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REVIEW

Oxidative stress as a crucial factor in liver diseases

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Abstract

Redox state constitutes an important background of numerous liver disorders. The redox state participates in the course of inflammatory, metabolic and proliferative liver diseases. Reactive oxygen species (ROS) are primarily produced in the mitochondria and in the endoplasmic reticulum of hepatocytes *via* the cytochrome P450 enzymes. Under the proper conditions, cells are equipped with special molecular strategies that control the level of oxidative stress and maintain a balance between oxidant and antioxidant particles. Oxidative stress represents an imbalance between oxidant and antioxidant agents. Hepatocytic proteins, lipids and DNA are among the cellular structures that are primarily affected by ROS and reactive nitrogen species. The process results in structural and functional abnormalities in the liver. Thus, the phenomenon of oxidative stress should be investigated for several reasons. First, it may explain the pathogenesis of various liver disorders. Moreover, monitoring oxidative markers among hepatocytes offers the potential to diagnose the degree of liver damage and ultimately to observe the response to pharmacological therapies. The present report focuses on the role of oxidative stress in selected liver diseases.

Key words: Liver disease; Oxidative stress; Redox state

Core tip: This article focuses on the role of oxidative stress in liver diseases. Redox state constitutes the important background of numerous liver disorders. It participates in the course of inflammatory, metabolic and proliferative liver diseases. An oxidative stress stands for an imbalance between oxidant and antioxidant agents. It may explain pathogenetic aspects of chronic liver diseases. This paper is a review on newest literature in this field. In the light the above we hope that this article is interesting and may contribute to current knowledge.

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OXIDATIVE STRESS

Oxidative agents in humans

Free radicals are molecules that have an unpaired electron in their valence orbital. Free radicals and their related reactants are not equally toxic; the most reactive, and therefore damaging, products are assumed to be the oxygen-based hydroxyl radical and the nitrogen-based peroxynitrite anion. The generation of molecular oxygen in the form of reactive oxygen species (ROS) is a natural part of aerobic life that is responsible for the manifestation of cellular functions ranging from signal transduction pathways, defense against invading microorganisms and gene expression to the promotion of growth or death[1]. Redox signaling is of essential importance due to the abundance of oxygen in the Earth's atmosphere. Nevertheless, an excessive amount of ROS is highly toxic to cells. Oxidative stress affects the major cellular com-

ponents: proteins, lipids and DNA. The importance of oxidative stress is commonly emphasized in the pathogenesis of various degenerative diseases, such as diabetes, cancer, cardiovascular disorders or neurodegenerative diseases[2]. The described conditions are inseparably connected with the state of chronic oxidative stress. However, acute exposure to high levels of ROS may also result in serious damage within the human body, such as during ischemia/reperfusion (I/R) of the liver. Aside from the harmful effects, ROS are also perceived as molecular secondary messengers that are generated in response to growth factors, hormones, cytokines and extracellular ATP. Hence, the role of oxidant agents in cells is complex and depends on the balance between oxidant and antioxidant particles. Protective actions against ROS are performed by several enzymes (*e.g.*, superoxide dismutase (SOD), catalase and glutathione peroxidase) as well as nonenzymatic compounds (*e.g.*, tocopherol, vitamin E, beta-carotene, ascorbate and glutathione (GSH))[3-5]. When the capacity of this antioxidant system decreases, the level of inactivated ROS rises. Ultimately, a dangerous level of redox state is established, and the undesirable influences of oxidative agents appear. These consequences affect several amino acids, such as tyrosine, tryptophan, histidine and, particularly, cysteine. Proteins that are rich in these specific amino acids comprise the direct targets of ROS. ROS-mediated modification might alter both protein structure and function. Oxidized proteins are highly susceptible to proteolytic attack by proteasomes[6]. ROS generation also leads to altered mitochondrial permeability and transition potential. These changes induce the release of pro-apoptotic factors (*e.g.*, cytochrome C). Moreover, mitochondrial permeability increases caspase-3 activation among cells. Other phenomena connected with an increased redox state include decreased ATP synthesis and reduced mitochondrial protein synthesis, alteration of the oxidative phosphorylation system and damage to mitochondrial DNA[7-9]. Additionally, oxidative stress may induce reversible and irreversible changes in sensitive proteins. Reversible alterations usually involve cysteine and play a dual role; they can protect a cell from irreversible damage and modulate the function of a protein. Irreversible modifications caused by ROS, such as lysine and arginine carbonylation, di-tyrosine formation or protein-protein cross-linking, typically result in a permanent loss of function and may contribute both to the degradation of damaged proteins and to their accumulation into cytoplasmic inclusions. This process is often associated with neurodegenerative disorders[10].

