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OBM Hepatology and Gastroenterology

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Review

Zinc in Liver Fibrosis

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Academic Editor: Matthias Bartneck

Special Issue: Exploring Novel Treatment Options for Liver Fibrosis: Can Anti-Angiogenics Succeed ?

OBM Hepatology and Gastroenterology
2019, volume 3, issue 2
doi:10.21926/obm.hg.1902023

Received: March 15, 2019
Accepted: June 05, 2019
Published: June 13, 2019

Abstract

Acute and in particular chronic liver disease of viral, alcoholic and non-alcoholic genesis is a large, often unnoticed health hazard around the world. It can lead to cirrhosis and hepatocellular carcinoma (HCC) during the course of decades. Liver fibrosis, conversion of functional parenchyma to connective tissue (scar tissue) as a consequence of chronic liver damage, is a connecting pathogenic process in all chronic liver diseases. Zinc is an essential micronutrient in human health, playing a fundamental role in cellular metabolism, acting mostly through binding in a wide range of proteins and thus affecting a broad spectrum of biological processes. Thus, the liver is essential for zinc homeostasis of the human body. Zinc deficiency leads to the impairment of many hepatic functions. Liver diseases can alter zinc levels and, in turn, may be influenced by zinc deficiency. In spite of the vast increase in knowledge about the fibrotic wound healing process on both cellular and molecular levels, apoptotic signaling, epigenetic phenomena, and usage of stem cells, there is no effective anti-fibrotic medicine so far for use in humans. The diverse hepatoprotective effects of zinc documented in many experimental studies and initial clinical pilot projects should be reason enough to include zinc as a component in future studies on liver fibrosis, especially non-

alcoholic fatty liver hepatitis and alcoholic liver hepatitis. This review will address the evidence regarding the use of zinc in the treatment of liver fibrosis.

Keywords
Liver fibrosis; zinc; treatment

1. Introduction

Acute and in particular chronic liver disease of viral, alcoholic and non-alcoholic genesis is a large, often unnoticed health hazard around the world. It can lead to cirrhosis and hepatocellular carcinoma (HCC) during the course of decades. In the future, the main issue after management of hepatitis C to the greatest extent, or even its eradication, and the containment of hepatitis B, will be non-alcoholic fatty liver disease (NAFLD) and non-alcoholic fatty liver hepatitis (NASH) as a consequence in addition to alcoholic liver disease (ALD). The main causes are mostly the epidemic increase of "metabolic diseases of affluence", in particular obesity, type 2 diabetes mellitus and disorders of fat metabolism in industrialized states. These factors trigger the formation of fatty liver. Liver fibrosis, conversion of functional parenchyma to connective tissue (scar tissue) as a consequence of chronic liver damage, is a connecting pathogenic process in all chronic liver diseases [1, 2].

Zinc is an essential trace element for the human organism with a wide range of biological functions. It is of fundamental relevance for many cellular, molecular, metabolic, and immunological processes, including anti-oxidative, anti-inflammatory, and anti-apoptotic responses [3].

Chronic liver diseases coincide with reduced zinc values in the serum or plasma, independently of genesis. The exact definition of what constitutes a zinc deficiency remains problematic. More than 97% of the body`s zinc is bound to proteins and thus inaccessible to conventional laboratory methods. Still, determination of the zinc concentration in serum or plasma is a reliable and a reproducible parameter in routine clinical practice [3, 4]. The scope of zinc deficiency more so depends on the severity of the liver damage, fibrosis or cirrhosis, with or without decompensation or complication, and less so on genesis [4].

Here, we present essential aspects of liver fibrosis and the functions and effects of zinc, under special consideration of the liver as the main organ of zinc metabolism. The aim of the following statements is to determine approaches for using zinc in treatment of liver fibrosis, in combination with other active substances within the scope of adjuvant treatment, based on literature research on hepatoprotective effects of this trace element.

2. Liver Fibrosis

Chronic liver diseases are marked by a protracted wound healing reaction with progressive scarring (fibrosing), sometimes leading to complete destruction of the organ structure through scar tissue (cirrhosis). This process involves not only chronic inflammation, but also activation of the hepatic stellate cells (HSC) with subsequent angiogenesis (formation of new vessels) and

parenchyma loss through occluded vessels [1, 5-7]. There are severe changes to the hepatic microcirculation, characterized by a sinusoidal reshaping (depositing of extracellular matrix of proliferating activated HSC, resulting in a capillarization of the sinusoids), formation of intrahepatic shunts (through formation of new vessels and the loss of parenchyma cells, as well as hepatic endothelial dysfunction) [8]. The endothelial dysfunction is characterized by an insufficient release of vasodilators, of which nitric oxide is the most important [5].

