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Novel insight into the role of zinc in the pathogenesis of chronic liver diseases

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Abstract

Zinc (Zn) homeostasis is largely regulated by the liver, at the same time, Zn is necessary for the maintenance of normal liver function. Therefore, Zn deficiency results in the impairment of hepatocyte function, leading to chronic liver injuries such as hepatic inflammation, fibrosis and steatosis. Numerous metabolic abnormalities, including impaired glucose tolerance, dyslipidemia, hepatic encephalopathy, and sarcopenia, are also associated with these chronic liver injuries. Zn supplementation can recover these chronic liver injuries and related metabolic disorders. Recent advances in molecular biological techniques have enabled us to elucidate the putative mechanisms by which chronic liver disorders evoke varieties of metabolic abnormalities derived from Zn deficiency. This review focuses on the most recent discoveries regarding the role of Zn deficiency in chronic liver diseases, including chronic hepatitis, liver cirrhosis, nonalcoholic fatty liver disease, and autoimmune liver diseases. Moreover, we would like to verify Zn supplementation on these chronic liver diseases.

Key words: zinc, chronic liver disease, hepatic activity, hepatic fibrosis, hepatic steatosis, metabolic abnormalities

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Abbreviations:

ALT: alanine aminotransferase, BCAA: branched-chain amino acid, CLD: chronic liver disease, DAA: direct-acting antiviral agents, ECM; extracellular matrix, ER: endoplasmic reticulum, HBV: hepatitis B virus, HCC: hepatocellular carcinoma, HCV: hepatitis C virus, HDL-C: high-density lipoprotein cholesterol, HOMA-IR: homeostasis model for assessment of insulin resistance, IFN: interferon, IGF-1: insulin-like growth factor-1, LDL-C: low-density lipoprotein cholesterol, miRNA: microRNA, MMP-1: matrix metalloproteinase-1, MT: metallothionein, NAFLD: nonalcoholic fatty liver disease, NASH: nonalcoholic steatohepatitis; PPAR-α: peroxisome

proliferator-activated receptor-α, T2DM: type 2 diabetes mellitus, TG: triglyceride, TIMP-1: tissue inhibitor of metalloprotease, TGF-β: transforming growth factor-β, Zn: zinc

Introduction

Zinc (Zn) is an essential trace element which participates in wide range of biological functions, including cell differentiation and proliferation. Approximately 10% of the human proteome are recognized as Zn-containing proteins. So far, more than 300 enzymes that contain Zn ions within their catalytic domains have been identified [1,2]. Zn also regulates intracellular signaling in both innate and adaptive immune systems [3,4]. Moreover, the anti-inflammatory, anti-oxidant and anti-apoptotic properties of Zn have been fully established [5-7].

The liver turns out to be the main organ responsible for the maintenance of Zn homeostasis [8-10]. Zn homeostasis is primarily regulated by metallothionein (MT) and two types of Zn transporters: Zn transporters (ZnTs) and Zrt- and Irt-like proteins (ZIPs). These Zn transporter families participate in the absorption, excretion, transportation and intracellular storage of Zn [11]. The impairments of these Zn regulation processes eventually lead to Zn deficiency.

Zn deficiency is frequently observed in patients with chronic liver diseases (CLDs) such as chronic viral hepatitis, nonalcoholic fatty liver disease (NAFLD), alcoholic liver disease and especially liver cirrhosis [12-17]. Zn deficiency results in the exacerbation of fibrosis and steatosis in the liver, accompanying a variety of metabolic abnormalities including insulin resistance, dyslipidemia, iron overload, hepatic encephalopathy, and even sarcopenia [13,14,16,18]. Hence, Zn supplementation may be a potential therapeutic strategy to improve these metabolic abnormalities as well as the associated liver diseases [12,16,18]. Indeed, some clinical guidelines specially advocate the clinical significance of Zn supplementation in the management of patients with liver cirrhosis [19,20].

Zn status is ordinarily monitored by serum Zn concentration, because serum Zn concentration is proportional to hepatic Zn content [8,9]. However, the definition of Zn deficiency remained indefinite. The clinical diagnosis of Zn deficiency has been currently established in Japan as less than 60 μg/dL [21].

Recent advances in molecular biological techniques have provided many pieces of novel evidence on the roles of zinc in the pathogenesis of CLDs. These novel techniques have enabled us to investigate the close interactions between the liver and other organs such as the brain, lung, kidney, pancreas, gut and muscles. The impairment of other organs due to Zn deficiency affects liver function.

