic inflammatory diseases such as rheumatoid arthritis and systemic lupus erythematosus have been associated with higher risks of atherosclerotic diseases and arterial thromboembolic events including MI[33,34]. A procoagulant imbalance exists and is worse in NASH[18]. Since NASH is a severe inflammatory subtype of NAFLD we hypothesize that NASH is an independent risk factor MI. Previous cross-sectional studies have grouped NASH and NAFLD as a single disease entity. We believe by doing so, the authors might have created a null effect and subsequently contributed to the heterogeneity in outcomes reported. Hence, further methodologically stringent

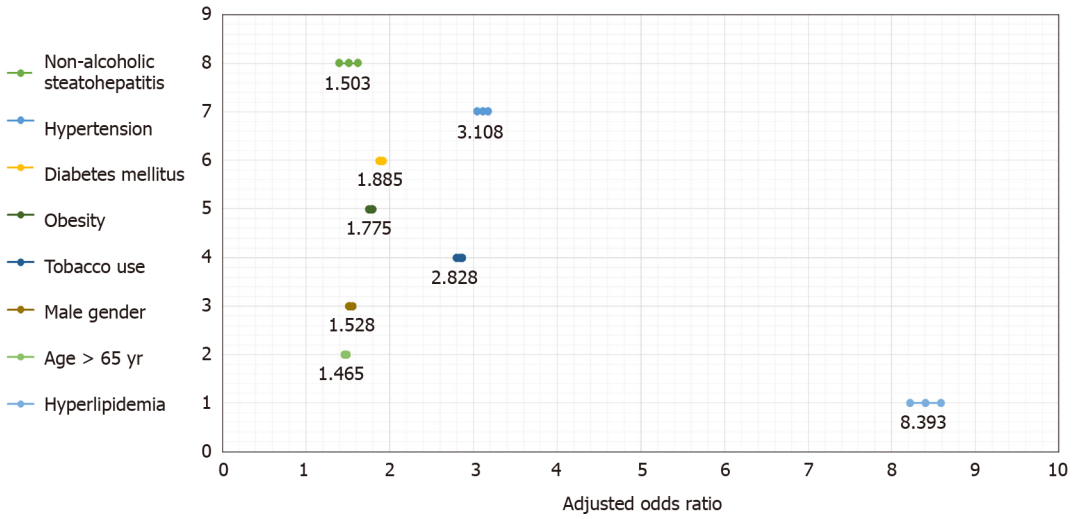

Figure 3 Forest plot showing adjusted odds ratio of having myocardial infarction. The dots represent the odds ratio and the horizontal line represents the 95% confidence interval.

studies with long-term follow up are needed to gain mechanistic insight into the pathology of NASH-MI axis.

NAFLD is part of a systemic disease with complex multidirectional relationships between MetS and CVD. The liver plays a crucial role in lipid and glucose metabolism. Even though dyslipidemia is an independent risk factor for CVD, abnormal lipid profiles are seen in NAFLD and are more deranged with increased histological inflammation[17]. A stepwise increase in lipid ratios correlates with histological severity of liver injury and is associated with increased risk of CVD and an atherogenic lipid profile[17]. Insulin resistance and alterations in lipid metabolism might also precede the development of hyperlipidemia and diabetes mellitus both of which are associated with increased risk of ischemic heart disease[11]. In our study, NASH was significantly associated with MI when these variables were partially controlled. Even though we observed an independent association between NASH and MI, to completely control these variables would blunt the contribution NASH might have on ischemic heart disease[19,20].

The prevalence of NASH is reported to be the greatest among subjects between the ages of 40-49 years[35]. It is conceivable to say that the presence of NASH in the younger population might increase their relative risk of MI compared to those without NASH (Figure 1). In our study the relative risk of MI was highest in the younger NASH population, suggesting increased inflammation and more aggressive disease in the younger group. Although age correlates with the duration of NAFLD, advanced disease may not be attributed to age alone. Furthermore, with aging, the non-NASH group might have accumulated traditional factors that may have reduced the relative contribution of inflammation on arthrosclerosis. Hence the contribution of NASH towards the total burden of cardiovascular risk maybe reduced with advancing age. This being said, we were not able to control for the duration or the severity of disease as this was an observational study. Nonetheless, we observed a step wise increase in the absolute risk of MI in both cohorts with increasing age (Figure 1).

There is increasing evidence that menopause is associated with the progression of NAFLD[36]. Although more data is needed, this is likely due the loss of estrogen's hepato-protective role post menopause. In the current study, premenopausal females with NASH had a higher relative risk of MI compared to post-menopausal females (Figure 2). Our findings maybe partially explained by the lower CVD risk profile at baseline of the younger NASH females compared to their older counterparts. This supports the growing body of evidence suggesting a pathological association between inflammation and ischemic heart disease at least in the younger patient group[13,26-30].

Across almost all age groups, the risk of MI was higher in females compared to their male counterparts. The relative risk of MI in females with NASH decreased with increasing age, while males maintained a relatively more stable relative risk of MI throughout their lifetime (Figure 2). Current data suggests young women with MI are more likely than their male counterparts to have comorbidities[37]. Additionally, although the risk of MI on average is higher in men than women, the presence of comorbidities has been shown to confer an excess greater risk of MI in women than

men and this risk doesn't attenuate with age[37]. However given that data on time trends in risk factors was not available in Explorys, we do not know whether there were sex-related differences in risk factor levels and risk factor control before MI and our findings indicate further studies are needed to investigate the phenomenon reported in the current study.

An important strength of our study is that we used a large national database and reported data on over 55 million active adult subjects. We also provided comprehensive epidemiological information on the risk of MI in patients with NASH based on classical risk factors for both entities. Our results are therefore consistent with established data suggesting that NASH might be significantly associated with acute CVD events such as MI.

There are several limitations to this study that should be addressed. Observational studies preclude us from addressing causality. Due to the design of Explorys, we could not establish temporal relationships between the duration of NASH, severity of the disease and impact of interventions on the risk of cardiovascular events in our cohort. Second, the prevalence and natural disease progression of NAFLD is difficult to estimate as accurate diagnosis requires tissue analysis. The prevalence of NASH is estimated to be between 1.5%-6.45% based on a few biopsy series[38]. As this is a retrospective study reliant on diagnosed SNOMED-CT codes, it is impossible to verify the accuracy of diagnoses and it is prone to coding errors. The reported prevalence of NASH in our study was 0.08%. We excluded those with the diagnosis of fatty liver disease in an attempt to increase accuracy in our reporting; as the latter may be caused by drugs, viruses or alcohol. But even though the NASH population was underrepresented in the current study, MI was significantly associated with this disease entity.

An important limitation of our study is the validity of diagnosis of acute MI. We were unable to differentiate between MI secondary to coronary artery disease and MI resulting from demand-related events (Type II MI). Validation was also not possible as patient information in this platform is de-identified. Further, direct temporal relationships between NASH and MI cannot be defined due to the inherent design of Explorys. We acknowledged this limitation and included only those with a diagnosis of acute MI within the last year of the study to circumvent this issue. However, using a large national population-based sample allows for the ability to generalize our results to the United States population and offset these limitations.

In our large United States-based cohort study, we found acute MI to be a prevalent diagnosis among patients with NASH. Although our findings need to be further confirmed by prospective studies, we propose that patients with NAFLD at risk of NASH, should be identified early and screened for associated cardiovascular risk factors. Current AASLD-AGA guidelines recommend modification of CVD risk factors in patients with NAFLD[39]. Whereas, the European association for the study of liver view CVD as an extra-hepatic manifestation of NAFLD[40]. As we have mentioned above, severe inflammation, as seen in patients with NASH, is associated with accelerated atherosclerosis. A major problem that hampers screening for CVD in this subgroup is the unawareness of health care providers of the relevance of this disease, and as a consequence, NASH is under diagnosed in the general population. Even in our study, out of 55 million patients, only 43170 (0.08%) of patients had an ICD-coded diagnosis of NASH, despite a reported prevalence of NAFLD as high as 24%[35].

In conclusion, there is a significant relationship between NASH and acute MI. The use of already existing non-invasive scoring systems to stratify disease severity in NAFLD can overcome the need for liver biopsy and allow for early detection and aggressive risk factor modification.

ARTICLE HIGHLIGHTS

Research background

Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease in the United States. The severity of NAFLD ranges from simple steatosis to non-alcoholic steatohepatitis which can progress to fibrosis, cirrhosis and hepatocellular carcinoma. Recent evidence suggests that the diagnosis of NAFLD may be associated with an increased risk of cardiovascular disease (CVD) independent of traditional risk factors. We believe that patients with non-alcoholic steatohepatitis (NASH) are at a higher risk of serious cardiovascular events such as myocardial infarction (MI).

Research motivation

There is an overlap between the risk factors that give rise to NAFLD and CVD. NASH remains underdiagnosed in the general population as tissue analysis is needed for accurate diagnosis. It is a more severe subtype of NAFLD associated with hepatic and systemic inflammation. Inflammation is implicated in the pathogenesis of atherosclerosis. Whether NASH is associated with serious cardiovascular events such as MI has major economic and public health implications.

Research objectives

The aim of this study was to assess the prevalence of acute MI among patients with NASH and to investigate the contribution of age and gender on the relative risk of MI in a large cohort of subjects in the United States.

Research methods

This was a large retrospective study that included over 50 million patients from over 50 states in the United States. Patients diagnosed with NASH between 1999-2019 and those with acute MI within 2018-2019 were identified. Traditional risk factors associated with both diseases were also collected. Univariable and multivariable analyses were performed to assess the association between NASH and MI.

Research results

After adjusting for traditional risk factors, there was an independent associated between NASH and MI (1.5, 95%CI: 1.40-1.62, $P < 0.0001$). The relative risk of MI in patients with NASH appeared to be the highest in the younger patient population (< 49 years old, RR 10.1, $P < 0.0001$) suggesting inflammation might be the driving force for this observation. Women with NASH had a higher relative risk of MI compared to their male counterparts (15.8 *vs* 11.6, $P < 0.0001$, respectively). Overall, the absolute risk of MI was higher in the older population.

Research conclusions

Acute MI is a prevalent diagnosis among patients with NASH. According to our dataset, NASH continues to be underdiagnosed in the United States population. Systemic inflammatory cascades are exaggerated in NASH and might be implicated in the pathogenesis of arthrosclerosis. Identification of NAFLD patients at high risk of NASH might allow for primary prevention and aggressive cardiovascular risk modification.

Research perspectives

Performing large scale prospective studies with long-term follow-up are needed to gain mechanistic insight into the pathology of NASH-MI axis.

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REFERENCES

1 **Sayiner M**, Koenig A, Henry L, Younossi ZM. Epidemiology of Nonalcoholic Fatty Liver Disease and Nonalcoholic Steatohepatitis in the United States and the Rest of the World. *Clin Liver Dis* 2016; **20**: 205-214 [PMID: 27063264 DOI: 10.1016/j.cld.2015.10.001]

2 **Kanwar P**, Kowdley KV. The Metabolic Syndrome and Its Influence on Nonalcoholic Steatohepatitis. *Clin Liver Dis* 2016; **20**: 225-243 [PMID: 27063266 DOI: 10.1016/j.cld.2015.10.002]

3 **Williams CD**, Stengel J, Asike MI, Torres DM, Shaw J, Contreras M, Landt CL, Harrison SA. Prevalence of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis among a largely middle-aged population utilizing ultrasound and liver biopsy: a prospective study. *Gastroenterology* 2011; **140**: 124-131 [PMID: 20858492 DOI: 10.1053/j.gastro.2010.09.038]

4 **Vernon G**, Baranova A, Younossi ZM. Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. *Aliment Pharmacol Ther* 2011; **34**: 274-285 [PMID: 21623852 DOI: 10.1111/j.1365-2036.2011.04724.x]

5 **Lazo M**, Hernaez R, Eberhardt MS, Bonekamp S, Kamel I, Guallar E, Koteish A, Brancati FL, Clark JM. Prevalence of nonalcoholic fatty liver disease in the United States: the Third National Health and Nutrition Examination Survey, 1988-1994. *Am J Epidemiol* 2013; **178**: 38-45 [PMID: 23703888 DOI:

10.1093/aje/kws448]
6 **Dixon JB**, Bhathal PS, O'Brien PE. Nonalcoholic fatty liver disease: predictors of nonalcoholic steatohepatitis and liver fibrosis in the severely obese. *Gastroenterology* 2001; **121**: 91-100 [PMID: 11438497 DOI: 10.1053/gast.2001.25540]
7 **Ekstedt M**, Hagström H, Nasr P, Fredrikson M, Stål P, Kechagias S, Hultcrantz R. Fibrosis stage is the strongest predictor for disease-specific mortality in NAFLD after up to 33 years of follow-up. *Hepatology* 2015; **61**: 1547-1554 [PMID: 25125077 DOI: 10.1002/hep.27368]
8 **Söderberg C**, Stål P, Askling J, Glaumann H, Lindberg G, Marmur J, Hultcrantz R. Decreased survival of subjects with elevated liver function tests during a 28-year follow-up. *Hepatology* 2010; **51**: 595-602 [PMID: 20014114 DOI: 10.1002/hep.23314]
9 **Angulo P**, Kleiner DE, Dam-Larsen S, Adams LA, Bjornsson ES, Charatcharoenwitthaya P, Mills PR, Keach JC, Lafferty HD, Stahler A, Haflidadottir S, Bendtsen F. Liver Fibrosis, but No Other Histologic Features, Is Associated With Long-term Outcomes of Patients With Nonalcoholic Fatty Liver Disease. *Gastroenterology* 2015; **149**: 389-97.e10 [PMID: 25935633 DOI: 10.1053/j.gastro.2015.04.043]
10 **Salvi P**, Ruffini R, Agnoletti D, Magnani E, Pagliarani G, Comandini G, Praticò A, Borghi C, Benetos A, Pazzi P. Increased arterial stiffness in nonalcoholic fatty liver disease: the Cardio-GOOSE study. *J Hypertens* 2010; **28**: 1699-1707 [PMID: 20467324 DOI: 10.1097/HJH.0b013e32833a7de6]
11 **Mellinger JL**, Pencina KM, Massaro JM, Hoffmann U, Seshadri S, Fox CS, O'Donnell CJ, Speliotes EK. Hepatic steatosis and cardiovascular disease outcomes: An analysis of the Framingham Heart Study. *J Hepatol* 2015; **63**: 470-476 [PMID: 25776891 DOI: 10.1016/j.jhep.2015.02.045]
12 **Lee YJ**, Shim JY, Moon BS, Shin YH, Jung DH, Lee JH, Lee HR. The relationship between arterial stiffness and nonalcoholic fatty liver disease. *Dig Dis Sci* 2012; **57**: 196-203 [PMID: 21750929 DOI: 10.1007/s10620-011-1819-3]
13 **Sookoian S**, Pirola CJ. Non-alcoholic fatty liver disease is strongly associated with carotid atherosclerosis: a systematic review. *J Hepatol* 2008; **49**: 600-607 [PMID: 18672311 DOI: 10.1016/j.jhep.2008.06.012]
14 **Ong JP**, Pitts A, Younossi ZM. Increased overall mortality and liver-related mortality in non-alcoholic fatty liver disease. *J Hepatol* 2008; **49**: 608-612 [PMID: 18682312 DOI: 10.1016/j.jhep.2008.06.018]
15 **Villanova N**, Moscatiello S, Ramilli S, Bugianesi E, Magalotti D, Vanni E, Zoli M, Marchesini G. Endothelial dysfunction and cardiovascular risk profile in nonalcoholic fatty liver disease. *Hepatology* 2005; **42**: 473-480 [PMID: 15981216 DOI: 10.1002/hep.20781]
16 **Sookoian S**, Gianotti TF, Rosselli MS, Burgueño AL, Castaño GO, Pirola CJ. Liver transcriptional profile of atherosclerosis-related genes in human nonalcoholic fatty liver disease. *Atherosclerosis* 2011; **218**: 378-385 [PMID: 21664615 DOI: 10.1016/j.atherosclerosis.2011.05.014]
17 **Alkhouri N**, Tamimi TA, Yerian L, Lopez R, Zein NN, Feldstein AE. The inflamed liver and atherosclerosis: a link between histologic severity of nonalcoholic fatty liver disease and increased cardiovascular risk. *Dig Dis Sci* 2010; **55**: 2644-2650 [PMID: 19960252 DOI: 10.1007/s10620-009-1075-y]
18 **Verrijken A**, Francque S, Mertens I, Prawitt J, Caron S, Hubens G, Van Marck E, Staels B, Michielsen P, Van Gaal L. Prothrombotic factors in histologically proven nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. *Hepatology* 2014; **59**: 121-129 [PMID: 24375485 DOI: 10.1002/hep.26510]
19 **Targher G**, Byrne CD, Lonardo A, Zoppini G, Barbui C. Non-alcoholic fatty liver disease and risk of incident cardiovascular disease: A meta-analysis. *J Hepatol* 2016; **65**: 589-600 [PMID: 27212244 DOI: 10.1016/j.jhep.2016.05.013]
20 **Baratta F**, Pastori D, Angelico F, Balla A, Paganini AM, Cocomello N, Ferro D, Violi F, Sanyal AJ, Del Ben M. Nonalcoholic Fatty Liver Disease and Fibrosis Associated With Increased Risk of Cardiovascular Events in a Prospective Study. *Clin Gastroenterol Hepatol* 2019 [PMID: 31887443 DOI: 10.1016/j.cgh.2019.12.026]
21 **IBM Explorys-Accelerating Insights from Real-World Evidence**. 2019. Available from: http://www-01.ibm.com/common/ssi/cgi-bin/ssialias?htmlfid=HL912350U-SEN
22 **Kaelber DC**, Foster W, Gilder J, Love TE, Jain AK. Patient characteristics associated with venous thromboembolic events: a cohort study using pooled electronic health record data. *J Am Med Inform Assoc* 2012; **19**: 965-972 [PMID: 22759621 DOI: 10.1136/amiajnl-2011-000782]
23 **Satapathy SK**, Sanyal AJ. Epidemiology and Natural History of Nonalcoholic Fatty Liver Disease. *Semin Liver Dis* 2015; **35**: 221-235 [PMID: 26378640 DOI: 10.1055/s-0035-1562943]
24 **Perseghin G**. The role of non-alcoholic fatty liver disease in cardiovascular disease. *Dig Dis* 2010; **28**: 210-213 [PMID: 20460913 DOI: 10.1159/000282088]
25 **Sung KC**, Ryan MC, Wilson AM. The severity of nonalcoholic fatty liver disease is associated with increased cardiovascular risk in a large cohort of non-obese Asian subjects. *Atherosclerosis* 2009; **203**: 581-586 [PMID: 18774133 DOI: 10.1016/j.atherosclerosis.2008.07.024]
26 **Targher G**, Day CP, Bonora E. Risk of cardiovascular disease in patients with nonalcoholic fatty liver disease. *N Engl J Med* 2010; **363**: 1341-1350 [PMID: 20879883 DOI: 10.1056/NEJMra0912063]
27 **Chalasani N**, Deeg MA, Crabb DW. Systemic levels of lipid peroxidation and its metabolic and dietary correlates in patients with nonalcoholic steatohepatitis. *Am J Gastroenterol* 2004; **99**: 1497-1502 [PMID: 15307867 DOI: 10.1111/j.1572-0241.2004.30159.x]
28 **Stoner L**, Lucero AA, Palmer BR, Jones LM, Young JM, Faulkner J. Inflammatory biomarkers for predicting cardiovascular disease. *Clin Biochem* 2013; **46**: 1353-1371 [PMID: 23756129 DOI: 10.1016/j.clinbiochem.2013.05.070]
29 **Wieckowska A**, Papouchado BG, Li Z, Lopez R, Zein NN, Feldstein AE. Increased hepatic and circulating interleukin-6 levels in human nonalcoholic steatohepatitis. *Am J Gastroenterol* 2008; **103**: 1372-1379 [PMID: 18510618 DOI: 10.1111/j.1572-0241.2007.01774.x]
30 **Hamirani YS**, Katz R, Nasir K, Zeb I, Blaha MJ, Blumenthal RS, Kronmal RN, Budoff MJ. Association between inflammatory markers and liver fat: The Multi-Ethnic Study of Atherosclerosis. *J Clin Exp Cardiolog* 2014; 5 [PMID: 25598995 DOI: 10.4172/2155-9880.1000344]
31 **Neuschwander-Tetri BA**. Hepatic lipotoxicity and the pathogenesis of nonalcoholic steatohepatitis: the central role of nontriglyceride fatty acid metabolites. *Hepatology* 2010; **52**: 774-788 [PMID: 20683968 DOI:

10.1002/hep.23719]
32 **Racanelli V**, Rehermann B. The liver as an immunological organ. *Hepatology* 2006; **43**: S54-S62 [PMID: 16447271 DOI: 10.1002/hep.21060]
33 **Solomon DH**, Reed GW, Kremer JM, Curtis JR, Farkouh ME, Harrold LR, Hochberg MC, Tsao P, Greenberg JD. Disease activity in rheumatoid arthritis and the risk of cardiovascular events. *Arthritis Rheumatol* 2015; **67**: 1449-1455 [PMID: 25776112 DOI: 10.1002/art.39098]
34 **Manzi S**, Meilahn EN, Rairie JE, Conte CG, Medsger TA Jr, Jansen-McWilliams L, D'Agostino RB, Kuller LH. Age-specific incidence rates of myocardial infarction and angina in women with systemic lupus erythematosus: comparison with the Framingham Study. *Am J Epidemiol* 1997; **145**: 408-415 [PMID: 9048514 DOI: 10.1093/oxfordjournals.aje.a009122]
35 **Sanyal AJ**; American Gastroenterological Association. AGA technical review on nonalcoholic fatty liver disease. *Gastroenterology* 2002; **123**: 1705-1725 [PMID: 12404245 DOI: 10.1053/gast.2002.36572]
36 **Venetsanaki V**, Polyzos SA. Menopause and Non-Alcoholic Fatty Liver Disease: A Review Focusing on Therapeutic Perspectives. *Curr Vasc Pharmacol* 2019; **17**: 546-555 [PMID: 29992886 DOI: 10.2174/1570161116666180711121949]
37 **Arora S**, Stouffer GA, Kucharska-Newton AM, Qamar A, Vaduganathan M, Pandey A, Porterfield D, Blankstein R, Rosamond WD, Bhatt DL, Caughey MC. Twenty Year Trends and Sex Differences in Young Adults Hospitalized With Acute Myocardial Infarction. *Circulation* 2019; **139**: 1047-1056 [PMID: 30586725 DOI: 10.1161/CIRCULATIONAHA.118.037137]
38 **Younossi ZM**. The epidemiology of nonalcoholic steatohepatitis. *Clin Liver Dis (Hoboken)* 2018; **11**: 92-94 [PMID: 30992797 DOI: 10.1002/cld.710]
39 **Chalasani N**, Younossi Z, Lavine JE, Charlton M, Cusi K, Rinella M, Harrison SA, Brunt EM, Sanyal AJ. The diagnosis and management of nonalcoholic fatty liver disease: Practice guidance from the American Association for the Study of Liver Diseases. *Hepatology* 2018; **67**: 328-357 [PMID: 28714183 DOI: 10.1002/hep.29367]
40 **Ratziu V**, Bellentani S, Cortez-Pinto H, Day C, Marchesini G. A position statement on NAFLD/NASH based on the EASL 2009 special conference. *J Hepatol* 2010; **53**: 372-384 [PMID: 20494470 DOI: 10.1016/j.jhep.2010.04.008]

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META-ANALYSIS

Effect of zinc treatment on clinical outcomes in patients with liver cirrhosis: A systematic review and meta-analysis

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Abstract

BACKGROUND

Zinc is an essential trace element integral to many cellular and immune functions. Zinc deficiency is highly prevalent in patients with cirrhosis and related to disease severity.

AIM

To evaluate whether zinc supplementation improves clinical outcomes (disease severity and mortality) in patients with cirrhosis.

METHODS

This prospectively registered systematic review (PROSPERO reference: CRD42018118219) included all studies in Medline, Embase or Cochrane database with inclusion criteria of adult human studies, comparing zinc supplementation of at least 28 d with standard care or placebo in patients with cirrhosis. Mortality and clinical severity score data were extracted. Random effects meta-analyses compared mortality at 6 mo and 2 years. Risk of bias was assessed using the National Institutes of Health quality assessment tool.

RESULTS

Seven hundred and twelve articles were identified of which four were eligible. Zinc formulations and doses varied (elemental zinc 3.4-214 mg daily) for different

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intervention periods in patients with differing etiology and severity of cirrhosis. Two studies were considered to be at high risk of bias. There was no significant difference in 6-mo mortality between patients treated with zinc versus controls [risk ratio 0.98 (0.90-1.05)]. Changes in severity scores were not reported in any study.

CONCLUSION
Zinc supplementation is not associated with reduced mortality in patients with cirrhosis. Findings are limited by the small number of eligible studies and significant heterogeneity in intervention and patient population.

Key words: Zinc; Cirrhosis; Mortality

Core tip: Zinc deficiency is highly prevalent in patients with cirrhosis and may contribute to disease progression and mortality. This systematic review aimed to determine whether zinc supplementation was associated with clinical outcomes in patients with cirrhosis. Meta-analysis of data from four eligible studies found that zinc supplementation was not associated with reduced mortality at 6 mo. No study reported changes in disease severity or complications. Eligible studies were highly heterogeneous with different zinc formulations, dosage and duration applied to varying patient populations. Further well-designed prospective studies are required to determine whether zinc supplementation improves long-term clinical outcome in patients with cirrhosis.

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INTRODUCTION

Chronic liver disease is a serious problem both in the United Kingdom and globally. Approximately 0.1% of the European population is affected by cirrhosis which account for 1.8% of all deaths corresponding to 170000 deaths per year[1].