There are a variety of causes that trigger chronic liver damage: Hepatitis viruses (mostly HBV and HCV), alcohol and other hazardous substances, cholestasis, insulin resistance leading to liver fattening and NASH, and genetic disposition (among other things, haemochromatosis). Quick progression of fibrosis to cirrhosis is often marked by the presence of several causes (Hits) such as chronic hepatitis C (Hit 1) and alcoholic liver damage or NASH (Hit 2). The typical sequence of progression from chronic liver disease to chronic inflammation to fibrosis and cirrhosis is characterized by a complex interaction of various cell types and soluble factors [6]. Sustained liver damage leads to the release of danger signals (Pathogen/Danger-Associated Molecular Patterns, PAMPs/DAMPs) from hepatocytes and non-parenchymatous liver cells (stellar cells, Kupffer cells, vascular endothelial cells). PAMPs and DAMPs are recognized by immune cells that activate inflammatory signaling pathways and release soluble mediators of the inflammation reaction (cytokines, chemokines) [1, 6, 7]. As a consequence, there is a massive recruiting of inflammation cells from the blood (monocytes, granulocytes, and lymphocytes, among others). In particular liver cells (Kupffer cells) and infiltrating immune cells (mostly macrophages) generate a highly inflammatory and fibrinogenic environment that leads to activation of resting HSC and their transformation into myofibroblasts. These myofibroblasts are mainly matrix producing cells [1, 2, 9]. They also have immunomodulatory properties. Other cells may also transdifferentiate into fibrogenic myofibroblasts. This includes stem cells from the bone marrow, portal fibroblasts, hepatocytes, and cholangiocytes [7]. Collagens are the main components of the extracellular matrix in fibrosis. Beyond this, the matrix also contains many other mediators that are used as indicators or therapeutic targets in liver fibrosis [1]. Collagen I is the main fibrin of the extracellular matrix, the synthesis of which increases fibrosis and the degradation of which reduces it [10]. Reduction of the extracellular matrix depends on the activity of matrix metalloproteinases (MMPs) [11]. The MMPs, divided into 5 categories, are regulated by the tissue inhibitors of metalloproteinase (TIMs). The imbalance between the activities of MMPs and TIMPs is the decisive factor for deposition of collagen [10]. Transforming growth factor ß (TGF-ß) is a profibrotic cytokine that can stimulate cellular synthesis and deposition of components of the extracellular matrix such as collagen types I, II, IV, elastin, tenascin, osteonectin, biglucan, and proteoglucan core protein polysaccharide [10]. HSC are the main origin of TGF-ß, but macrophages, liver cells, and thrombocytes can secrete this cytokine, too.

Increased interest in the pathogenesis of liver fibrosis concerns hemostasis with interferences of hepatic microcirculation, coagulation, and in particular, thrombin formation. An uncontrolled activation of the coagulation cascade influences fibrogenesis by affecting the different cell types and biochemical processes and triggering increased thrombin formation [9]. In the liver, it particularly affects the hepatic stellar cells (HSC) and the sinusoidal cells [9]. The term "immunothrombosis", coined by Engelmann et al. [12], defines thrombosis as an uncontrolled, changed immune response to tissue damage. This means that tissue damage caused by exogenous noxae causes immune reactions connected to impairment of the coagulation system and in

particular also the thrombocytes [12, 13]. Newer findings [14, 15] support and expand earlier examinations, according to which a restriction of the clearance function of the reticuloendothelial system (RES) of the liver that is mostly represented by the Kupffer cells, causes intestinal bacteria and their products, e.g. endotoxins, to enter the systemic circulation in the course of chronic liver disease. In this way, endotoxins and other bacterial products influence the pathogenesis of chronic liver diseases [16, 17, 18]. In chronic liver disease, it should be considered that the intestinal venous blood can bypass the liver RES due to shunt formation so that the antigen-clearance is impaired independently of the current functional status of the RES.

In the last decade, several genetic variations were identified to be associated with an increased risk for the development of liver fattening [19]. This includes polymorphisms in the genes PNPLA3, TM6SF2, and MBOAT7 [19]. The proteins coordinated by these genes serve, among other functions, an important role in liver fat metabolism. Genetically caused loss of function leads to an accumulation of certain lipids in hepatocytes. Additionally, the formation of extracellular connective tissue structure is induced [19]. This damages the cell and promotes the progression of disease into fibrosis and finally cirrhosis.

In spite of the vast increase in knowledge about the fibrotic wound healing process on both cellular and molecular levels, apoptotic signalling, epigenetic phenomena, and usage of stem cells, there is no effective anti-fibrotic medicine so far for use in humans [7, 20].

This is not in the contradiction to the successful regression of cirrhosis in an increasing number of patients, in which the underlying liver disease, in particular hepatitis C, but also B, can be treated effectively [21].