This review article highlights the current understanding of Zn metabolism in the pathogenesis of CLDs. In addition, we also discuss the clinical efficacy of Zn supplementation in the management of these metabolic abnormalities as well as in the associated liver diseases.

The effects of Zn supplementation in CLDs

1. Antiviral effects of Zn on hepatotropic viruses

A large amount of evidence has accumulated showing that Zn possesses a variety of direct and indirect antiviral properties in relation to numerous viruses [22]. Zn has even been shown to exhibit antiviral effects on severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection [23]. Thus, lower serum Zn levels may predict an unfavorable prognosis in patients with severe corona virus disease 2019 (COVID-19) [24].

Similarly, Zn plays crucial roles in the inhibition of hepatitis C virus (HCV) replication [22,25]. It is well established that persistent HCV infection evokes oxidative stress and subsequently inflammation and fibrosis in the liver, leading to chronic liver damage such as chronic hepatitis, liver cirrhosis, and ultimately hepatocellular carcinoma (HCC) [26]. Read et al. revealed that zinc sulfate (ZnSO4) could inhibit HCV replication *in vitro* [27]. The authors speculated that MTs induced by Zn possessed antiviral activities either directly or indirectly. Moreover, they also documented a single-nucleotide polymorphism of interferon-lambda 3 (IFN-λ3), which is recognized as an anti-viral and pro-inflammatory cytokine, potentially participating in the initiation of hepatic MT through increased systemic Zn levels [28]. In a previous randomized controlled trial (RCT), the combination treatment of Zn with IFN significantly improved the rate of viral clearance in patients with chronic hepatitis C [29], although other RCTs did not confirm the antiviral effects of Zn in such patients [30,31]. Therefore, it may make sense that the eradication of HCV by treatment with direct-acting antiviral agents (DAAs) resulted in the increased serum Zn levels in patients with

HCV-related CLD [32,33].

Hepatitis B virus (HBV) infection often causes serious public health problems throughout the world, especially in East Asia and Africa [34]. Mother–to-child transmission is the most common cause of persistent HBV infection. Individuals who are persistently infected with HBV also develop chronic liver disease, including chronic hepatitis and liver cirrhosis, and HCC [26]. No evidence that Zn impedes the replication of HBV has been provided. However, zinc ion supplementation was sufficient to initiate assembly of the HBV capsid protein *in vitro* [35]. In addition, Zn-saturated lactoferrin significantly inhibited the amplification of HBV-DNA in a dose-dependent manner, although lactoferrin by itself did not affect HBV-DNA copies at all [36]. Lactoferrin caused a larger conformational change if it was saturated with zinc. Such a conformational change might exhibit antiviral effects on HBV.

In a clinical study, serum Zn status at entry became a useful predictor for the outcome of IFN treatment [37], which is widely used as a treatment for chronic hepatitis B. However, a RCT did not provide the evidence that combination treatment of Zn with IFN affected the load of HBV DNA in such patients [38]. On the other hand, similar to results in patients with chronic hepatitis C who achieved the clearance of HCV (sustained virological response: SVR) by treatment with DAAs, patients with persistent HBV infection had elevated hepatic Zn contents after treatment with nucleic acid analogs [39].

2. Verification of Zn supplementation on hepatic inflammation in CLDs

It is well recognized that serum Zn concentrations are inversely correlated with serum aspartate aminotransferase (AST) and/or alanine aminotransferase (ALT) levels or histological activities in patients with chronic hepatitis [37,40,41], or those with NAFLD [42], as Zn has beneficial properties to inhibit inflammation in the liver. Therefore, we previously confirmed the administration of polaprezinc, a complex of zinc and L-carnosine, resulted in the significant improvement of serum ALT levels in patients with HCV-related CLD [43], which was approximately consistent with the results obtained by other studies in such patients [44,45] and even in patients with NAFLD [46] (**Table 1**). The improvement of serum ALT levels in patients with HCV-related CLD by Zn supplementation may be attributed to an indirect effect rather than a direct action on HCV. However, other studies did not confirm the improvement of serum ALT levels by additional polaprezinc supplementation to the IFN-based treatment in patients with HCV-related CLD [30,31]. In the future, the anti-inflammatory effects of other Zn compounds should be investigated in patients with CLD.