Liver cirrhosis constitutes the third most common cause of premature death in the United Kingdom[2]. The incidence of liver cirrhosis has been increasing in the United Kingdom at a faster rate than the top four most-commonly diagnosed cancers (lung, breast, bowel and prostate)[2]. It is estimated that over 600000 people in the United Kingdom have serious liver disease, and 60000 of those have cirrhosis. Over 14000 people die of liver disease each year in the United Kingdom. This figure has increased by 400% since the 1970s[3].

Regardless of the underlying etiology of liver disease, malnutrition becomes a significant clinical problem as it progresses[4]. Protein-calorie malnutrition is well documented and accounts for more than 60% of patients with advanced alcohol-related cirrhosis[5]. Micronutrient deficiency including trace element deficiency has also been documented, which may play a role in disease pathogenesis through regulating antioxidant, anti-inflammatory and anti-fibrotic pathways[6].

Zinc is the second most abundant trace element in the body after iron. It forms part of more than 300 enzymes in the body[7]. It plays an indispensable role in cell growth, cell differentiation and human metabolism[7]. Therefore, zinc deficiency can contribute to oxidative stress, growth disorder, cognitive disorder and immune dysfunction[7]. Attesting to zinc's biological importance is its known association with the activity of proteins, including the enzymes needed for the production and destruction of collagen, thus directly affecting the process of fibrosis[7]. It also possesses anti-inflammatory and antioxidant characteristics that may indirectly affect hepatic stellate cells[8].

The global prevalence of zinc deficiency ranges from 4% in countries rich in animal protein and up to 73% in countries with plant-based diets[8]. However, up to 83% of

cirrhotic patients have zinc deficiency which is associated with disease severity[8]. Zinc deficiency in liver disease is multifactorial[9]. Changes in carbohydrate-lipid metabolism precipitates protein calorie and micronutrient malnutrition in patients with chronic liver disease[9]. Zinc is bound to albumin, alpha 2-macroglobulin and acids so the rate of zinc absorption is due largely to albumin concentrations[10]. As liver disease progresses, the level of albumin decreases and this may lead to decreased absorption of zinc, resulting in progression of liver disease and an increased risk of hepatocellular carcinoma[10]. Other factors that are responsible for zinc deficiency in liver cirrhosis include disturbed zinc absorption by the digestive tract as a result of the effects of cytokines, mainly interleukin-6 and endotoxins on gut blood flow[11]. This changes the small bowel intestinal mucosa and decreases zinc absorption[11]. Diuretic therapy also plays a factor as it increases renal zinc excretion and reduces serum albumin and the binding capacity for zinc.

Zinc supplementation has beneficial effects on antioxidant and inflammatory pathways and therefore may delay or prevent progression of cirrhosis[12,13]. We performed a systematic review of the published literature to determine whether zinc supplementation was associated with improved clinical outcomes and long-term survival in patients with cirrhosis. A systematic review of the role of zinc supplementation in the management of chronic liver diseases has been recently published[14]. The review evaluated its effect on response to hepatitis C virus treatment, hepatic encephalopathy and changes in biochemistry but did not assess the effect of zinc supplementation on overall long-term survival[14].

MATERIALS AND METHODS

The protocol for this systematic review was prospectively registered on the PROSPERO database (reference: CRD42018118219) including the literature search strategy.

Types of studies

We included all interventional clinical trials in humans including randomized controlled trials and open-label trials or observational cohort studies that compared zinc supplementation of at least 28 d with those of standard intervention, or placebo in patients with cirrhosis. Trials were included irrespective of publication status, year of publication or language. We excluded non-human studies and laboratory studies using non-clinical samples.

Patients

All adults (> 18 years old) with liver cirrhosis of any etiology, diagnosed using by liver histology, imaging or non-invasive methods. We excluded studies with patients under 18 years of age and with known solid organ cancer including hepatocellular carcinoma.

Intervention

Studies that compared more than 28 d of zinc supplementation *via* any route (oral or parenteral) with placebo or other standard intervention for the management of patients with cirrhosis.

Outcome measures

The primary outcome was 1-year mortality. Secondary outcomes were 6-mo mortality, 2-year mortality, change in severity scores [Child Pugh/ model for end-stage liver disease (MELD) score], complication rate from cirrhosis (hepatic encephalopathy, new ascites, variceal bleed, new jaundice or hepatocellular carcinoma). Studies had to report at least one of these outcomes to be considered for inclusions in the systematic review.

Search strategy

Electronic searches *via* MEDLINE (PubMed) 1961-present, EMBASE (1974-present), the Cochrane library (Cochrane Database of Systematic reviews), Cochrane Central Register of Controlled Trials, conference abstracts from 1980 for the following annual meetings: American Gastroenterology Association, American Association for the Study of Liver Disease, European Association for the Study of the Liver, United European Gastroenterology, British Society for Gastroenterology and British

Association for the Study of the Liver.

Comprehensive searches of the following biomedical electronic databases were also conducted: MEDLINE, EMBASE, PubMed and TRIPS. The search strategy included subject headings and keywords related to "alcohol", "zinc" and "liver". The full search strategy is presented in Supplementary material. The references in all identified review articles and studies were also inspected to identify other trials. Two authors independently assessed the eligible studies.

Selection of studies

Titles and abstracts of studies retrieved using the search strategy were screened independently by two authors (Tan HK and Dhanda AD) to identify eligible studies. For potentially relevant articles or in cases of disagreement between the two reviewers, the full text article was obtained and inspected independently by a third reviewer. Where an eligible study failed to report data on the primary or secondary outcomes, this information was requested from the corresponding author by email. A follow-up email was sent after 2 wk if no response was obtained.

Data extraction and management

Data were extracted independently by two authors (HT and AD) using a standardized, pre-piloted form for assessment of study quality and evidence synthesis. We studied the following data: Study setting and target population, study methodology, details of intervention, primary and secondary outcomes and method of measurement and information of bias. Extracted data were discussed and this discussion was documented.

Assessment of risk of bias in included studies

Two authors independently assessed risk of bias in the trials without masking the trial names using a standard checklist. Risk of bias was assessed using the National Institutes of Health risk of bias tools for controlled trials or observational studies[15]. Any discrepancies or unusual patterns were checked with an independent reviewer.

Controlled intervention studies were assessed for randomization, allocation method, blinding, and similarity of baseline characteristics, drop-out rate, protocol adherence, outcome measures and method of analysis.

Observational studies were assessed for whether there was a defined population, the participation of eligible population, appropriate outcome measures and analysis methods, loss to follow-up rates and confounding factors.

Data synthesis

We provide a narrative synthesis of the findings from the included studies, structured around the target population, timing of intervention, and type of outcome.

The survival (until death) rates from each study contributed to a meta-analysis of the efficacy of zinc supplementation in reducing mortality. If heterogeneity was deemed to be at least moderate as determined by the I^2 statistic exceeding 30% detected at least at the 5% level, then the data would be meta-analyzed with a random effects model, otherwise a fixed effects model would be considered, if there were too few studies to detect heterogeneity. If any subset of studies were found to share characteristics that contributed to heterogeneity across all the studies, then further meta-analyses would be conducted on those subsets. Analysis was performed using the *meta package*[16] with a current installation in R.

RESULTS

Study selection

Seven hundred and twelve records were identified; 49 of them were retrieved and assessed for eligibility. Six studies met selection criteria but outcome data could not be obtained from one and another was an uncontrolled cohort study. Therefore, a total of four randomized controlled trials were included in this systematic review (Figure 1)[17-20]. Study characteristics are presented in Table 1.

Characteristics of included studies

All four included studies were randomized controlled trials (RCTs). All but one study documented both gender and age. None of the studies reported ethnicity of their patient cohort. All four studies selected different clinical populations including

Table 1 Characteristics of studies included in systematic review, *n* (%)

	Bresci *et al*[19]	Vilar Gomez *et al*[18]	Hayashi *et al*[20]	Takuma *et al*[17]
Country	Italy	Cuba	Japan	Japan
Number of participants	90	100	40	79
%male (control group)	23	18	13	40
%male (intervention group)	33	22	10	
Age (control group)	49	56.6 (8.4)	65.1 (11.3)	66.5 (7.4)
Age (intervention group)	51	58.5 (8.9)	66.0 (9.9)	66.5 (5.7)
Inclusion criteria	Cirrhosis (any aetiology) with encephalopathy	Decompensated HCV cirrhosis	Cirrhosis of any aetiology	Cirrhosis (any aetiology) with encephalopathy
MELD (control group)	N/A	13.3 (4.7)	N/A	11.8 (3.2)
MELD (intervention group)	N/A	12.5 (3.7)	N/A	11.8 (3.2)
Control group treatment	Placebo	Placebo	BCAAs	BCAAs
Intervention group treatment	Zinc acetate	Viusid	Zinc sulfate + BCAAs	Polaprezinc + BCAAs
Zinc preparation and dosage	Zinc acetate 600 mg/d	Viusid 3 tablets/d	Zinc sulfate 200-600 mg/d	Polaprezinc
Elemental Zinc dosage (mg Zn/d)	214	3.4	45-136	51
Duration of intervention	6 mo	96 wk	5-6 mo	6 mo
Primary Outcome	Hepatic encephalopathy assessments	Overall survival rate	Change in biochemistry	Hepatic encephalopathy assessments
Results	No significant improvement in hepatic encephalopathy assessments	Significant improvement in overall survival, time to disease progression and cumulative incidence of HCV	Significant improvement in blood ammonia levels	Significant improvement in the physical component scale, but not the mental component scale
Duration of follow up	1 yr	96 wk	5-6 mo	6 mo

Continuous variables expressed as mean ± SD where available. BCAA: Branched chain amino acid; HCV: Hepatitis C virus; MELD: Model for end-stage liver disease; Zn: Zinc; N/A: Not applicable.

patients with cirrhosis with hepatic encephalopathy, decompensated hepatitis C cirrhosis, and cirrhosis with etiology not documented. All studies were conducted in community settings. The definition of cirrhosis was based on histological and biochemical confirmation in three studies; the remaining study did not specify the method of diagnosis of cirrhosis. Only one study was designed to test the effect of zinc supplementation on mortality.

Only two studies (Takuma *et al*[17] and Vilar Gomez *et al*[18]) reported severity of cirrhosis with mean Child Pugh scores of 6.0 and 6.3 and MELD scores of 11.8 and 12.9.

Bresci *et al*[19] examined the effect of long-term oral zinc supplementation on recurrent hepatic encephalopathy. Ninety cirrhotic patients with recurrent encephalopathy were treated with 600 mg of oral zinc acetate daily for 30 d in addition to standard therapy. The final values of psychometric tests were better in the zinc group compared to the standard therapy group, but the differences were not statistically significant. Three deaths were reported within 6 mo (one in the placebo and two in the intervention group).

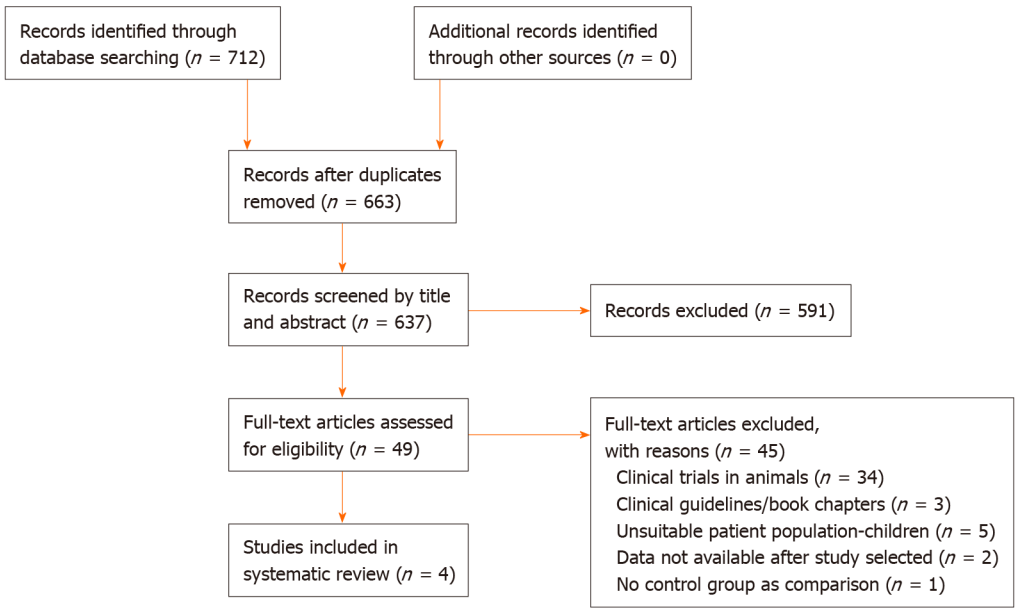

Figure 1 Flow diagram of study identification and selection.