3. Functions of Zinc

Zinc is an essential micronutrient in human health and plays a fundamental role in cellular metabolism, acting mostly through binding in a wide range of proteins and thus affecting a broad spectrum of biological processes [22]. By influencing the molecular functions of many proteins in cell metabolism and signal transduction, zinc is involved in proliferation, differentiation, and apoptosis of cells with profound implications for healthy growth, renewal, and repair of cells [23]. It is an essential component of more than 300 different enzymes and owes its catalytic effects to its direct involvement in substrate conversion and the stabilization of enzyme structure. Zinc exerts structural effects on several transcription factors and regulates hormones, hormone receptors, and gene expression [4]. It is an enzymatic cofactor in the regulation of carbohydrate, fat, and protein metabolism.

Zinc plays an important role as a second messenger, as a signalling ion, and affects the redox metabolism. Though zinc is part of the copper-zinc superoxide dismutase, zinc can also alter oxidative stress. Cell-damaging oxidative stress as a result of zinc deficiency is a fundamental principle [24].

There are very close relationships between zinc deficits and oxidative cellular stress. Cellular stress (impaired biological processes and sequences in the cell) causes zinc loss that may lead to a cellular zinc deficit which then increases not only oxidative, but also endoplasmic, stress, resulting in a vicious cycle [24].

Although the Zn^2 ion is redox inert, it has important anti-oxidative properties [25]. Zinc-finger structures act as redox-sensitive molecular switches controlling several crucial cellular processes

[26]. Kröncke et al. [26] hypothesized that almost all inflammatory reactions accompanied by oxidative or nitrosative stress or both and resulting in a shift of the intracellular redox balance to a more oxidative state will lead to a cellular dyshomeostasis with increased intracellular concentrations of non-protein-bound Zn^2.

Moreover, zinc competes with iron and copper in the cell membrane, inhibits the NADPH-oxidase enzyme, and reduces chronic inflammation and hyperglycemia [27, 28, 29]. Therefore, maintaining adequate concentrations of zinc in cell compartments is essential for proper functioning of the anti-oxidant system [27]. Zinc is bound to metallothionein (MT), an acute-phase protein, under normal physiological conditions. Under oxidative stress conditions, the micronutrient is released from its complex with MT and redistributed in the cell to serve anti-oxidant actions [30]. While it is important that zinc also attenuates oxidative stress by acting as an anti-inflammatory nutrient, the precise mechanism remains unclear [27].

Moreover, zinc is important in the metabolism of neurotransmitters and growth, sex, and thyroid hormones, as well as the synthesis, secretion, and storage of insulin in the Langerhans cells of the pancreas [31]. In addition, zinc has extensive roles in both the adaptive (specific) and the innate (non-specific) immune responses at multiple steps, including gene expression as well as differentiation and development of immune cells [3, 32]. It has been suggested that zinc is involved in nutritional immunity, acts as a hepatoprotective agent or a differentiation signal for innate immune cells, or supports the synthesis of acute-phase proteins [32].

According to the World Health Organization (WHO), an estimated 30% of the world's population is zinc deficient, and inadequate intake contributes to 800,000 deaths worldwide [33]. Although severe zinc deficiency is rare, mild to moderate deficiency is prevalent throughout the world [33].

Zinc homeostasis following dietary intake is highly regulated, with body stores and tissue concentrations affected by intestinal absorption, gastrointestinal and urinary losses, and cellular retention. Dietary zinc is absorbed through enterocytes predominantly in the proximal small intestine. After enteric absorption, zinc enters the small plasma pool (0.1% of total body zinc), which is rapidly turning-over. The total amount of zinc in the body comprises 2 - 4g with a plasma concentration of 12 - 16 µmol. The plasma zinc pool is small but mobile and thus important for distribution of zinc in the body. Zinc distribution within the body is widespread, with the highest amounts found in muscles, bones, the liver and skin corresponding of whole body zinc. There is a steady state of the intake and excretion [34, 35].

Intracellular zinc homeostasis is subject to control by numerous proteins. The existence of such control underscores the crucial importance of zinc [36]. MTs and zinc transporters are the main components in this process. MTs are important for the storage and re-distribution of zinc. A distinct group of zinc transporters facilitates the influx (ZIP, SLC39A family) and the efflux (ZnT, SLC30A family) of zinc through cell membranes [37]. Both transporter types have a specific tissue expression with different tasks in the regulation of nutritionally-induced zinc deficiency or oversupply and in the regulation of physiological stimuli, such as hormones or cytokines. Moreover, zinc transporters coordinate the subcellular, cellular, and organelle-based translocation of zinc ions [38].

4. Zinc Metabolism in the Liver

As shown in Figure 1, it exist important interactions between the zinc status of the human body and the liver. Thus, the liver is essential for zinc homeostasis of the human body. Zinc deficiency leads to the impairment of many hepatic functions. Liver diseases can alter zinc levels and in turn may be influenced by zinc deficiency [4, 39].