However, it was of interest that a positive correlation between serum Zn and ALT levels were observed in obese and young females, indicating that serum ALT levels were increased due to the higher Zn levels in such individuals [47]. Bai et al. also demonstrated that dietary Zn was correlated with serum ALT levels in adolescents. Especially, young females seemed to be susceptible to elevated ALT levels by higher Zn intake [48]. Taking these results into consideration, higher intake of Zn may be deleterious to the liver.

The role of a Zn transport in the pathogenesis of liver injury has been explored. Su et al. revealed that knockout of ZnT8, which is an important Zn transporter and was highly expressed in pancreatic islets, resulted in the attenuation of acetaminophen-induced liver injury through the upregulation of hepatic Zn and MT [49]. The data elucidated by their study provided novel insights on the organ-organ interactions between the liver and pancreas.

3. Verification of Zn supplementation on hepatic fibrosis in CLDs

Hepatic fibrosis is widely accepted as the final stage of chronic liver injury, and is mediated by hepatic stellate cell activation and extracellular matrix (ECM) secretion and deposition [50]. The activation of hepatic stellate cells is mediated by the release of transforming growth factor-β (TGF-β) from Kupffer cells. The ECM in the normal liver mainly consists of type I and type III collagens [51]. As the stage of hepatic fibrosis progresses, these collagens are increased due to the inactivation of matrix metalloproteinase-1 (MMP-1) [52], which is a Zn-containing endopeptidase, and plays a crucial role in the degradation of ECM. In addition, serum Zn concentrations were gradually decreased in proportion to the severity of hepatic fibrosis in patients with HCV-related CLD [40,53,54] and those with NAFLD [55,56]. Taken together, these data may indicate that the state of Zn deficiency results in the impairment of MMP-1 activity, leading to the promotion of hepatic fibrosis. Kang et al. confirmed that zinc regulated the synthesis of collagen in hepatic stellate cells through the inhibition of TGF-β signaling *in vitro* [57]. Interestingly, the authors also revealed that a decrease in MT synthesis by Zn deficiency caused apoptosis of hepatic stellate cells

Table 1. | Verification of zinc supplementation on hepatic inflammation, fibrosis and steatosis in patients with CLDs

Reference	Study design	Assigned paients	Formulation	Dosage and duration	Outcomes
Inflammation					
Himoto et al. [43]	retrospective study (end of point vs. base line)	HCV-related CLD (n=14)	polaprezinc	225mg, 6 months	decrease in serum ALT level decrease in serum ferritin level no effect on load of HCV RNA
Murakami et al. [44]	RCT (Zn+ IFN-based treatment vs. IFN-based treatment)	chronic hepatitis C (n=11)	polaprezinc	150mg, 48 weeks	decrease in serum ALT level
Matsuoka et al. [45]	RCT (Zn group vs. untreated group)	HCV-related CLD (n=62)	polaprezinc	150mg, 3 years	decrease in serum ALT level
Fathi et al. [46]	RCT (Zn+calorie-restriction vs. calorie-restriction)	NAFLD (n=25)	zinc gluconate	220mg, 12 weeks	decrease in serum ALT and γ-GT level decrease in waist circumferance
Suzuki et al. [30]	RCT (Zn+ IFN-based treatment vs. IFN-based treatment)	chronic hepatitis C genotype 2 (n=41)			no effect on serum ALT level no effect on load of HCV eradication
Kim et al. [31]	RCT (Zn+ IFN-based treatment vs. IFN-based treatment)	chronic hepatitis C (n=16)	polaprezinc	150mg, 48 weeks	no effect on serum ALT level no effect on load of HCV eradication
Fibrosis					
Takahashi et al. [67]	case control study (Zn-responder group, Zn-nonresponder group vs. untreated group)	liver cirrhosis (n=17)	polaprezinc	150mg, 24 weeks	decrease in serum TIMP-1 level
Moriya et al. [68]	case control study (Zn-responder group vs. Zn-nonresponder group)	AIH (n=27)	polaprezinc	150mg, 2 years	decrease in serum procollagen type III and type IV collagen 7S levels
Attallah et al. [69]	RCT (Zn+DAA vs DAA)	chronic hepatitis C (n=147)	zinc sulfate	440mg, 12 or 24 weeks	improvement of liver stiffness (genotype CC) improvement of SVR (genotype CT/TT)
Steatosis					
Fathi et al. [46]	RCT (Zn+calorie-restriction vs. calorie-restriction)	NAFLD (n=25)	zinc gluconate	220mg, 12 weeks	no effect on hepatic steatosis

γGT; γ-glutamyl transferase, AIH: autoimmune hepatitis, SVR: sustained virological response

in an *in vitro* experiment [58]. By contrast, the administration of Zn turned out to suppress the activity of prolyl-hydroxylase, which acts as an enzyme for collagen synthesis. [59] (**Figure 1**).