Role of melatonin in oxidative stress

In humans, one unique antioxidant warrants separate discussion from other antioxidants because of its multiple actions. This special molecule is melatonin (N-acetyl-5-methoxytryptamine), a serotonin derivative produced by the pineal gland and, to a lesser degree, by the retina, gastrointestinal tract and bone marrow. Melatonin was first thought to only play a role in sleep and circadian rhythm regulation[11]. Nevertheless, numerous studies have indicated that it also influences the immune system and is an endogenous substance that shows antioxidative, anti-inflammatory and anti-apoptotic properties[12,13]. The exceptional nature of melatonin centers on its ability to target both ROS and reactive nitrogen species. As a result, the antioxidant power of melatonin is significantly greater than that of vitamin E or C and is 5 to 15 times higher than that of glutathione. Melatonin may act as a direct scavenger of free radicals, and one molecule can bind two hydroxyl radicals. This connection yields a cyclic 3-hydroxymelatonin as a product. This molecule in turn is excreted into the urine, and its level is proportional to the level of oxidative stress in the organism. Furthermore, melatonin increases the amount of antioxidant enzymes by augmenting messenger RNA levels of superoxide dismutase and gamma-glutamylcysteine synthase, which intensify the formation of glutathione and glutathione peroxidase[14]. Additionally, the diffusion of melatonin is not limited by any barrier, including cell compartments. In contrast, α-tocopherol cannot cross the blood-brain and placental barriers. The indirect role of melatonin in antioxidant activity is reflected by its protective influence on cell membranes, cell proteins and both the genomic and mitochondrial DNA[15,16]. Many investigations have demonstrated that the administration of melatonin improves liver function after ischemia/reperfusion in cases of alcoholic liver injury and cirrhosis induced by carbon tetrachloride[17-19]. Melatonin could be effective in nonalcoholic fatty liver disease (NAFLD) in consideration of the pathogenetic mechanisms involved in the development of NAFLD, especially nonalcoholic steatohepatitis (NASH).

Redox susceptible targets in the liver

Mitochondria are the main source of cellular ROS within non-phagocytic human cells. Oxygen metabolites are produced during the course of oxidative phosphorylation. Under physiological circumstances, up to approximately 1% of the mitochondrial electron flow contributes to the generation of superoxide anion, which is formed by the univalent reduction of molecular oxygen. This reaction is catalyzed by enzymes such as NADP(H) or xanthine oxidase[20]. The reduction of molecular oxygen may also occur nonenzymatically as a result of redox-active compounds such as the semi-ubiquinone compound of the mitochondrial electron transport chain. Physiologically, synthesized oxygen free radicals play a positive role in the cell; they are responsible for signal transduction, gene expression and defense against invading pathogens. However, interference with electron transport can increase superoxide production to such an extent that their role in cells becomes harmful. In addition to the mitochondria, the endoplasmic reticulum can also produce ROS in the liver *via* the cytochrome P450 enzymes, and this reaction can occur in macrophages and neutrophils[21,22]. Chronic liver diseases are nearly always characterized by increased oxidative stress, regardless of the cause of the