Zinc release from hepatocytes is differentially regulated. Turnover studies using 65Zn showed that the exchange of zinc in hepatocytes occurs in less than two days [40]. The liver represents a fast exchangeable zinc pool with an important role in the metabolism of zinc and other trace elements [41]. The regulatory processes depend almost completely on hormonal control by insulin, glucagon, and the glucocorticoids [42]. Depending on the metabolic situation, these substrates trigger a transient dysregulation of zinc metabolism with subsequent plasma zinc deficiency. Stress or several mediators, like pro-inflammatory cytokines or lipopolysaccharides, can induce similar effects.

Changes in zinc status directly influence gene expression. Zinc deficiency affects different hepatic functions and, because of the liver's central role in metabolism, especially carbohydrate, lipid, and protein metabolism, this impacts metabolic processes in other organs [43-46].

There is a tight interaction between MT and zinc. MT, a class of small and cysteine-rich proteins, participates in the detoxification of heavy metals and the absorption, distribution, and intracellular accumulation of zinc and other trace elements [42]. In addition, an increased intake of zinc triggers an increase in MT synthesis [4]. Close interactions have been identified between zinc and interleukin-6 (IL-6), a pro-inflammatory cytokine regulating the acute-phase genes. The effects of zinc in the hepatic synthesis of acute-phase proteins, in the regulation of gluconeogenesis, and in the control of reactive substances (e.g. nitric oxide) or hydrophilic radicals, as well as the control of microbial growth, are all functions of IL-6. It regulates the zinc transporter ZIP 14 in the liver, thus contributing to hypozincemia during the acute-phase reaction [4, 47].

At the onset of systemic injury and infection the acute phase response is activated, leading to the mobilization of zinc from the blood compartment into tissues and cells. The transport of zinc into the cells is facilitated by the zinc transporters ZIP 8 and ZIP 14, the expression of which is induced by cytokines. These alterations in zinc metabolism are critical in guiding the initial host response that influences both innate and adaptive immune function. If the host is zinc-sufficient, the chance of recovery is improved, whereas zinc deficiency increases morbidity and mortality [48]. The cellular consequences of zinc deficiency are manifested through oxidant stress, modulation of inflammation, and in extreme situations, premature cell death [48].

The scope of zinc deficiency is not determined as much by its genesis (alcohol, viruses, etc.) but rather by the severity of liver damage, fibrosis or cirrhosis, with or without metabolic or portal decompensation and/or the presence of hepatocellular carcinoma (HCC) [43]. Zinc deficiency or an altered zinc metabolism in patients with liver diseases is caused by a variety of factors, such as inadequate intake, changes in protein and amino acid metabolism, diminished hepatic extraction, portosystemic shunts, alcohol induced impaired absorption, effects of cytokines (mainly IL-6), and the catabolism associated with malnutrition [43]. Zinc deficiency can result in a spectrum of clinical manifestations, such as poor appetite, loss of body hair, altered smell and taste, testicular atrophy, cerebral dysfunction, and diminished drug elimination capacity, which are also common symptoms in patients with chronic liver diseases [4].