Several lines of novel evidence on the beneficial effects of Zn compounds have been provided by experimental animal models of hepatic fibrosis. The administration of zinc sulfate significantly inhibited hepatic fibrosis induced by bile duct ligation through the selective alleviation of M1 macrophages [60]. Surprisingly, the polarization of M1 macrophages was correlated with the Notch 1 signaling, which might participate in the process of hepatic fibrosis [61]. Another study elucidated the inhibitory effect of Zn on hepatic fibrosis induced by bile duct ligation through the enhancement of MMP-13 synthesis [62]. Supplementation with polaprezinc improved hepatic fibrosis through the attenuation of tissue inhibitor of metalloproteinase-1 (TIMP-1) in an experimental model of nonalcoholic steatohepatitis (NASH) [63] and in a thioaccetamide-induced model of hepatic fibrosis [64]. Polaprezinc also inhibited the synthesis of fibrotic markers such as collagen I, fibronectin and α-smooth muscle actin (α-SMA) in human hepatic stellate cells via the attenuation of human hepatic stellate cells' proliferation and migration [65]. In addition, the anti-fibrotic action of supplementation with zinc oxide nanoparticles via the alleviation of oxidative stress was confirmed in

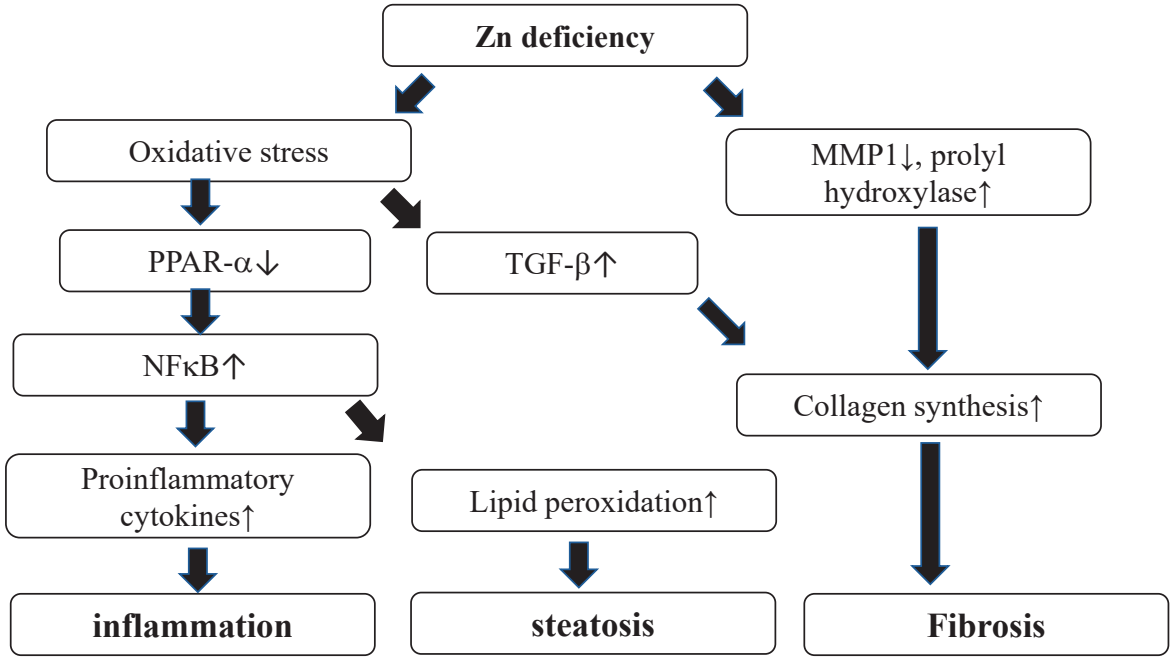

Figure 1. | Putative mechanisms by which Zn deficiency causes liver damage in patients with CLDs.

a thioacetamide-induced hepatic fibrosis model [66].