Vilar Gomez *et al*[18] evaluated the efficacy of Viusid for 96 wk in reducing mortality in 100 patients with hepatitis C-related decompensated cirrhosis. Viusid was chosen because it is a nutritional supplement with recognized anti-inflammatory and antioxidant properties. It contains 11 active compounds including zinc sulphate and glycyrrhizic acid. Glycyrrhizin is the most active ingredient of Viusid and has anti-inflammatory, immune-modulating and antiviral properties. The total amount of daily elemental zinc participants received was 3.4 mg, the lowest zinc content of any of the included studies. The study demonstrated a significant improvement in overall survival, time to disease progression and cumulative incidence of hepatocellular carcinoma. No differences were observed between groups for incidence of liver decompensation (including hepatic encephalopathy, spontaneous bacterial peritonitis, hepatorenal syndrome and gastrointestinal bleeding).

Hayashi *et al*[20] randomized 40 patients with cirrhosis to receive a combination of branched chain amino acids and zinc sulfate 600 mg daily for 6 mo. No deaths were reported during the study. They demonstrated a significant improvement in blood ammonia levels in the combination group, but there was no further investigation done to determine the mechanism of action and long-term clinical efficacy.

Takuma *et al*[17] investigated the effectiveness of oral polaprezinc (51 mg zinc and 174 mg of L-carnosine daily) in 79 patients with hepatic encephalopathy. They concluded that oral polaprezinc did significantly improve the physical component scale, but not the mental component scale in patients treated with zinc supplementation. One death occurred in the placebo treated arm only. This study was limited by short-term follow-up (6 mo) and non-blinded treatment allocation.

Survival

No studies reported 1-year mortality, which was the primary outcome of this systematic review. Three studies (Bresci *et al*[19], Hayashi *et al*[20], and Takuma *et al*[17]) reported 6-mo survival and one (Vilar Gomez *et al*[18]) reported 2-year survival. There is a substantial amount of heterogeneity across the selected study trials in terms of zinc formulation, dosage and patient characteristics. The heterogeneity includes dose quantity and frequency, formulation and types of patients.

Risk of bias assessment

We used the National Institutes of Health risk of bias tools to assess the quality of included studies[16]. Two studies (Vilar Gomez *et al*[18] and Hayashi *et al*[20]) were of low risk of bias and two studies of uncertain risk of bias (Bresci *et al*[19] and Takuma *et al*[17]). Risk of bias assessments are presented in Supplementary Table 1. The two studies scored low as a result of lack of blinding and non-similarity in baseline characteristics. Three out of four studies reported information on patient characteristics. Thus, multivariate meta-regression was not appropriate. Selection bias is likely as two

studies included only male subjects. Lack of information precluded a proper evaluation of all the risk of bias in the studies.

Meta-analysis

The I^2 statistic measuring heterogeneity across all included RCTs was estimated to be 66% and significant at the 5% level (P = 0.03). The overall effect of zinc supplements from the included RCTs was estimated to reduce the risk of mortality by 0.98 [95% confidence interval (CI): 0.90-1.05; Figure 2]. Three out of the four included studies had a 6-mo follow-up. The study by Vilar Gomez *et al*[18] reported the largest effect (risk ratio = 0.82), although uncertainty around the point estimate was relatively wide (95%CI: 0.68-0.99). Notably it had a longer 2-year follow-up, and so the meta-analysis was repeated without this particular study, which according to its weight, contributed the least to the overall estimated effect. The effect of all the studies measuring mortality within a 6-mo follow-up were similarly located close to, and not significantly different from, the null effect. Repeating the meta-analysis on these resulted in a risk ratio of unity (95%CI: 0.90-1.05) for the effect of zinc supplementation on the risk of mortality over a 6-mo follow-up (Figure 3).

DISCUSSION

This systematic review identified just four studies that assessed whether zinc supplementation is associated with improved clinical outcomes in patients with cirrhosis. All four eligible studies were highly heterogeneous in terms of patient characteristics, treatment formulation and duration. Four different zinc preparations, with daily elemental zinc doses ranging from 3.4 to 214 mg daily, were tested. Two of these (Viusid, polaprezinc) contained other active compounds, which could have contributed to the positive clinical outcome, for example Viusid contains only 3.4 mg of elemental zinc along with 10 other active ingredients. Three studies tested zinc supplementation in combination with other intervention (branch chain amino acids and nutritional support).

A further limitation is the low mortality rate in all eligible studies. With such a low event rate, any effect of zinc on mortality is difficult to determine. This suggests that either severity of cirrhosis was mild or follow-up duration was insufficient. Only two studies reported severity of cirrhosis with the majority of subjects classified as Child Pugh class A. Two out of four studies were designed to determine the benefit of zinc on hepatic encephalopathy and not powered to detect differences in mortality.

Both Vilar Gomez *et al*[18] and Takuma *et al*[17] suggested the most likely beneficiaries from zinc supplementation would be those with portal hypertension and leaky gut with its resultant risk of sepsis. Preclinical and *in vivo* evidence suggests that zinc may reduce gut permeability[21]. Zinc carnosine (polaprezinc) improved *in vitro* gut epithelial cell migration and proliferation[21]. In healthy volunteers, zinc carnosine treatment reversed both indomethacin-induced and extreme exercise-induced gut leakiness[22]. To test the benefit of a combination strategy of zinc supplementation and treatment of gut leakiness in advanced cirrhosis, long-term studies in patients with portal hypertension are required.

Given the limited data, we are unable to determine whether zinc supplementation improves survival or reduces disease severity in patients with cirrhosis. Research is still needed to confirm the preclinical benefits seen with zinc supplementation on anti-fibrotic, anti-inflammatory and antioxidant processes. To determine whether zinc supplementation improves clinical outcomes of patients with cirrhosis, further high quality studies are required to ascertain the optimal zinc formulation, dose, duration of treatment and patient population to treat.

In conclusion, this systematic review has highlighted the paucity of high quality studies investigating the effect of zinc supplementation in patients with cirrhosis. Eligible studies were of variable design and quality. The primary analyses all had substantial heterogeneity reflecting the differences in study design, inclusion criteria and primary outcome. The difference in etiology and severity of liver cirrhosis also make the effect of zinc supplementation difficult to interpret. With a plausible rationale for benefit from zinc supplementation, there is a strong argument to develop well designed studies in patients stratified clearly by severity of cirrhosis and presence of portal hypertension to determine the long term outcome of zinc supplementation.

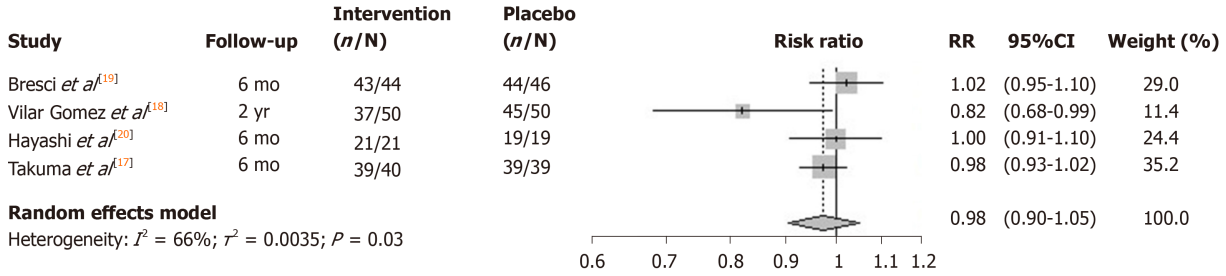

Figure 2 Forest plot of all included randomized controlled trials using a random effects model. Risk ratio 0.98 (95% confidence interval: 0.90-1.05); Heterogeneity: I^2 = 66%; P = 0.03. CI: Confidence interval.

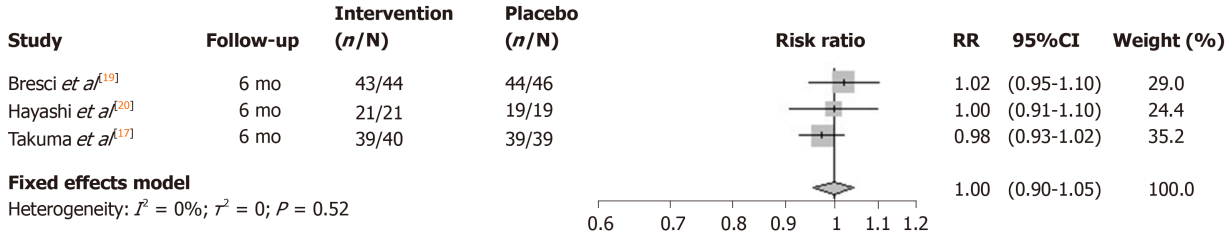

Figure 3 Forest plot of included randomized controlled trials reporting 6 mo mortality using a random effects model (Vilar Gomez *et al*[18] excluded). Risk ratio 1.00 (95% confidence interval: 0.96-1.05), Heterogeneity: I^2 = 0%; P = 0.52. CI: Confidence interval.

ARTICLE HIGHLIGHTS

Research background

Zinc is an essential trace element integral to many cellular and immune functions. Zinc deficiency is highly prevalent in patients with cirrhosis and related to disease severity.

Research motivation

Zinc supplementation has been used to treat complications of cirrhosis including hepatic encephalopathy. However, it is unknown whether zinc supplementation in patients with cirrhosis results in a change in the risk of progression of cirrhosis or death.

Research objectives

This study aimed to evaluate whether zinc supplementation improves clinical outcomes and long-term survival in patients with cirrhosis.

Research methods

A systematic review was performed including all studies in Medline, Embase or Cochrane database with inclusion criteria of adult human studies, comparing zinc supplementation of at least 28 d with standard care or placebo in patients with cirrhosis. Mortality and clinical severity score data were extracted. Random effects meta-analyses determined risk of mortality in patients receiving zinc supplementation versus comparator at 6 mo and 2 years. Risk of bias was assessed using the National Institutes of Health quality assessment tool.

Research results

Seven hundred and twelve articles were identified of which four were eligible. Zinc formulations and doses varied for different intervention periods in patients with differing etiology and severity of cirrhosis. Two studies were considered to be at high risk of bias. There was no significant difference in 6-mo mortality between patients treated with zinc versus controls. Changes in severity scores were not reported in any study.

Research conclusions

Findings are limited by the small number of eligible studies and significant heterogeneity in intervention and patient population. Zinc supplementation is not

statistically associated with reduced mortality or improved long term outcome in patients with cirrhosis.

Research perspectives

There is substantial heterogeneity in study design, inclusion criteria and primary outcome. The difference in etiology and severity of liver cirrhosis also make the effect of zinc supplementation difficult to interpret. Further well-designed prospective studies are required to determine whether zinc supplementation improves long-term clinical outcome in patients with cirrhosis.

REFERENCES

1 **World Health Organization**. Age-standardized death rates of liver cirrhosis 2016. [accessed 9 September 2019]. Available from: https://www.who.int/gho/alcohol/harms_consequences/deaths_liver_cirrhosis/en/

2 **Williams R**, Alexander G, Aspinall R, Batterham R, Bhala N, Bosanquet N, Severi K, Burton A, Burton R, Cramp ME, Day N, Dhawan A, Dillon J, Drummond C, Dyson J, Ferguson J, Foster GR, Gilmore I, Greenberg J, Henn C, Hudson M, Jarvis H, Kelly D, Mann J, McDougall N, McKee M, Moriarty K, Morling J, Newsome P, O'Grady J, Rolfe L, Rice P, Rutter H, Sheron N, Thorburn D, Verne J, Vohra J, Wass J, Yeoman A. Gathering momentum for the way ahead: fifth report of the Lancet Standing Commission on Liver Disease in the UK. *Lancet* 2018; **392**: 2398-2412 [PMID: 30473364 DOI: 10.1016/S0140-6736(18)32561-3]

3 **Williams R**, Aspinall R, Bellis M, Camps-Walsh G, Cramp M, Dhawan A, Ferguson J, Forton D, Foster G, Gilmore I, Hickman M, Hudson M, Kelly D, Langford A, Lombard M, Longworth L, Martin N, Moriarty K, Newsome P, O'Grady J, Pryke R, Rutter H, Ryder S, Sheron N, Smith T. Addressing liver disease in the UK: a blueprint for attaining excellence in health care and reducing premature mortality from lifestyle issues of excess consumption of alcohol, obesity, and viral hepatitis. *Lancet* 2014; **384**: 1953-1997 [PMID: 25433429 DOI: 10.1016/S0140-6736(14)61838-9]

4 **Johnson TM**, Overgard EB, Cohen AE, DiBaise JK. Nutrition assessment and management in advanced liver disease. *Nutr Clin Pract* 2013; **28**: 15-29 [PMID: 23319353 DOI: 10.1177/0884533612469027]

5 **Mendenhall C**, Roselle GA, Gartside P, Moritz T. Relationship of protein calorie malnutrition to alcoholic liver disease: a reexamination of data from two Veterans Administration Cooperative Studies. *Alcohol Clin Exp Res* 1995; **19**: 635-641 [PMID: 7573786 DOI: 10.1111/j.1530-0277.1995.tb01560.x]