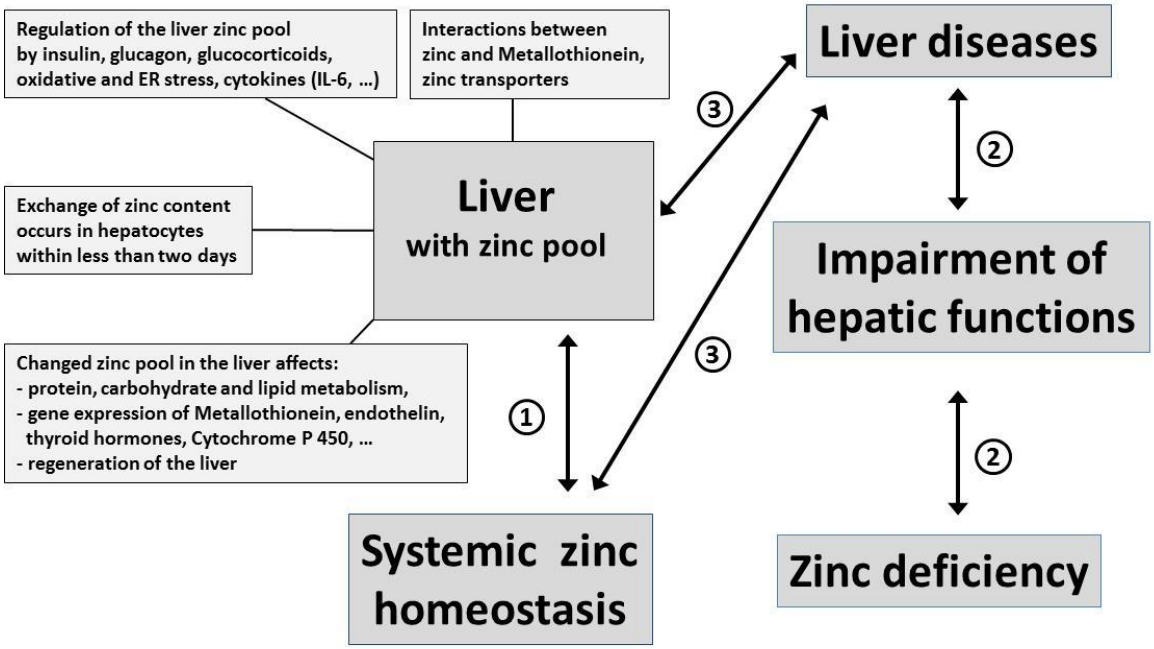

Figure 1 Schematic depiction of interactions between systemic zinc homeostasis and the liver and the possible influence of zinc deficiency and/or liver diseases. The arrows indicate interactions between (1) the essential zinc pool of the liver and the systemic zinc homeostasis, (2) zinc deficiency and hepatic functions as well as liver diseases, and (3) liver diseases and both systemic zinc homeostasis and the liver zinc pool.

5. Hepatoprotective Effects of Zinc and Its Possible Role in Liver Fibrosis

As depicted in Table 1, the hepatoprotective effects of zinc are shown against various chemical substances such as carbon tetrachloride (CCl4), thioacetamide (TAA), bromobenzene, acetaminophen, and various metals [49, 50, 51]. The first promising success pointing to the possible usage of zinc as an anti-fibrotic drug was reported by Antinnen et al. [52]. In experimental studies, the authors were able to limit the scope of CCl4-induced fibrosis of the liver by administration of zinc due to the zinc-dependent inhibition of the iron-dependent proline hydroxylase. In the TAA rat model, Müller et al. [53] found an anti-inflammatory effect of zinc, a clear reduction of hepatic collagen that even surpassed the effect of colchicine in this animal model. Other authors examined the effect of zinc on liver damage induced by galactosamine and found inhibition of lipid peroxidation and an increase of protein synthesis [54]. Gimenez et al. [55, 56] described inhibition of fibrosis in early stages and reduction of fibrotic activity in later stages of liver damage induced by CCl4 in rats. They found a delay of the fibrotic process and thus cirrhosis development and reduction of lipid peroxidation by oral administration of zinc in CCl4-induced liver cirrhosis in rats. The beneficial effects were connected to inhibition of the hepatic expression and activity of cytochrome P450 and a stimulation of MT.

Since the turn of the millennium, increased research efforts on zinc have contributed to a number of noticeable new findings concerning the hepatoprotective properties of this trace element. Souzo et al. [57] found that zinc protects HSC from the cytotoxic damage of cadmium. This is on the one hand explained by its membrane-stabilizing effects and on the other hand by

preservation of the intracellular redox balance. Similarly, Zhou et al. [58] and Sakaguchi et al. [59] explained the protective effect of zinc against thermal damage or endotoxins (ie against oxidative stress), as anti-oxidative properties are displayed when interacting with MT. In contrast to these findings, Krones et al. [60] were unable to document a protective effect of zinc in experimentally triggered acute endotoxemia. Instead, in simultaneous infusion with lipopolysaccharides (LPS), it led to further deterioration of the situation through increased synthesis of pro-inflammatory cytokines, in particular TNF-alpha and IL-6. For this reason, the authors advise against prophylactic zinc administration in the early stage of sepsis. In another study on mice, the early administration of zinc led to an increased intracellular availability of zinc in liver cells, which was connected to a reduction of the accumulation of superoxide and necrotic cell death in the liver after injection of LPS [61]. In the course of sepsis, zinc is redistributed from the serum. Several studies discuss the correlation between zinc and the consequences of sepsis [32]. Jiang and Kang [62] demonstrated in a mouse model of a liver fibrosis triggered with CCl4 that fibrosis was reversed by induction of MT genes, with higher MT levels after discontinuation of CCl4 administration being associated with higher reversibility. The increase of MT led to an increase of the activity of collagenases in the liver. As shown already, MT plays an important role in the regulation of cellular zinc homeostasis and can be induced by oxidative stress. It also plays an important role when protecting against chemically induced liver damage [56, 63].

Examinations by Liang et al. [64], on a diabetes mellitus 1 mouse model characterized by significant liver damage including mild inflammation, lipid accumulation, oxidative damage and partial cell death, as well as an increase of ALAT, appear of special current interest for the effect of a 3-month zinc treatment or supplementation. Diabetic liver damage was reversed almost entirely. The mice showed a significant increase of MT in the liver, a significant reduction of ER stress and oxidative stress, inflammation, and steatosis. These results show that a zinc deficit increases diabetes-induced liver damage, while also indirectly confirming the critical role of stress in inducing hepatocellular cell destruction. The authors see decisive factors for this occurrence in improvement of zinc status and activation of MT synthesis.