There have been a few human studies to investigate the effect of Zn supplementation on hepatic fibrosis. The administration of polaprezinc resulted in the suppression of serum TIMP-1 concentrations in patients with liver cirrhosis with Zn elevation [68]. Likewise, serum procollagen type III and type IV collagen-7S levels were significantly decreased by treatment with polaprezinc in autoimmune hepatitis patients with Zn elevation [68]. It was of interest that additional zinc sulfate administration significantly improved hepatic fibrosis in patients with HCV-related CLD who possessed interleukin28 (IL28) rs12979860 polymorphism CC genotype and received a DAA treatment [69] (**Table 1**).

4. Verification of Zn supplementation on hepatic steatosis in CLDs

Hepatic steatosis is characterized by an abundance of lipid droplets in hepatocytes. Zn participates in the activation of peroxisome proliferator-activated receptor-α (PPAR-α), a regulator of lipid homeostasis [70]. Zn deficiency thus results in the attenuation of PPAR-α activity, and subsequently promotes lipid peroxidation, finally leading to the exacerbation of hepatic steatosis (**Figure 1**). Indeed, dietary Zn deficiency exacerbated ethanol-induced hepatic steatosis in mice [71]. In human studies, serum Zn levels were gradually reduced in HCV-related CLD patients as the grade of hepatic steatosis, which was one of the common histological features in those patients [72], became more progressive from mild to severe [73,74]. Moreover, we elucidated the close correlation between insulin resistance and the grade of hepatic steatosis in such patients. Surprisingly, no correlation between serum Zn levels and the grade of hepatic steatosis in patients with NAFLD was found [42]. However, these studies were conducted with small sample sizes. A large-scaled multi-centered trial should be performed to verify the relationship between serum Zn levels and grades of hepatic steatosis in patients with HCV-related CLD and those with NAFLD.

The favorable effects of Zn compounds on hepatic steatosis were exhibited in several animal studies. Alcohol-induced steatosis was reversed by the administration of zinc sulfate in mice via activated PPAR-α and hepatocyte nuclear factor-4α [75]. Interestingly, the activation of PPAR-α induced by zinc sulfate supplementation also caused lipophagy [76]. Xu et al. provided the data that Zn supplementation resulted in the alleviation of hepatic steatosis in lipid disturbance rabbits, accompanied by decreased triglyceride (TG) and increased high-density lipoprotein cholesterol (HDL-C) levels [77]. Type 2 diabetes mellitus (T2DM)-induced hepatic

steatosis in mice was also improved by the treatment with zinc sulfate through the promotion of nuclear factor-erythroid 2-related factor 2 (Nrf2)-metallothionein pathway [78]. The administration of zinc sulfate [79-82], zinc glycine [83], or zinc oxide nanoparticles [84] reversed hepatic steatosis in both *in vitro* and *in vivo* experimental animal models of NAFLD. However, polaprezinc did not alter the grade of hepatic steatosis in a mouse model of NASH [63].

Unfortunately, there have been few studies that have verified the efficacy of Zn compounds on hepatic steatosis in human clinical trials. Fathi et al. revealed that the administration of zinc gluconate (30mg of Zn) did not affect the grade of hepatic steatosis after 12 weeks in overweight/obese patients with NAFLD who adhered to a calorie-restricted diet [46] (**Table 1**). Instead, the authors confirmed that insulin resistance was improved by the same treatment in those patients [85]. When the evidence that Zn deficiency resulted in the exacerbation of hepatic steatosis in patients with NAFLD is provided, Zn supplementation may be promising for the attenuation of hepatic steatosis in such patients. In the near future, the optimal dose of Zn supplementation, the types of Zn compounds and the duration of Zn supplementation in those patients are urgent matters for researchers to address.

5. Verification of Zn supplementation on impaired glucose metabolism in CLDs

It is fully established that Zn is indispensable for the synthesis, storage, and release of insulin, indicating that Zn plays crucial roles in glucose metabolism [86]. In addition, Zn has been considered to show insulin-like action by activating the Akt/PKB signaling pathway [87]. Accordingly, Zn deficiency induces insulin resistance and ultimately leads to the occurrence of T2DM. Zn is stored in the endoplasmic reticulum (ER) of cells, and it is essential for maintaining the homeostatic function of this organelle. Hence, Zn depletion results in the activation of ER-localized chaperon BiP, and the impairment of protein folding in the ER, known as ER stress [88]. This response is closely linked to the different processes involved in the development of insulin resistance and T2DM [89]. The downregulation of hepatic MT expression induced by Zn deficiency has been proposed as the putative mechanism by which Zn depletion initiates ER stress [90]. A recent study suggested that impairment of the Zn transporter, ZIP7 might be responsible for insulin resistance in patients with T2DM [91].