6 **Nangliya V**, Sharma A, Yadav D, Sunder S, Nijhawan S, Mishra S. Study of trace elements in liver cirrhosis patients and their role in prognosis of disease. *Biol Trace Elem Res* 2015; **165**: 35-40 [PMID: 25613584 DOI: 10.1007/s12011-015-0237-3]

7 **Vallee BL**, Falchuk KH. The biochemical basis of zinc physiology. *Physiol Rev* 1993; **73**: 79-118 [PMID: 8419966 DOI: 10.1152/physrev.1993.73.1.79]

8 **Sengupta S**, Wroblewski K, Aronsohn A, Reau N, Reddy KG, Jensen D, Te H. Screening for Zinc Deficiency in Patients with Cirrhosis: When Should We Start? *Dig Dis Sci* 2015; **60**: 3130-3135 [PMID: 25742912 DOI: 10.1007/s10620-015-3613-0]

9 **Maret W**. Zinc and human disease. *Met Ions Life Sci* 2013; **13**: 389-414 [PMID: 24470098 DOI: 10.1007/978-94-007-7500-8-12]

10 **Grüngreiff K**, Reinhold D, Wedemeyer H. The role of zinc in liver cirrhosis. *Ann Hepatol* 2016; **15**: 7-16 [PMID: 26626635 DOI: 10.5604/16652681.1184191]

11 **Stamoulis I**, Kouraklis G, Theocharis S. Zinc and the liver: an active interaction. *Dig Dis Sci* 2007; **52**: 1595-1612 [PMID: 17415640 DOI: 10.1007/s10620-006-9462-0]

12 **Hennig B**, Meerarani P, Toborek M, McClain CJ. Antioxidant-like properties of zinc in activated endothelial cells. *J Am Coll Nutr* 1999; **18**: 152-158 [PMID: 10204831 DOI: 10.1080/07315724.1999.10718843]

13 **Shen H**, Oesterling E, Stromberg A, Toborek M, MacDonald R, Hennig B. Zinc deficiency induces vascular pro-inflammatory parameters associated with NF-kappaB and PPAR signaling. *J Am Coll Nutr* 2008; **27**: 577-587 [PMID: 18845708 DOI: 10.1080/07315724.2008.10719741]

14 **Diglio DC**, Fernandes SA, Stein J, Azeredo-da-Silva A, de Mattos AA, Tovo CV. Role of zinc supplementation in the management of chronic liver diseases: A systematic review and meta-analysis. *Ann Hepatol* 2020; **19**: 190-196 [PMID: 31611064 DOI: 10.1016/j.aohep.2019.08.011]

15 **National Institutes of Health**. Study quality assessment tools. [accessed 1 March 2019]. Available from: https://www.nhlbi.nih.gov/health-topics/study-quality-assessment-tools

16 **Schwarzer G**, Carpenter JR, Rücker G. Small-Study Effects in Meta-Analysis. In: Meta-Analysis with R. Use R! Switzerland: Springer Cham, 2015: 107-141. [DOI: 10.1007/978-3-319-21416-0_5]

17 **Takuma Y**, Nouso K, Makino Y, Hayashi M, Takahashi H. Clinical trial: oral zinc in hepatic encephalopathy. *Aliment Pharmacol Ther* 2010; **32**: 1080-1090 [PMID: 20822500 DOI: 10.1111/j.1365-2036.2010.04448.x]

18 **Vilar Gomez E**, Sanchez Rodriguez Y, Torres Gonzalez A, Calzadilla Bertot L, Arus Soler E, Martinez Perez Y, Yasells Garcia A, Abreu Vazquez Mdel R. Viusid, a nutritional supplement, increases survival and reduces disease progression in HCV-related decompensated cirrhosis: a randomised and controlled trial. *BMJ Open* 2011; **1**: e000140 [PMID: 22021873 DOI: 10.1136/bmjopen-2011-000140]

19 **Bresci G**, Parisi G, Banti S. Management of hepatic encephalopathy with oral zinc supplementation: a long-term treatment. *Eur J Med* 1993; **2**: 414-416 [PMID: 8258031]

20 **Hayashi M**, Ikezawa K, Ono A, Okabayashi S, Hayashi Y, Shimizu S, Mizuno T, Maeda K, Akasaka T, Naito M, Michida T, Ueshima D, Nada T, Kawaguchi K, Nakamura T, Katayama K. Evaluation of the effects of combination therapy with branched-chain amino acid and zinc supplements on nitrogen

metabolism in liver cirrhosis. *Hepatol Res* 2007; **37**: 615-619 [PMID: 17517070 DOI: 10.1111/j.1872-034X.2007.00095.x]

21 **Mahmood A**, FitzGerald AJ, Marchbank T, Ntatsaki E, Murray D, Ghosh S, Playford RJ. Zinc carnosine, a health food supplement that stabilises small bowel integrity and stimulates gut repair processes. *Gut* 2007; **56**: 168-175 [PMID: 16777920 DOI: 10.1136/gut.2006.099929]

22 **Davison G**, Marchbank T, March DS, Thatcher R, Playford RJ. Zinc carnosine works with bovine colostrum in truncating heavy exercise-induced increase in gut permeability in healthy volunteers. *Am J Clin Nutr* 2016; **104**: 526-536 [PMID: 27357095 DOI: 10.3945/ajcn.116.134403]

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CASE REPORT

Diagnosis and management of hepatic artery in-stent restenosis after liver transplantation by optical coherence tomography: A case report

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Abstract

BACKGROUND

Percutaneous transluminal angioplasty and stenting represent an effective treatment for hepatic artery stenosis after liver transplantation. In the first year after stenting, approximately 22% of patients experience in-stent restenosis, increasing the risk of artery thrombosis and related complications, and 50% experience liver failure. Although angiography is an important tool for diagnosis and the planning of therapeutic interventions, it may raise doubts, especially in small-diameter arteries, and it provides low resolution rates compared with newer intravascular imaging methods, such as optical coherence tomography (OCT).

CASE SUMMARY

manuscript, interpreted the imaging findings; Almeida MD, Garcia RG and Wolosker N were responsible for revision of the manuscript for important intellectual content; All authors issued final approval for the version to be submitted.

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A 64-year-old male developed hepatic artery stenosis one year after orthotropic liver transplantation and was successfully treated with percutaneous transluminal angioplasty with stenting. Five months later, the Doppler ultrasound results indicated restenosis. Visceral arteriography confirmed hepatic artery tortuosity but was doubtful for significant in-stent restenosis (ISR) and intrahepatic flow reduction. To confirm ISR, identify the etiology and guide treatment, OCT was performed. OCT showed severe stenosis due to four mechanisms: Focal and partial stent fracture, late stent malapposition, in-stent neointimal hyperplasia, and neoatherosclerosis.

CONCLUSION
Intravascular diagnostic methods can be useful in evaluating cases in which initial angiography results are not sufficient to provide a proper diagnosis of significant stenosis, especially with regard to ISR. A wide range of diagnoses are provided by OCT, resulting in different treatment options. Interventional radiologists should consider intravascular diagnostic methods as additional tools for evaluating patients when visceral angiography results are unclear.

Key words: Liver transplantation; Hepatic artery restenosis; Tomography; Optical coherence; Case report; Endovascular procedures; Angiography

Core tip: This is the first case report of optical coherence tomography in the evaluation of in-stent restenosis in a transplant hepatic artery. In this case, optical coherence tomography proved to be valuable in grading the significance of stenosis, identifying its possible causes, and providing measures for choosing appropriate devices for re-treatment. In this case, this additional and modern tool helped in the diagnosis and the therapeutic planning after a doubtful angiography result.

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INTRODUCTION

Hepatic artery stenosis (HAS) is one of the most common vascular complications of liver transplantation and is a leading cause of hepatic artery thrombosis and allograft dysfunction[1]. HAS treatment consisting of percutaneous transluminal angioplasty with stenting (PTAS) has been largely adopted and proven to be effective, although it carries a risk of up to 22% for in-stent restenosis (ISR) in the first year and poses a therapeutic challenge related to the fact that up to 50% of cases result in liver failure[2].

ISR after liver transplantation is frequently attributed to neointimal proliferation. Doppler ultrasound (DUS) is the standard method for screening diagnosis combined with laboratory abnormalities[3]. Angiography is an important tool for confirming the diagnosis and planning optimum therapeutic intervention. However, because angiography only provides a two-dimensional view of a three-dimensional structure, it poorly estimates plaque volume, morphology and lesion severity. Moreover, angiography sometimes overestimates lumen dimensions and provides low resolution rates compared with newer intravascular imaging methods, such as intravascular ultrasound (IVUS) and optical coherence tomography (OCT), especially in small-diameter arteries[4]. Additionally, considerable intra- and interobserver variability in the interpretation of stenosis severity has been observed with arteriography[5].

Several factors contribute to stent failures, and OCT can assess them with great imaging quality. One of these factors is neoatherosclerosis, which is observed by OCT as the presence of clusters of lipid-laden foamy macrophages with or without necrotic core formation and/or calcification within the neointimal tissue of stented segments[6].

Other possible findings are: Stent malapposition which is defined by the separation of at least one stent strut from the intimal surface of the arterial wall with evidence of blood behind the strut[7] and stent fracture which is complete separation of stent segments or separated stent struts without displacement[8].

OCT provides the highest resolution of all invasive imaging modalities, resulting in high-quality cross-sectional tomographic images of vessel architecture, and it demonstrates superiority compared with IVUS or angiography in terms of assessing lesions, particularly identifying thrombus, plaque erosion and rupture[9].

To our knowledge, this is the first case report of OCT used to evaluate ISR in a transplant hepatic artery. In this case, OCT proved to be valuable for grading the significance of stenosis, identifying its possible causes, and providing measures for choosing appropriate devices for retreatment.

CASE PRESENTATION

Chief complaints

A 64-year-old man developed, during clinical and ultrasound follow up, hepatic artery intra-stent restenosis.

History of present illness

The patient had a history of orthotopic liver transplantation and was successfully treated with PTAS. Five months later, the patient presented with increased alkaline phosphatase and alanine aminotransferase levels and DUS indicated restenosis.

History of past illness

The patient had alcoholic liver cirrhosis previous to liver transplantation.

Physical examination

The patient was conscious, with good general health and normal vital signs, anicteric, eupneic and without fever. The abdomen was distended without signs of ascites.

Laboratory examinations

Alkaline phosphatase level was 186 U/L and alanine aminotransferase level was 390 U/L.

Imaging examinations

DUS of the intrahepatic arteries showed a low resistance index, ranging from 0.4 to 0.5, indicating restenosis (Figure 1).

Further diagnostic work-up

Visceral arteriography confirmed the tortuosity of the hepatic artery but showed doubtful significant intrastent diameter reduction and intrahepatic flow reduction (Figure 2). Two experienced interventional radiologists disagreed on the angiography results. To confirm ISR, identify the etiology and guide treatment, OCT was performed.

OCT showed severe stenosis (vessel luminal area: 9.8 mm^2/minimal luminal area: 1.7 mm^2/82% of area reduction) due to four mechanisms: Focal and partial stent fracture, late stent malapposition, in-stent neointimal hyperplasia, and neoatherosclerosis (Figure 2).

FINAL DIAGNOSIS

Hepatic artery intra-stent restenosis.

TREATMENT

PTAS with a new drug-eluting stent (Sirolimus) Orsiro 3 mm × 30 mm (Biotronik) resulted in complete resolution of restenosis with improvements in intrahepatic perfusion (Figure 3).