Several studies showed that zinc regulates nuclear factor kappa (NF-κB) transcription influencing the anti-inflammatory protein A20 and the receptor signalling pathway activated by peroxisome proliferator-alpha (PPAR-α) [65]. The NF-κB signalling pathway has particular relevance to several liver diseases including viral hepatitis, liver fibrosis, cirrhosis, and HCC [66]. There are many regulators involved in the inflammation-fibrosis-cancer process, and the NF-κB signalling pathway appears to have a central function in liver homeostasis, pathophysiology, and regulation of the inflammation-fibrosis-cancer axis [66]. Furthermore, the NF-κB signalling pathway is a potential target for development of hepatoprotective agents. Several drugs including selective estrogen receptor modulators (SERMs), anti-oxidants, proteasome inhibitors, and nucleic acid-based decoys have been shown to interfere with NF-κB activity at different levels and may be useful for the treatment of liver diseases. NF-κB also plays an important hepatoprotective function that needs to be taken into consideration during development of new therapeutic regimes [66].

Table 1 Hepatoprotective effects of zinc.

• Stabilization of cell membranes and intestinal permeability	[57, 59, 78]
• Induction of metallothionein synthesis	[30, 56, 62-64]
• Diminishing the activity of cytochrome P450	[44, 80]
• Improvement of protein synthesis in the liver	[43, 64]
• Inhibition of lipid peroxidation	[56, 64]
• Anti-oxidative effects against cellular, mitochondrial and ER stress	[24, 58, 59, 81]
• Inhibition of metalloproteinases (TIMP-1 and other)	[10, 11]
• Influence on both the innate and adaptive immune system	[3, 32, 77, 82]
• Anti-inflammatory effects of higher zinc concentrations	[51, 77, 82]
• Impairment of TGF-ß signalling	[10]

6. Alcoholic Liver Damage (ALD)

Alcohol is consumed around the world and excessive alcohol consumption leads to ALD, the essential clinical symptoms of which are fatty liver, fatty liver hepatitis (ASH), fibrosis, and cirrhosis. ALD is a multifactorial disease that includes pro-inflammatory interactions between the intestinal microbiome and the liver, fatty tissue dysfunctions, genetic polymorphisms, epigenetic changes, ER stress, and various mitochondrial changes [67].

Kang et al. [68] consider fatty liver to be the fundamental metabolic damage in the progression towards alcoholic liver disease. According to these authors, zinc deficit is the most frequent biochemical and nutrition-specific marker in manifestation of chronic liver disease. The authors examined the effects of zinc supplementation in mice with an experimentally generated zinc deficit, in which previously administrated alcohol was connected to an increase of the ß-oxidation of the fatty acids and VLDL secretion.

Based on previous studies by Fischer et al. [69] and Tomita et al. [70] which demonstrated that alcoholic stress is connected to both accumulation of fat in the liver and an increase of triglycerides in fatty tissue, Kang et al. [68] found that dysregulation of lipid homeostasis is involved in the development of alcoholic fatty liver both in the liver and the free fatty tissue. According to the results of this study, zinc administration is connected to reduction of the fatty liver and fat collection in the adipose tissue. Zinc administration causes increase of the hepatic VLDL secretion and normalization of the triglyceride plasma level, through increase of the hepatic fat utilization and export and acceleration of fat deposition and/or inhibition of lipolysis in the fatty tissue. The authors describe the effect of zinc on various "pathways" and a therapeutic efficacy in alcoholic fatty liver. Accordingly, the decisive targets of zinc are in reactivation of the Hepatocyte Nuclear Factor-4α (HNF-4α) and the Peroxisome Proliferators Activated Receptor-alpha (PPAR-alpha), as well as inhibition of oxidative stress.

Alcohol is metabolized in the liver, accelerates the reduction of glutathione, and at the same time increases lipid peroxidation, which damages mitochondria [71, 72, 73] and limits local lymphocyte function [74]. Alcohol has a central position in fibrogenesis, through increased expression of collagen in the course of HSC activation [75]. Additionally, alcohol consumption inhibits the anti-fibrotic activity of natural killer (NK) cells [76], increases intestinal permeability, and, connected to this, the translocation of bacterial products, such as LPS, that are involved in