We previously documented that serum Zn concentrations were inversely correlated with the homeostasis model for the assessment of insulin resistance (HOMA-IR) values, the hallmark for insulin resistance in patients with HCV-related CLD, indicating that insulin resistance may derived from Zn deficiency in those patients [54,73]. The inverse correlation was also confirmed in NAFLD patients [55]. In addition, our study elucidated an inverse correlation between the serum Zn level and the insulin-like growth factor (IGF-1)/IGF-binding protein-3 (IGFBP-3) ratio, which is a surrogate for circulating free IGF-1 level in patients with HCV-related CLD [92] (**Figure 2**).

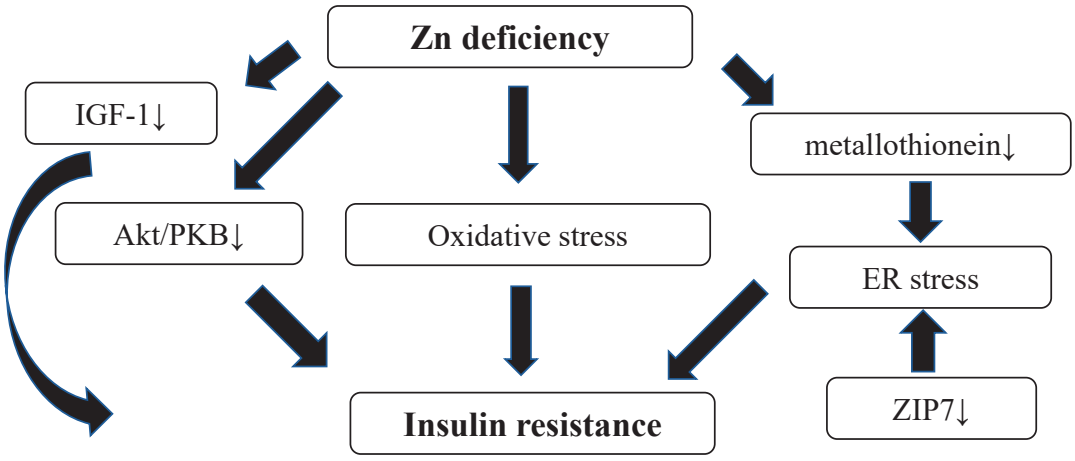

Figure 2. | Putative mechanisms by which Zn deficiency evokes insulin resistance in patients with CLDs.

The beneficial effects of Zn on impaired glucose tolerance have been revealed in several animal studies. Co-supplementation of zinc sulfate and sodium selenite resulted in the improvement of HOMA-IR values in a rat model of NAFLD [79]. In another rat model of NAFLD, zinc sulfate administration and strength exercise increased insulin signaling activity and improved hepatic steatosis [82]. In contrast, Yu et al. documented that zinc sulfate did not affect glucose metabolism, although it significantly alleviated hepatic steatosis in diabetic mice [78]. In a human study, the administration of zinc gluconate (30mg of Zn) for 12 weeks resulted in the improvement of insulin resistance in overweight/obese patients with NAFLD [85] (**Table 2**).

6. Verification of Zn supplementation on impaired lipid metabolism in CLDs

Numerous studies have demonstrated that Zn is involved in lipid metabolism [93]. Indeed, Zn deficiency exacerbates hepatic lipid metabolism. A Zn-deficient diet significantly elevated plasma cholesterol and triglyceride (TG) levels in low-density lipoprotein (LDL)-receptor-deficient mice by way of increases in very low-density lipoprotein (VLDL) and high-density lipoprotein (HDL) fractions [94]. Marginal Zn deficiency reduced plasma HDL-C and altered fatty acid profiles in healthy men [95]. Zn deficiency resulted in dysregulation of plasma concentrations of ω3 fatty acids concentration in HCV-related patients with heavy drinking habits [96].

Favorable outcomes of Zn supplementation on impaired lipid metabolism have been revealed in animal studies and *in vitro* studies. High-dose Zn potentially initiated the upregulation of stearoyl-CoA desaturase-1 (SCD-1) expression in hepatocytes and thereby facilitated fatty acid synthesis [97]. In an animal study of NAFLD, co-administration of zinc sulfate and sodium selenite improved the LDL-C/HDL-C ratio and the TG/HDL-C ratio [79]. Zn supplementation caused a decrease in serum TG levels and an increase in serum HDL-C levels in a rabbit model of NAFLD, although it did not alter the serum LDL-C level in the animals [77]. In contrast, few clinical studies investigating the efficacy of Zn treatment on impaired lipid metabolism have been conducted. The administration of zinc gluconate (30mg of Zn) for 12 weeks led to the improvement of serum LDL-C and TG levels in overweight/obese patients with NAFLD [85] (**Table 2**).