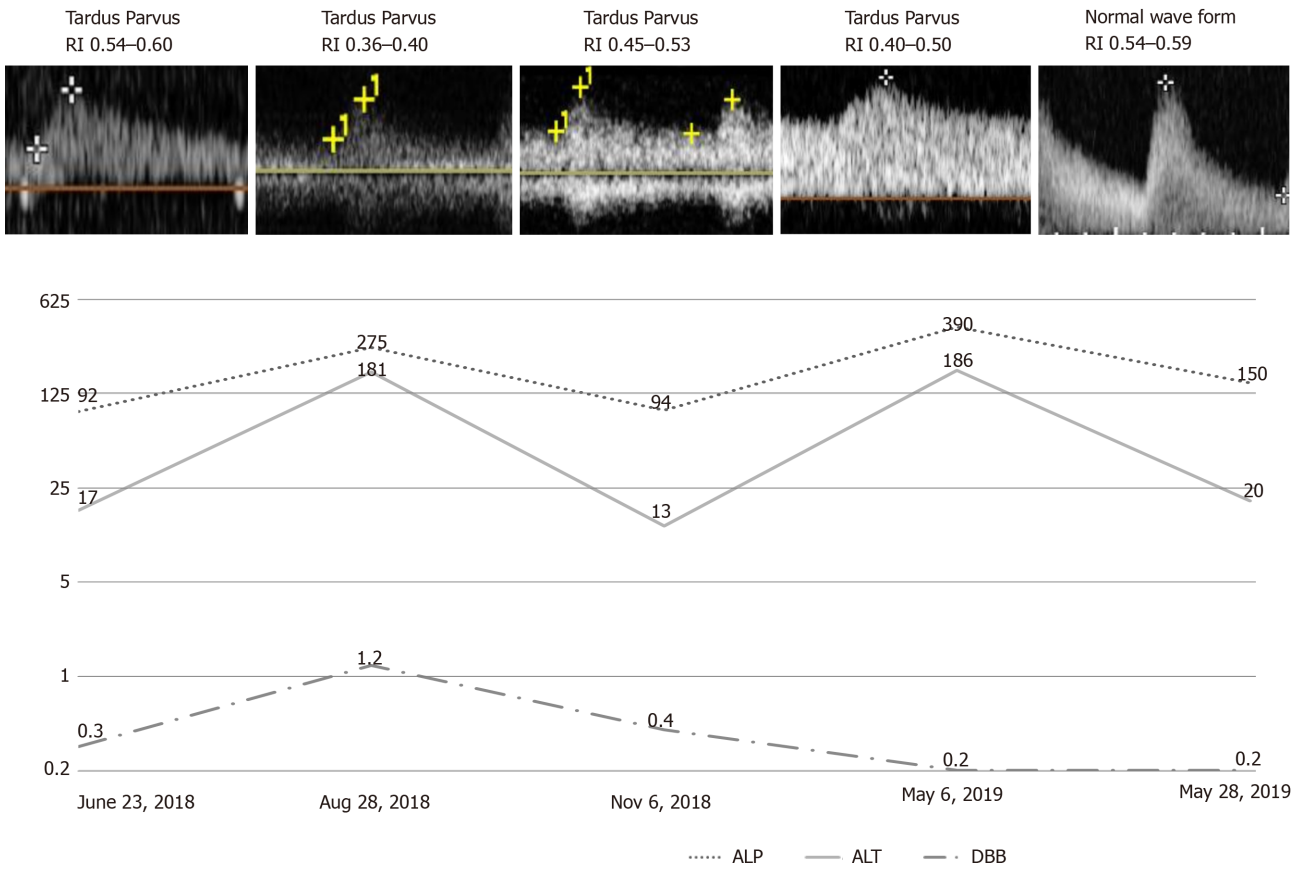

Figure 1 Graphic showing patient's serum levels of direct bilirubin (mg/dL), alkaline phosphatase (U/L) and alanine aminotransferase (U/L); Doppler wave forms and resistive index along time. DBB: Direct bilirubin; ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; RI: Resistance index.

OUTCOME AND FOLLOW-UP

Twenty days later, liver enzymes were normalized, and DUS waveforms were normal for the first time after liver transplantation. The resistance index ranged from 0.54 to 0.59 (Figure 1). In a 10-mo outpatient follow-up, the patient was free from symptoms, with normal liver enzymes.

DISCUSSION

The endovascular approach has become an alternative for the treatment of HAS due to its low morbidity, high patency rates similar to those of open surgery[10], low graft loss, and low mortality rates. Recent retrospective studies have shown that primary stenting may be the first option, with primary assisted patency rates up to 97% within 12 mo and 93% within 24 mo as well as a decrease in the need for reintervention[3,11].

ISR and occlusion are known complications of endovascular procedures that justify clinical follow-up and DUS in these patients. The development of intravascular diagnostic methods has shown that restenosis etiologies are not as simple as they were believed to be. IVUS and OCT enable better evaluation of ISR, and they should be considered for the detection of stent-related mechanical problems leading to restenosis in patients with coronary revascularization, therefore leading to class IIA and level C recommendations[12,13]. The wide range of differential diagnoses for ISR are due to endovascular diagnostic methods, including stent fracture, neoatherosclerosis, neointimal hyperplasia, late acquired stent malapposition, stent underexpansion, evagination, stent crush and edge dissection. Treatment options differ according to the diagnosis of ISR[13].

In our case, the patient presented with biochemical alterations and DUS parameters that suggested new arterial stenosis and probable ISR. Angiography showed tortuosity of the treated vessel, but this tool was limited in terms of measuring intrastent luminal reduction and intrahepatic contrast enhancement. Furthermore, angiography could

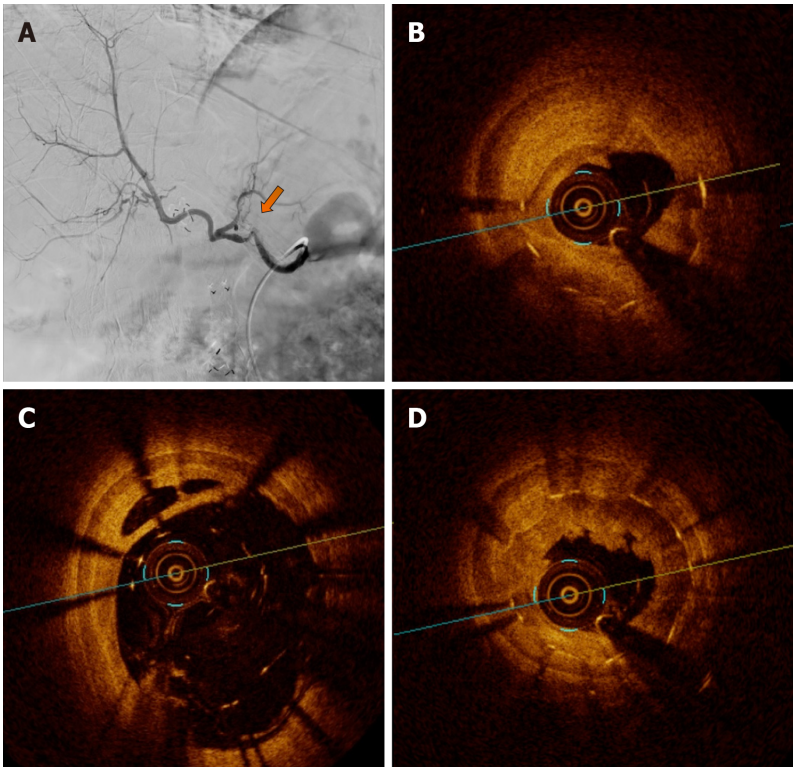

Figure 2 Visceral arteriography and optical coherence tomography. A: Visceral arteriography diagnosis with hepatic artery tortuosity and doubtful significant intra-stent diameter reduction and intrahepatic flow reduction. The yellow arrow indicates the possible stenosis area, to be confirmed further with an intravascular method; B: OCT diagnosis of severe stenosis due neointimal hyperplasia and focal and partial stent fracture; C: OCT diagnosis of stent late malapposition; D: OCT diagnosis of in-stent neointimal hyperplasia and neoatherosclerosis. OCT: Optical coherence tomography.

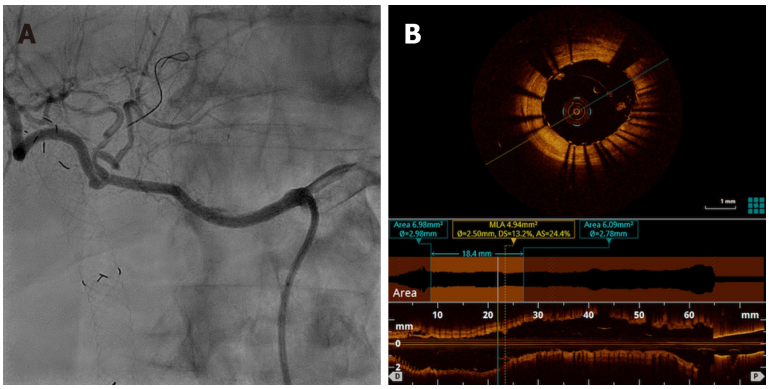

Figure 3 Visceral arteriography after percutaneous transluminal angioplasty and optical coherence tomography posttreatment. A: Visceral arteriography after percutaneous transluminal angioplasty with drug-eluting stent (Sirolimus) Orsiro 3 mm × 30 mm (Biotronik) showing complete resolution of restenosis and improvement in intrahepatic perfusion; B: Optical coherence tomography posttreatment showing acute gain in luminal area due to complete stent expansion.

not categorize the severity of the lesion, the presence of hemodynamic repercussion or the etiology of restenosis. OCT showed severe stenosis caused by stent fracture, late stent malapposition, neointimal hyperplasia and probable neoatherosclerosis tissue. These diagnoses and OCT measurements guided the treatment and stent choice.

Posttreatment angiography undoubtedly showed ISR resolution and improvements in hepatic perfusion with faster parenchymal enhancement and washout. Both interventional radiologists agreed with these findings. Similar to angiography, OCT confirmed an increased vessel area without residual stenosis and provided the finding of adequate stent apposition. DUS performed immediately postprocedure demonstrated normal intrahepatic wave forms and resistance index, and it was used as a basic follow-up test. Consistent with the results of the imaging exams, the patient progressed and demonstrated clinical and laboratory improvements.

The disagreement on the angiography evaluation by two experienced interventional radiologists and the urge to identify stent-related mechanical problems motivated the use of additional intravascular diagnostic methods. In our case, for the first time in the published medical literature, OCT has proven to be an effective additional method for elucidating doubtful lesions on visceral arteries after transplantation. Moreover, posttreatment OCT evaluation confirmed that the interventional radiologists were in agreement regarding the adequate results. Although OCT provides outstanding new information to the vascular territory, we understand that it has some limitations, such as the additional volume of contrast that is unavoidable, the imaging is not free from artifacts and it depends on the observer's experience. In addition, it has a high cost and poor availability[14].

CONCLUSION

Angiography is the gold standard for evaluating patients with suspected restenosis; however, in some cases, this procedure can cast doubt. Intravascular diagnostic methods can be useful when evaluating such cases, especially with regard to in-stent restenosis. A wide range of diagnoses are provided by OCT, which results in different treatment options. Interventional radiologists should consider intravascular diagnostic methods as additional tools for evaluating patients when visceral angiography results are unclear.

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REFERENCES

1 **Rostambeigi N**, Hunter D, Duval S, Chinnakotla S, Golzarian J. Stent placement versus angioplasty for hepatic artery stenosis after liver transplant: a meta-analysis of case series. *Eur Radiol* 2013; **23**: 1323-1334 [PMID: 23239061 DOI: 10.1007/s00330-012-2730-9]

2 **Sugi MD**, Albadawi H, Knuttinen G, Naidu SG, Mathur AK, Moss AA, Oklu R. Transplant artery thrombosis and outcomes. *Cardiovasc Diagn Ther* 2017; **7**: S219-S227 [PMID: 29399525 DOI: 10.21037/cdt.2017.10.13]

3 **Pulitano C**, Joseph D, Sandroussi C, Verran D, Strasser SI, Shackel NA, McCaughan GW, Crawford M. Hepatic artery stenosis after liver transplantation: is endovascular treatment always necessary? *Liver Transpl* 2015; **21**: 162-168 [PMID: 25378262 DOI: 10.1002/lt.24043]

4 **Nakamura S**, Mahon DJ, Maheswaran B, Gutfinger DE, Colombo A, Tobis JM. An explanation for discrepancy between angiographic and intravascular ultrasound measurements after percutaneous transluminal coronary angioplasty. *J Am Coll Cardiol* 1995; **25**: 633-639 [PMID: 7860907 DOI: 10.1016/0735-1097(94)00453-W]

5 **Thomas AC**, Davies MJ, Dilly S, Dilly N, Franc F. Potential errors in the estimation of coronary arterial stenosis from clinical arteriography with reference to the shape of the coronary arterial lumen. *Br Heart J* 1986; **55**: 129-139 [PMID: 3942647 DOI: 10.1136/hrt.55.2.129]

6 **Hong SJ**, Lee SY, Hong MK. Clinical Implication of Optical Coherence Tomography-Based Neoatherosclerosis. *J Korean Med Sci* 2017; **32**: 1056-1061 [PMID: 28581259 DOI: 10.3346/jkms.2017.32.7.1056]

7 **Karalis I**, Ahmed TA, Jukema JW. Late acquired stent malapposition: why, when and how to handle? *Heart* 2012; **98**: 1529-1536 [PMID: 23001063 DOI: 10.1136/heartjnl-2011-301220]

8 **Mohsen MK**, Alqahtani A, Al Suwaidi J. Stent fracture: how frequently is it recognized? *Heart Views* 2013; **14**: 72-81 [PMID: 23983912 DOI: 10.4103/1995-705X.115501]

9 **Hamby BA**, Ramirez DE, Loss GE, Bazan HA, Smith TA, Bluth E, Sternbergh WC 3rd. Endovascular treatment of hepatic artery stenosis after liver transplantation. *J Vasc Surg* 2013; **57**: 1067-1072 [PMID: 23332988 DOI: 10.1016/j.jvs.2012.10.086]

10 **Le L**, Terral W, Zea N, Bazan HA, Smith TA, Loss GE, Bluth E, Sternbergh WC 3rd. Primary stent placement for hepatic artery stenosis after liver transplantation. *J Vasc Surg* 2015; **62**: 704-709 [PMID: 26054583 DOI: 10.1016/j.jvs.2015.04.400]

11 **Vidjak V**, Novačić K, Matijević F, Kavur L, Slavica M, Mrzljak A, Filipec-Kanižaj T, Leder NI, Škegro D. Percutaneous Endovascular Treatment for Hepatic Artery Stenosis after Liver Transplantation: The Role of Percutaneous Endovascular Treatment. *Pol J Radiol* 2015; **80**: 309-316 [PMID: 26150902 DOI: 10.12659/PJR.893831]

12 **Secco GG**, Grattoni C, Parisi R, Oshoala K, Cremonesi A, Fattori R, Castriota F. Optical Coherence Tomography Guidance during Peripheral Vascular Intervention. *Cardiovasc Intervent Radiol* 2015; **38**: 768-772 [PMID: 24595662 DOI: 10.1007/s00270-014-0868-3]

13 **Neumann FJ**, Sousa-Uva M, Ahlsson A, Alfonso F, Banning AP, Benedetto U, Byrne RA, Collet JP, Falk

V, Head SJ, Juni P, Kastrati A, Koller A, Kristensen SD, Niebauer J, Richter DJ, Seferović PM, Sibbing D, Stefanini GG, Windecker S, Yadav R, Zembala MO. [2018 ESC/EACTS Guidelines on myocardial revascularization]. *Kardiol Pol* 2018; **76**: 1585-1664 [PMID: 30566213 DOI: 10.5603/KP.2018.0228]

14 **Roleder T**, Jąkała J, Kałuża GL, Partyka Ł, Proniewska K, Pociask E, Zasada W, Wojakowski W, Gąsior Z, Dudek D. The basics of intravascular optical coherence tomography. *Postepy Kardiol Interwencyjnej* 2015; **11**: 74-83 [PMID: 26161097 DOI: 10.5114/pwki.2015.52278]

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CASE REPORT

Is right lobe liver graft without main right hepatic vein suitable for living donor liver transplantation?