fibrogenesis. Alcohol consumption induces apoptosis in various tissues, specifically in liver and lymphatic tissue, among others. According to Szuster-Cisielska et al. [77], increased apoptosis in peripheral blood mononuclear cells (PMBC) of cirrhosis patients may be inhibited by administration of zinc. This is associated with a reduction in the number of CD4+lymphocytes. Lambert et al. [78] found that zinc pre-treatment clearly limits the scope of acute alcoholic liver damage. They explain this with zinc inhibition leading to the permeability impairment in the small intestine caused by alcohol. According to Zhou [79], excessive alcohol consumption impairs cellular zinc homeostasis with subsequent zinc depletion. The deactivation of zinc proteins caused by this due to zinc release is, according to the opinion of the authors, an essential molecular factor in the pathogenesis of alcoholic liver damage. In experimental investigations on mice, Zhou et al. [80] were able to document that zinc administration after ingestion of alcohol prevented the reduction of the liver zinc content and acute liver damage. The hepatoprotective effect of zinc is assigned to its anti-oxidative properties. Although it is a multifactorial process, the main effect of zinc is due to its inhibiting influence on cytochrome P450 (CYP2E1) and its anti-oxidative properties. These findings show the therapeutic potential of zinc in prevention and treatment of liver damage of fatty liver hepatitis. According to Kang et al. [81], zinc supplementation reduces alcoholic liver damage by influencing oxidative stress, TNF-α-mediated hepatocyte damage, and the increase of hepatocyte regeneration.

Examinations of Zhao et al. [82] on rodents showed that zinc withdrawal increases the neutrophil infiltration of the hepatocytes, one of the central mechanisms of alcohol-induced hepatitis, via stimulation of IL-8 production.

According to examinations by Sun et al. [83] in rats, alcohol stress leads to a hepatic zinc deficit with subsequent interference with mitochondrial respiratory processes, which finally causes induction of the formation of free radicals during metabolism of fatty acids and acetaldehyde.

7. Non-Alcoholic Fatty Liver Disease (NAFLD)

In the multifactorial genesis of NAFLD with different converging influences of the environment, microbiome, comorbidities, in particular the metabolic syndrome and genetic factors, the close relationship between NAFLD and diabetic metabolic situation are of central importance. There is a vicious cycle. A fatty liver contributes to insulin resistance and therefore to type 2 diabetes mellitus; insulin resistance increases the fatty liver [31, 84]. NASH is defined as fatty liver with a progressive development through inflammation and typical hepatocellular damage, accompanied by pericellular fibrosis that may lead to cirrhosis [2]. The progression from NAFLD to NASH and fibrosis is a complex multifactorial process that involves multiple cellular and molecular signals [85, 86]. Although this has long been suspected, only recently has evidence been shown indicating that the bacterial settlement of the intestine is metabolically controlled and involved in the pathogenesis of chronic liver diseases, in particular ALD and NAFLD. Apart from this, there are close relationships with liver cirrhosis and its complications. For the genesis of metabolic syndrome and therefore NAFLD, increasing interest is also given to the circadian rhythm. The processes and behaviors influenced by daily work, such as eating and sleeping habits, in particular in the industrial world, (i.e. increasing deprivation of sleep), and individual activities interfere with the original circadian life rhythms determined by light and dark, which impairs metabolic processes and also considerably contributes to the development of a metabolic syndrome and

therefore also NAFLD [85]. Many signal pathways in the body, including the main processes of metabolic homeostasis, are synchronized by a circadian hormonally controlled clock. Loss of the original day and night rhythm (activity and recovery) by a considerable increase of the "lit nightly darkness" has changed earning behavior (possibly around the clock). This and many other factors interfere with the daily rhythm of biological processes, which is connected to changes and impairments of the metabolic processes [85].

Further clarification of the role of metabolic, genetic, and other factors in the genesis of NAFLD may lead to subtypes, the course and prognosis of which is more easily foreseeable and permits more effective, customized treatment [2, 87]. According to Finck [84], the key in treatment of NAFLD, and specifically of NASH, is in identifying an ideal target and compound with minimal side effects to improve hyperglycemia.

Initial experimental tests with zinc in treatment of NAFLD brought hopeful results. Sugino et al. [88] reported that administration of Polaprezinc led to reduced fibrosis via reduced inflammation and lipid peroxidation in the early stage of NASH in the mouse model. At a later time, fibrinolysis was accelerated due to inhibition of the hepatic expression of TIMP.

In another experimental study in rats, Sidfar et al. [89] examined the effect of a combined administration of zinc and selenium on the progression of NAFLD. Fatty liver was generated by administration of a high-fat diet. The glucose, lipid profile, liver enzymes, and histology improved under administration of zinc. The authors came to the conclusion, however, that the results from this animal model cannot be directly transferred to humans.