Table 2. | Verification of zinc supplementation on metabolic abnormalities in patients with CLDs

Reference	Study design	Assigned paients	Formulation	Dosage and duration	Outcomes
Impaired glucose and lipid metabolism					
Fathi et al. [85]	RCT (Zn+calorie-restriction vs. calorie-restriction)	NAFLD (n=29)	zinc gluconate	220mg, 12 weeks	decrease in HOMA-IR value decrease in serum LDL-C and TG levels decrease in HbA1c
Hyperammonemia					
Hayashi et al. [105]	double blind RCT (Zn+BCAA vs. BCAA)	liver cirrhosis (n=19)	zinc sulfate	200mg or 600mg, 5-6 months	decrease in blood ammonia level no effect on serum Alb level
Takuma et al. [106]	RCT (Zn+standard treatment vs. standard treatment)	liver cirrhosis (n=39)	polaprezinc	225mg, 6 months	decreased in blood ammonia level improvement of hepatic encepalopathy grade increase in serum Alb level
Mousa et al. [107]	double blind RCT (Zn+vitamin A,C,E +lactulose vs. lactulose)	liver cirrhosis (n=31)	zinc gluconate	175mg, 3 months	decrease in blood ammonia level improvement of minimal hepatic encepalopathy decreased in serum ALT level
Ozeki et al. [108]	retrospective study (end of point vs. base line)	liver cirrhosis (n=60)	zinc acetate	200mg, 3 months	decrease in blood ammonia level
Katayama et al. [109]	double blind RCT (Zn vs. placebo)	liver cirrhosis (n=7)	zinc acetate	150mg, 3 months	decrease in blood ammonia level (not significant)
Horiguchi et al. [110]	retrospective study (end of point vs. base line)	liver cirrhosis (n=12)	zinc acetate	50mg, 3 months	no effect on blood ammonia level no effect on sarcopenia

HbA1c; hemoglobin A1c, Alb; albumin

7. Verification of Zn supplementation on hyperammonemia

Hepatic encephalopathy is a serious neuropsychiatric complication of liver disease which derives from fulminant hepatitis or decompensated liver cirrhosis [98]. The accumulation of ammonia is considered as a cause of the disease. Organs which largely participate in ammonia metabolism are the liver and the muscle. In the liver, ornithine transcarbamylase converts ammonia to urea [99]. On the other hand, glutamine synthetase metabolizes ammonia to glutamic acid in the muscle [100]. These enzymes require Zn as a cofactor for the ammonia metabolism. Therefore, Zn deficiency results in a marked increase in blood ammonia level, leading to hepatic encephalopathy [101].

Riggio et al. first confirmed that Zn supplementation reduced plasma ammonia level in cirrhosis rats [102]. Several human studies elucidated the efficacy of Zn supplementation on hepatic encephalopathy [103,104]. Additional administration of zinc sulfate [105], polaprezinc [106], or zinc gluconate [107] to the standard treatment for hepatic encephalopathy showed more favorable effects than the standard treatment alone. The administration of zinc acetate also reduced the blood ammonia level in patients with liver cirrhosis [108]. However, some studies did not confirm the effect of Zn supplementation on the improvement of blood ammonia levels in cirrhotic patients [109,110] (**Table 2**).

8. Verification of Zn supplementation on sarcopenia in CLDs

Sarcopenia is well defined as skeletal muscle volume loss and low muscle strength [111], and it is frequently observed in patients with liver cirrhosis [112]. Zn deficiency was also associated with sarcopenia in patients with CLDs [113,114]. Sarcopenia in such patients might be caused by an indirect rather than a direct effect of Zn deficiency. Zn supplementation is recommended as one of the nutritional interventions in CLD patients with sarcopenia [18]. Unfortunately, no evidence that Zn supplementation reverses skeletal muscle volume in patients with CLD has been provided. The involvement of Zn deficiency in sarcopenia is conflicting [110]. Further examinations should be performed to clarify the effect of Zn on sarcopenia in those patients.