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Author contributions: Akbulut S, Koc C and Cicek E designed the report; Akbulut S, and Dirican A performed surgical procedures, Cicek E and Akbulut S collected the patient's clinical data; Akbulut S, Demyati K and Yilmaz S analyzed the data and wrote the paper.

Informed consent statement: The patients involved in this study gave their informed written consent prior to study enrollment, authorizing the use and disclosure of protected health information.

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Abstract

BACKGROUND

Since the first living donor liver transplantation (LDLT) was performed by Raia and colleagues in December 1988, LDLT has become the gold standard treatment in countries where cadaveric organ donation is not sufficient. Adequate hepatic venous outflow reconstruction in LDLT is essential to prevent graft congestion and its complications including graft loss. However, this can be complex and technically demanding especially in the presence of complex variations and congenital anomalies in the graft hepatic veins.

CASE SUMMARY

Herein, we aimed to present two cases who underwent successful right lobe LDLT using a right lobe liver graft with rudimentary or congenital absence of the right hepatic vein and describe the utility of a common large opening drainage model in such complex cases.

CONCLUSION

Thanks to this venous reconstruction model, none of the patients developed postoperative complications related to venous drainage. Our experience with venous drainage reconstruction models shows that congenital variations in the hepatic venous structure of living liver donors are not absolute contraindications for LDLT.

Key words: Living donor liver transplantation; Congenital-absence of right hepatic vein; Common large opening drainage model; Case report

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Core tip: In this study, we aimed to present two cases who underwent successful right lobe living donor liver transplantation using a right lobe liver graft with rudimentary or congenital absence of the right hepatic vein and describe the utility of a common large opening drainage model in such complex cases. Thanks to this venous reconstruction model, none of the patients developed postoperative complications related to venous drainage.

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INTRODUCTION

Since the first successful liver transplantation (LT) performed in 1967, LT has become the gold standard treatment for many liver diseases in adult and pediatric patients[1]. In socioculturally developed western countries, most of the liver graft requirements are provided from the cadaveric organ pool, while in Asian and Middle Eastern countries, a significant portion of the organ requirements are provided from the living donor pool[1,2]. In deceased donor liver transplantation, whole size liver graft is harvested with the inferior vena cava (IVC) and then venous anastomosis can be performed easily between the IVC of the liver graft and IVC of the recipient using conventional, piggyback, or modified piggyback techniques[1,2]. In contrast, variations in the vascular structure of the liver graft obtained from a living liver donor (LLD) cause difficulties during vascular reconstruction in LDLT, especially hepatic venous reconstruction. Venous drainage of the right lobe (RL) is more complex compared to the left lobe of the liver. To both benefit from liver graft optimally and avoid congestion-related complications, all large venous structures including inferior right hepatic vein (IRHV), segment 5 vein (V5) and segment 8 vein (V8) should be integrated into the venous drainage system[3]. In other words, meticulous assessment of the vascular structures of the LLD candidates by preoperative radiological instruments and thus identification of variations is critical for both LLDs safety and planning of graft implantation techniques.

To evaluate the hepatic vascular structures of LLD candidates, Doppler ultrasonography (US), multidetector computed tomography (MDCT) and, if necessary, conventional hepatic angiography are the most commonly used techniques[2]. Variations detected in the liver vascular anatomy of the potential LLD candidates either result in rejecting the candidate or the surgical team considers alternative surgical techniques such as various venous drainage models.

Congenital absence of the right hepatic vein (RHV) is one of the rarest hepatic vascular anomalies. This anomaly is usually associated with multiple large IRHVs or wider middle hepatic vein (MHV) tributaries. To our knowledge, no clinical studies or case reports related to this RHV anomaly have been published in the English literature except autopsy studies. To our knowledge, a successful LDLT using the RL liver graft without the RHV was performed by our clinic for the first time in the world[2]. After that, Ray and colleagues reported that they performed successful LDLT using a RL liver graft without a RHV orifice. Herein, we present hepatic venous drainage reconstruction models of RL liver grafts obtained from two LLDs with congenital RHV anomalies.

CASE PRESENTATION

Chief complaint and history of present illness

Case 1: A 25-year-old healthy male (BMI: 20.2 kg/m^2, total liver volume: 1136 cc, RL: 786 cc, remnant liver: 34%) was admitted to our liver transplant institute to give a part of his liver to his 26-year-old sister with Budd Chiari Syndrome. He had no chronic disease.

Case 2: A 31-year-old healthy male (BMI: 23.7 kg/m^2, total liver volume: 1428 cc, RL: 1000 cc, remnant liver: 30%) was admitted to our liver transplant institute to give a part of his liver to his 56-year-old uncle with alcoholic liver cirrhosis.

Physical examination

Case 1 and Case 2: Physical examination revealed that vital signs were within normal limits. The LLD candidates were examined according to the donor evaluation algorithm applied in our liver transplant institute.

Laboratory and imaging examinations

Case 1: Biochemical blood tests and viral markers were within normal limits. Contrast-enhanced MDCT showed that the RHV was rudimentary and that the RL was drained by three IRHVs, one of them was located in the hepatocaval ligament. As our institute is experienced in RL drainage models, cadaveric organ donation was insufficient, and the recipient could not provide another potential donor candidate; thus, we decided to accept the LLD candidate.

Case 2: The potential LLD was examined according to the donor evaluation algorithm applied in our institute. Contrast-enhanced MDCT showed congenital absence of the RHV and that the RL was drained by two large IRHVs.

FINAL DIAGNOSIS

Case 1

The healthy individual who had a rudimentary RHV was accepted as a suitable LLD candidate.

Case 2

The healthy individual who had no RHV was accepted as a suitable LLD candidate.

TREATMENT

Case 1

RL hepatectomy was performed as previously described in our institute. Three IRHVs of 5-6 mm diameter, which drained the RL into the IVC, were preserved until the parenchymal transection was completed. Parenchymal transection was performed using the CUSA (Cavtron Ultrasonic Surgical Aspirator, Integra, United States) without Pringles maneuver. During transection, two V5 and one V8 were marked and preserved to be integrated into the venous drainage model. Bloodless RL graft volume and graft-recipient weight ratio were measured as 765 g and 1.03%, respectively.

Case 2

RL hepatectomy was performed as previously described in our institute, all three IRHVs were preserved until the parenchymal transection was completed and transection was performed using the CUSA without Pringles maneuver (Figure 1). During transection, two V5 and two V8 were marked and preserved to be integrated into the venous drainage model. Bloodless RL graft volume and graft-recipient weight ratio were measured as 1000 g and 1.02%, respectively. The drainage model was found to be successful by postoperative MDCT. Finally, the recipient was discharged without postoperative complications.

OUTCOME AND FOLLOW-UP

Case 1

The LLD had an uneventful postoperative clinical course. The drainage model was found to be successful by postoperative MDCT. Finally, the recipient was discharged on postoperative day 21 without complications.

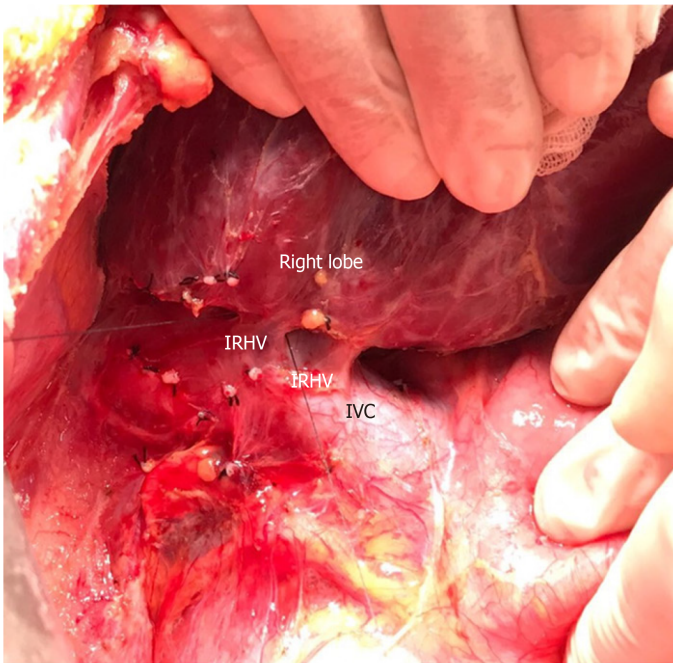

Figure 1 Dissection plan between the right lobe of the liver and inferior vena cava. Intraoperative view of two inferior right hepatic veins draining the right lobe posterior sector. IRHV: Inferior right hepatic vein; IVC: Inferior vena cava.

Case 2

The LLD had an uneventful postoperative clinical course. The drainage model was found to be successful by postoperative MDCT. Finally, the recipient was discharged with minimal biliary complications.

DISCUSSION

Definition of back-table reconstruction techniques for both patients

The perfusion and washing of the liver grafts with preservation solutions on the back-table stage were performed as described previously[2]. Using the cryopreserved vascular graft materials, a common large opening drainage model was created to include three IRHVs and V8. The rudimentary RHV was also integrated into the drainage model. For this common large opening drainage model, an aortic vascular graft was used as a quilt, while a saphenous vein graft was used to both create a circumferential fence and extend V8 to the main drainage model. Both V5 orifices on the cut surface were first created as a single orifice and then anastomosed directly to the recipient's left hepatic vein stump using an expanded polytetrafluoroethylene vascular graft (Figures 2 and 3). The liver implantation techniques used in both cases were not different from other LT recipients with normal RHV. Postoperative US and MDCT were performed to determine whether the venous drainage model was successful in both recipients (Figure 4).

LDLT has been expanded to overcome the graft shortage and disparity between supply and demand in patients on the LT waiting list. However, unlike a whole size deceased donor liver graft, most of the living liver grafts require reconstruction of the venous structures including RHV, IRHVs and MHV tributaries to restore venous drainage of the corresponding segments to prevent any postoperative congestion. Hepatic venous structures may be delineated using modern imaging techniques: Doppler US, MDCT, and conventional angiography are particularly useful for observing the venous structures. Variations or congenital anomalies in hepatic venous structure in LLD candidates can disqualify the candidate or alter surgical choice. One such hepatic venous anomaly is congenital absence of the RHV or a rudimentary RHV. As vascular anomalies and variations in LLD candidates may cause unexpected complications and difficulties, these vascular anomalies and variations must be evaluated and documented clearly by imaging techniques before surgery. In our cases, the rudimentary or congenitally absent RHV and presence of the IRHVs were identified easily on preoperative MDCT, which allowed us to plan the surgery.

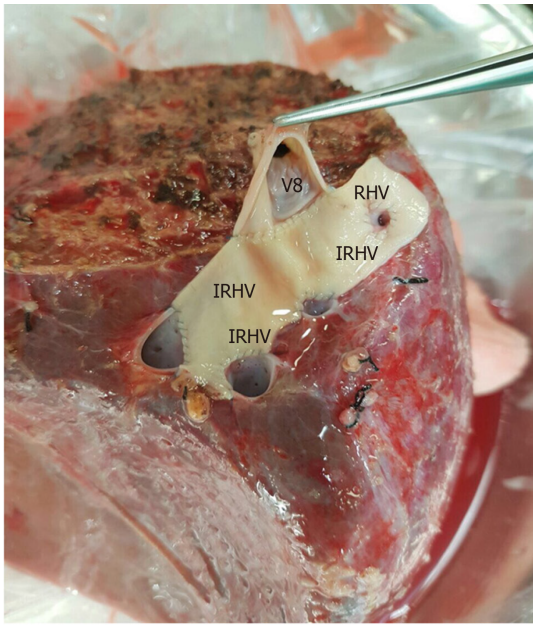

Figure 2 The cryopreserved aortic vascular graft patch was placed between the four orifices as a quilt. After that the segment 8 hepatic vein orifice was extended to the drainage model using a cryopreserved saphenous vein graft. IRHV: Inferior right hepatic vein; V8: 8 vein; RHV: Right hepatic vein.