8. Clinical Studies of Liver Fibrosis

Takahashi et al. [90] found improved concentrations of fibrosis markers in the serum in a pilot study on 17 patients with liver cirrhosis or early fibrosis after administration of 150 mg polaprezinc (35 mg zinc) for 24 weeks. At the same time, as the zinc level increased, the serum levels of collagen IV and the activity of the tissue inhibitor of metalloproteinase (TIMP-1) reduced significantly. TIMPs are important factors in hepatic fibrogenesis, not only through inhibition on the matrix degradation, but also through their influence on cell proliferation and apoptosis [90]. Kang et al. [10] described an inhibiting effect of zinc on collagen synthesis due to reduced TGF-ß signalling. Martinez et al. [91] examined, using a cohort study of 487 patients with HIV mono infection or HIV/HCV-coinfection, the influence of zinc plasma concentration on mitochondrial stress and the development of liver fibrosis. They found that lower zinc concentrations coincided with higher oxidative stress and faster fibrosis development.

In a real-life study, Omran et al. [92] examined the relationship between the serum zinc level and degree of fibrosis in 297 patients with chronic hepatitis C as measured by Fibroscan. They found that lower zinc levels correlated with a higher degree of fibrosis. A negative correlation was found to INR. No correlation was found with ALAT, ASAT, bilirubin, or albumin. They conclude that zinc concentrations reduced significantly with progression of fibrosis and consider zinc supplementation essential in the anti-viral treatment of chronic hepatitis C. Based on experimental experience in a murine model, Song et al. [93] found a reduction of the Child-Pugh and MELD scores in the zinc group in an interim analysis after 3 months conducted in the scope of a randomized placebo-controlled study set up for 2 years and spanning 22 patients with alcoholic liver cirrhosis, while the scores increased in the placebo group. In the lab parameters, TIMP-1

showed a significant increase in the placebo group, while there was no change in the initial value before treatment commenced in the zinc group. The serum procollagen III N-terminal propeptide (P3NP) tended to increase under the placebo, but not under administration of zinc. Hyaluronic acid (HA) acted in the opposite way, tending to reduce under zinc but not under the placebo. The authors concluded from these results that zinc supplementation reduced fibrosis. However, developments after completion of the study remain to be seen.

Most recently, Szabo et al. [94] published initial results from a multicentre, double-blind, randomized study among 103 patients with severe alcoholic hepatitis. While 50 patients received 32 mg methyl prednisolone orally for 28 days, 53 patients were treated with a combination of a recombinant human IL-1 receptor antagonist (IL-1RA, anakinra) 100 mg/day subcutaneously for 14 days, pentoxifylline (PTX) 3x 400 mg/day orally for 28 days, and zinc 220 mg orally/day for 180 days. The treatment in this combination had the following pathogenetic targets: Anakinra: inflammation; PTX: protection from cell damage; and Zinc: stabilization of the intestinal permeability (leakiness). After the first evaluation, this combination led to an effect comparable to the current common treatment with methyl prednisolone. The authors expect comparable benefits of this treatment combination for severe alcoholic hepatitis in long-term survival as well.

9. Conclusions

Several epidemiological, experimental, and clinical data clearly suggest that cellular stress, zinc deficiency in connection with impairment of zinc homeostasis, and excessive inflammation are not only causatively associated with metabolic diseases, but also with the development of various chronic diseases such as arteriosclerosis, obesity, diabetes types 1 and 2, cardiovascular diseases, liver, kidney, pancreas, and lung diseases, rheumatoid arthritis, tumours (e.g. pancreas, mammary, prostate and also liver carcinomas), as well as with severe microbial and viral infections [24 , 31, 95, 96]. While modern anti-viral treatment of hepatitis C, and within limitations also of hepatitis B, shows that fibrosis, and even cirrhosis, can recede, there is no promising medicine to reduce fibrosis yet in NASH and ASH. One main obstacle for a single active substance is that it will usually only align with a singular target (enzyme, receptor, or cell, among others) in the pathogenicity of a disease, and acts accordingly. The multifactorial genesis of NASH, and also of ASH, however, has many different pathogenetic paths, structures, and situations that contribute to development of the disease. Therefore, this heterogeneity requires a multiple treatment program targeting various paths. The diverse hepatoprotective effects of zinc documented in many experimental studies and initial clinical pilot projects should be a reason to include zinc as a component in future studies on NASH and ASH, similar to what De Wier et al. [97] described for flavonoids in NASH, in particular since zinc is most probably not toxic with long-term administration at quantities below 45 mg/day, and is also inexpensive. Due to the close interaction of the signal paths of ER stress, oxidative stress, and inflammatory response in the pathogenicity processes of many chronic diseases, zinc is a good component for pharmacological intervention. These future ideas do not mean that controlled zinc supplementation should take place, considering the current knowledge on the meaning of zinc for biological processes in general and of zinc deficits as a pathogenic factor for a number of chronic diseases, in particular when typical zinc deficit symptoms occur, and a zinc deficit is documented in the blood of a patient. This data suggest that zinc acts through multiple pathways to provide therapeutic efficacy in liver fibrosis.

Author Contributions

KG, TG and DR contributed to the literature review, drafting and editing of the manuscript.

Competing Interests

The authors have declared that no competing interests exist.

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