Branched-chain amino acids (BCAAs) are also recognized as a candidate for the treatment of sarcopenia in cirrhotic patients. It is of interest that the BCAA supplementation resulted in the elevation of serum Zn levels as well as the increase in muscle mass volumes in such patients [115]. As one of the reasons for Zn elevation by treatment with BCAA, the authors speculated that the BCAA contained sachet contained 52mg of Zn. Another reason might be attributed to an increase in serum albumin level by BCAA supplementation. The combined treatment of Zn with BCAA will be promising in cirrhotic patients with sarcopenia [116], because these agents are effective to decrease plasma ammonia levels which induce the synthesis of myostatin in patients with hepatic encephalopathy [117].

It is of noteworthy that deletion of the Zn transporter, Zip14, caused the impaired function of intestinal barrier and the subsequent leakage of endotoxin into the systemic circulation. Endotoxemia induced the activation of transcription factors including NF-κB and Mef2c in the skeletal muscle of Zip14 knockout mice, finally leading to the skeletal muscle wasting [118].

9. Verification of Zn supplementation on gut dysbiosis in CLDs

Gut dysbiosis, which causes a variety of critical complications including endotoxemia and hepatic encephalopathy, was frequently observed in patients with liver cirrhosis. It appears to be responsible for small intestinal bacterial overgrowth and/or an increase in intestinal permeability, known as “leaky gut” [119]. Endotoxinemia due to increased intestinal permeability has been also implicated in the development of NASH [120].

Zn deficiency may be associated with gut dysbiosis in patients with CLDs. Zhong et al. documented that Zn deficiency was involved in the dysfunction of intestinal barrier in a mouse model of alcohol-induced steatohepatitis [121]. Later, the authors revealed that Zn regulated bactericidal activity of Paneth cells in mice of alcohol-induced steatohepatitis and that dietary Zn deficiency caused dysfunction of Paneth cells [122]. Another study revealed that the administration of zinc sulfate preserved intestinal barrier function and improved endotoxinemia in rats of alcohol-induced steatohepatitis [123]. It is of interest that the Zn transporter, ZIP14 is involved in maintenance of intestinal tight junctions [124].

10. Inhibitory effects of Zn supplementation on hepatocarcinogenesis in CLDs

Zn is considered to be involved in antioxidant defense, DNA repair and activation of transcriptional factors for cancer prevention [125]. Therefore, Zn deficiency leads to increased occurrence of malignant diseases. Recent studies revealed that

lower serum Zn concentration might become an indicator for the development of HCC [126,127]. Another study documented that lower serum Zn levels in HCC patients who underwent initial hepatectomy might predict unfavorable prognosis [128]. Similarly, early HCC patients who treated curatively and were Zn deficient showed worse overall survival [129].

Several studies have provided the evidence that Zn supplementation, including zinc sulfate [130] and polaprezinc [65], suppressed the proliferation of HCC *in vitro*. These Zn compounds exerted remarkable anti-tumor effects by inducing cell cycle arrest and apoptosis of HCC cells. In a RCT study, the administration of polaprezinc produced more favorable outcomes in patients with chronic hepatitis C by inhibiting cumulative incidence of HCC [131]. Likewise, a retrospective study revealed that supplementation of zinc sulfate resulted in the maintenance of favorable hepatic reserve and the prevention of HCC development in patients with CLDs [132].

microRNAs (miRNAs), which are small, single stranded non-coding RNAs of 19-25 nucleotide in length, negatively regulate gene expression via translational inhibition or messenger RNA (mRNA) degradation [133]. miRNAs play essential roles in diverse biological processes, including cell differentiation, proliferation, migration, and survival [134]. A recent study identified some miRNAs which promoted the development of esophageal cancer in Zn-deficient rats [135]. miRNAs which regulate the development of CLDs in Zn-deficient state should be also explored.

Conclusion

Recent advances in various types of molecular biological technologies have led to novel evidence that Zn deficiency participates in the metabolic abnormalities as well as the pathogenesis of CLD. Many studies have elucidated the favorable outcomes of Zn supplementation, both in terms of the improvement of metabolic abnormalities and histological recovery in the liver, using experimental animal model of CLDs. However, the beneficial therapeutic effects of Zn supplementation on humans have not been fully established in clinical trials. The evidence levels in the human studies on the efficacy of Zn supplementation remain low. The dosage and duration of Zn administration, and the type of Zn compound administration should be investigated in patients with CLD in order to optimize the potential effects of Zn supplementation. Further prospective multicenter cohort studies should be conducted to verify the usefulness of Zn supplementation in CLD patients.

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