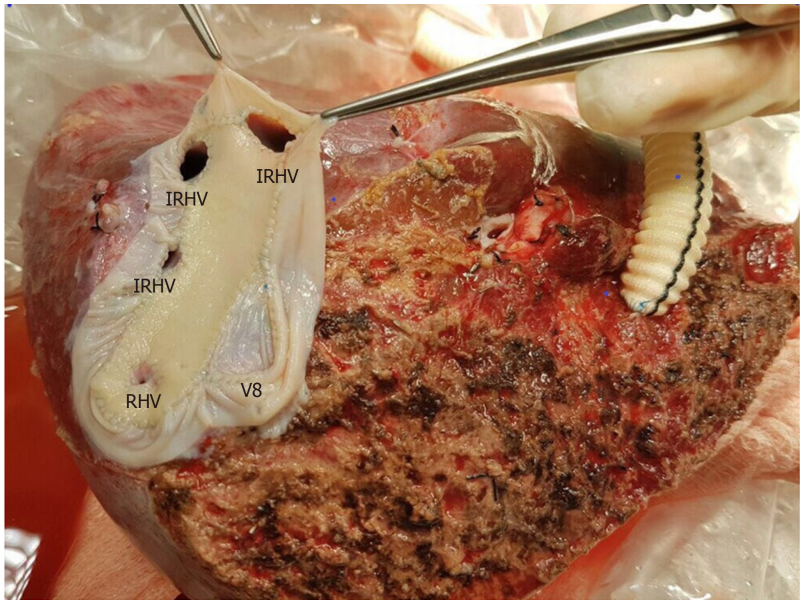

Figure 3 A common large opening drainage model was created using the cryopreserved saphenous vein graft. IRHV: Inferior right hepatic vein; V8: 8 vein; RHV: Right hepatic vein.

Difficulties in hepatic venous drainage in LDLT has been addressed by many studies with many technical considerations and modifications investigated[1-12]. While controversy exists regarding the ideal criteria and method of incorporating the IRHVs into the graft's drainage system and the ideal method of draining segments 5 and 8, it is agreed that venous congestion due to inadequate outflow reconstruction impairs regeneration, and is associated with increased complications including graft loss[8,12].

In our patients with absent or rudimentary main RHV and the presence of multiple major IRHVs, a common large opening drainage model allowed for a wider ostium, which achieved faster and easier anastomosis with the IVC reducing the warm ischemia time with an ostium tolerating compression with less risk of compression and obstruction. We reported a similar case from the same center in 2013, but did not find similar reported cases in the English literature[2]. One case was reported with a liver transplant in the absence of a RHV ostium with the right hepatic vein present and drained into the IVC through a single ostial opening by the middle and left hepatic

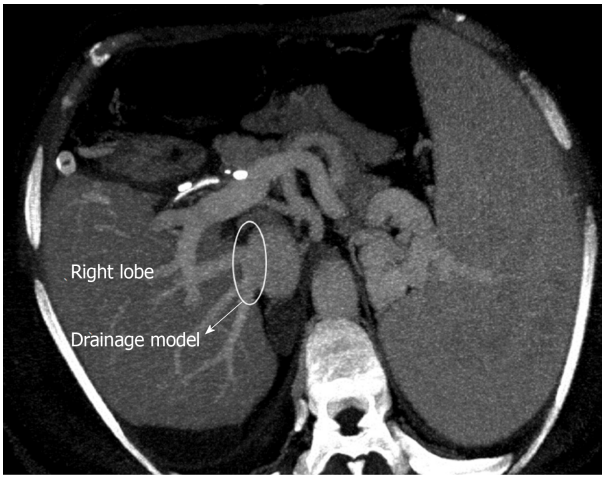

Figure 4 Postoperative contrast axial multidetector computed tomography image shows that the venous drainage model is functional.

veins, in that case a subtotal MHV was to be taken leaving behind the proximal MHV with drainage of the segment 4b and RHV vein into it, as the patient's RHV joined the MHV intra-hepatically[13].

The venous outflow reconstruction is technically challenging for RL liver grafts with an undrained anterior sector, along with the presence of multiple IRHVs with vulnerability of congestion if not adequately reconstructed. Adding to the complexity is the presence of a wide variability in the pattern of branching of hepatic veins, difficulty in determining the optimal anastomotic site and direction especially in the presence of major IRHVs to anastomose which requires further time[14,15]. Authors recommend that short hepatic veins with a diameter ≥ 4 mm should be integrated into the drainage system, which is our approach[2,3,15]. The need for IRHVs to be integrated into the drainage system is even more essential in the absence of adequate drainage through the RHV due to its absence or in cases where it is rudimentary, where in these cases the major IRHVs dominate the venous outflow.

A common large opening reconstruction technique diminishes morbidity as well as potential mortality associated with compromised graft outflow and has been proved to be safe[1,4-6]. A single, wide orifice is achieved by various venoplasty techniques during back-table procedures using cryopreserved conduits, or the recipient's saphenous vein, or synthetic vascular grafts[3,5-7]. The technique used to perform a back-table venoplasty to form a single, large orifice remains an easy procedure without added risks[5]. Also, in the presence of dense adhesions due to previous surgeries, reduced available length of IVC, and multiple collaterals, the outflow reconstruction becomes technically less complex with this technique in addition to reducing the warm ischemia time with one single anastomosis to the IVC.

With regard to the MHV tributaries, which drain the central region of the liver, our approach is to leave the MHV in the donor's side in cases without a segment 4b vein. In cases with a segment 4b vein, the decision to include the MHV in the graft is made with respect to the remnant liver volume. If the remnant liver volume is ≤ 30%, the MHV should be left in the donor's side. If the remnant liver volume is > 30%, the decision is made with respect to the diameters of veins draining segment 5 and 8[2,6].

CONCLUSION

In conclusion, rudimentary or congenital absence of RHV is not an absolute contraindication for RL-LDLT in centers with experience in venous outflow reconstruction and various drainage models. However, it is important to meticulously examine the vascular structures of donor candidates using preoperative radiological instruments.

REFERENCES

1 **Ara C**, Akbulut S, Ince V, Aydin C, Gonultas F, Kayaalp C, Unal B, Yilmaz S. Circumferential Fence With

the Use of Polyethylene Terephthalate (Dacron) Vascular Graft for All-in-One Hepatic Venous Reconstruction in Right-Lobe Living-Donor Liver Transplantation. *Transplant Proc* 2015; **47**: 1458-1461 [PMID: 26093742 DOI: 10.1016/j.transproceed.2015.04.069]

2 **Akbulut S**, Yilmaz M, Eris C, Kutlu R, Yilmaz S. Living-donor liver transplant using the right hepatic lobe without the right hepatic vein: solving the drainage problem. *Exp Clin Transplant* 2013; **11**: 278-282 [PMID: 23767945 DOI: 10.6002/ect.2012.0060]

3 **Hwang S**, Ha TY, Ahn CS, Moon DB, Kim KH, Song GW, Jung DH, Park GC, Namgoong JM, Jung SW, Yoon SY, Sung KB, Ko GY, Cho B, Kim KW, Lee SG. Reconstruction of inferior right hepatic veins in living donor liver transplantation using right liver grafts. *Liver Transpl* 2012; **18**: 238-247 [PMID: 22140053 DOI: 10.1002/lt.22465]

4 **Thorat A**, Jeng LB, Yang HR, Li PC, Li ML, Yeh CC, Chen TH, Hsu SC, Poon KS. Outflow reconstruction for right liver allograft with multiple hepatic veins: "V-plasty" of hepatic veins to form a common outflow channel versus 2 or more hepatic vein-to-inferior vena cava anastomoses in limited retrohepatic space. *Liver Transpl* 2016; **22**: 192-200 [PMID: 26390259 DOI: 10.1002/lt.24342]

5 **Jeng LB**, Thorat A, Li PC, Li ML, Yang HR, Yeh CC, Chen TH, Hsu CH, Hsu SC, Poon KS. "V-Plasty" technique using dual synthetic vascular grafts to reconstruct outflow channel in living donor liver transplantation. *Surgery* 2015; **158**: 1272-1282 [PMID: 25920910 DOI: 10.1016/j.surg.2015.03.018]

6 **Thorat A**, Jeng LB, Li PC, Li ML, Yang HR, Yeh CC, Chen TH, Hsu SC. "Single Oval Ostium Technique" using Polytetrafluoroethylene Graft for Outflow Reconstruction in Right Liver Grafts with venous Anomalies in Living Donor Liver Transplantation. *Hepatogastroenterology* 2015; **62**: 698-702 [PMID: 26897957]

7 **Barut B**, Akbulut S, Kutluturk K, Koc C, Gonultas F, Kayaalp C, Kutlu R, Yilmaz S. Eligibility of Circumferential Fence With the Autologous Peritoneal Patch for Venous Reconstruction in Right Lobe Living-Donor Liver Transplant: A Case Control Study. *Exp Clin Transplant* 2019 [PMID: 31718532 DOI: 10.6002/ect.2018.0325]

8 **Ozbilgin M**, Unek T, Egeli T, Agalar C, Ozkardeşler S, Altay C, Astarcioglu I. Comparison of Patients With and Without Anterior Sector Venous Drainage in Right Lobe Liver Transplantation From Live Donors in Terms of Complications, Rejections, and Graft Survival: Single-Center Experience. *Transplant Proc* 2019; **51**: 1127-1133 [PMID: 31101185 DOI: 10.1016/j.transproceed.2019.01.103]

9 **Sakamoto K**, Ogawa K, Matsui T, Utsunomiya T, Honjo M, Ueno Y, Tamura K, Inoue H, Nakamura T, Watanabe J, Takai A, Tohyama T, Takada Y. Reconstruction of Middle Hepatic Vein Tributaries With Artificial Vascular Grafts in Living Donor Liver Transplant Using Right Lobe Grafts: A Case Series. *Transplant Proc* 2019; **51**: 1506-1510 [PMID: 31010699 DOI: 10.1016/j.transproceed.2019.01.118]

10 **Pravisani R**, Soyama A, Takatsuki M, Hidaka M, Adachi T, Ono S, Hara T, Hamada T, Kanetaka K, Eguchi S. Relationship Between Venous Drainage Patterns and Regeneration of Segments 5 and 8 in Right Lobe Grafts in Adult Living-Donor Liver Transplant Recipients. *Exp Clin Transplant* 2019; **17**: 529-535 [PMID: 30995896 DOI: 10.6002/ect.2018.0155]

11 **Ali MA**, Yong CC, Eng HL, Wang CC, Lin TL, Li WF, Wang SH, Lin CC, Yap A, Chen CL. Cryopreserved arterial grafts as a conduit in outflow reconstruction in living donor liver transplantation. *J Hepatobiliary Pancreat Sci* 2015; **22**: 498-504 [PMID: 25783415 DOI: 10.1002/jhbp.240]

12 **Pravisani R**, Soyama A, Takatsuki M, Hidaka M, Adachi T, Ono S, Hara T, Hamada T, Eguchi S. Impact of the Inferior Right Hepatic Veins on Right Liver Lobe Regeneration in Living-Donor Liver Transplant: 3-Dimensional Computed Tomography Scan Analyses in Donors and Recipients. *Exp Clin Transplant* 2019; **17**: 768-774 [PMID: 30968763 DOI: 10.6002/ect.2018.0254]

13 **Ray S**, Anila T, Jha SK, Rawat S, Rawat KS, Singhvi SK. Is the absence of Right Hepatic Vein opening into Inferior Vena Cava a contraindication for right lobe liver donation in Living Donor Liver Transplantation? Common hepatic venous trunk-A rare hepatic vein anomaly: A case report and review. *Int J Surg Case Rep* 2017; **30**: 159-161 [PMID: 28012335 DOI: 10.1016/j.ijscr.2016.10.039]

14 **Shilal P**, Tuli A. Anatomical variations in the pattern of the right hepatic veins draining the posterior segment of the right lobe of the liver. *J Clin Diagn Res* 2015; **9**: AC08-AC12 [PMID: 25954610 DOI: 10.7860/JCDR/2015/8736.5671]

15 **Hwang S**, Lee SG, Park KM, Kim KH, Ahn CS, Moon DB, Ha TY. Quilt venoplasty using recipient saphenous vein graft for reconstruction of multiple short hepatic veins in right liver grafts. *Liver Transpl* 2005; **11**: 104-107 [PMID: 15690544 DOI: 10.1002/lt.20314]

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