liver disorder. Multiple studies have shown that patterns of protein expression may be modulated in mammalian cells in response to hydroperoxide stress. This modulation occurs due to the activation of redox-sensitive transcription factors such as Egr-1, NF-kappaB and AP-1 as well as G proteins. Cellular kinases, especially those in the mitogen-activated protein kinase family, also have an essential role[23]. Alterations in protein expression emphasize the importance of oxidative-dependent cellular signaling pathways. This pathological chain reaction exposes the liver to severe oxidative stress and results in hepatocyte apoptosis. The mechanism of this process remains incompletely understood, and investigations are on-going. Sensor polypeptides specific to ROS have not yet been identified. Such a finding would be extremely helpful for the development of new, effective therapies for various liver injuries. Although the data remain largely insufficient, several specific genes and their products are crucial in controlling cellular function in cases of oxidative stress. Apurinic/apyrimidinic endonuclease (APE)/redox factor (Ref)-1 constitutes a basic example of this mechanism. The enzyme APE/Ref1 is a key protein in the base excision repair pathway, which exhibits both repair and redox control properties. Its redox activities increase rapidly in a redox state. Previously reported data have substantiated a relationship between the activation of oxidative agents and the expression of APE/Ref-1. Thus, a better understanding of the diagnostic capabilities of APE/Ref-1 in oxidative-stress-based liver pathologies is extremely important[10,24] and would allow the observation of the initiation and development of oxidative stress. Furthermore, better knowledge of the role of APE/Ref-1 may enable scientists to find new therapeutic strategies for liver disorders. Because of its metabolic activity, the liver constitutes an organ that is particularly susceptible to oxidative stress. The liver is therefore equipped with a special defense mechanism to scavenge ROS, in which nuclear factor E2-related factor 2 (Nrf2) plays an important role. Nrf2 behaves as a cellular redox status sensor and is mostly bound to the cytoskeletal-anchoring protein Kelch-like ECH-associated protein 1 in the cytoplasm under normal circumstances. Elevated levels of ROS and electrophiles cause Nrf2 to release from sequestration and translocate to the nucleus, where it promotes the transcription of cytoprotective genes. Exposure to oxidative stress induces a series of antioxidant genes through the activation of the antioxidant response element (ARE) as a protective mechanism. ARE-containing gene expression is largely regulated by Nrf2 and affects the enzymes that are responsible for GSH homeostasis, NAD(P)H quinone oxidoreductase 1 and UDP-glucosyltransferase. Inappropriate expression of ARE-containing genes results in increased sensitivity of cells to oxidative stress[25,26]. Multiple studies have emphasized the involvement of Nrf2 in the course of liver diseases. Studies conducted on various animal models have indicated that the Nrf2-ARE loop counteracts alcoholic and nonalcoholic liver disease, viral hepatitis, fibrosis and cancer by activating gene expression. Moreover, this loop supports liver regeneration. Nrf2 knockout increases liver damage in response to toxins and a high-fat diet, with the consequence of elevated mitochondrial ROS production. Studying Nrf2 has expanded the understanding of oxidative stress and may enable the design of new therapies against liver disorders connected with redox state[27].

ROS undoubtedly play a crucial role in the development of numerous chronic liver diseases and stimulate their progression. Oxidative stress constitutes the background of viral and alcoholic liver diseases and participates in the liver fibrogenic response. The pathogenesis of the damage involves each cell type of the liver (*i.e.*, hepatocytes and stellate, endothelial and Kupffer cells) and contributes to ischemia/regeneration, necrosis and apoptosis. All mentioned changes result in altered gene expression and progressive liver damage[28-31].

REDOX STATE IN SELECTED LIVER DISEASES

Alcoholic liver disease

Alcoholic liver disease (ALD) constitutes a complex disorder that is a common cause of morbidity and mortality across the world. The disease spectrum includes hepatic steatosis, hepatitis and cirrhosis, which may lead to the development of hepatocellular carcinoma (HCC). A liver exposed to excessive amounts of alcohol undergoes numerous changes as a consequence of two major, linked phenomena: oxidative stress and inflammation. The induction of these two components is a key element in the pathogenesis of ALD. Alcohol-induced liver damage is undoubtedly connected to an excessive production of ROS and the presence of oxidative stress within hepatocytes[32]. The explanation of this mechanism may be found in the course of alcohol metabolism in liver, beginning with alcohol dehydrogenase (ADH), which forms acetaldehyde. Next, acetaldehyde is metabolized to acetate by acetaldehyde dehydrogenase (ALDH). This product is unstable and easily breaks down into water and carbon dioxide. However, the formation of acetaldehyde is destructive to liver cells; it is a reactive agent that can react with DNA and creates adducts that result in tissue injury. Acetaldehyde and its derivative-malondialdehyde (MDA) simultaneously bind to proteins and form hybrid malondialdehyde-acetaldehyde (MAA) adducts. These products are recognized by scavenger receptors in liver cells (*i.e.*, Kupffer cells, endothelial cells and stellate cells) that stimulate the up-regulation of cytokines and trigger an inflammatory response during ALD[33]. The second pathway of ethanol degradation is through the microsomal system catalyzed by cytochrome P450 enzymes. The 2E1 isoform of cytochrome P450 (CYP2E1) is specifically responsible for the breakdown of alcohol under conditions of chronic consumption. Activated CYP2E1 causes the release of ROS (specifically superoxide anions and hydroxyl radicals), leading to oxidative stress and cell death. However, this pathway is

not the exclusive mechanism of damage by ROS; oxygen radicals may also sensitize hepatocytes to lipopolysaccharide and tumor necrosis factor (TNF) alpha toxicity. Thus, the correlation between oxidative stress and inflammation in the course of ALD is indisputable. Moreover, an enzymatic chain reaction leading to the formation of acetate from ethanol increases the NADH/NAD+ ratio in the mitochondria and cytoplasm. Excess NADH causes the inhibition of mitochondrial beta-oxidation and accumulation of intracellular lipids[34]. Furthermore, ROS generated by CYP2E1 can peroxidize the mitochondrial and peroxisomal enzymes that are involved in beta-oxidation (*e.g.*, acyl-CoA dehydrogenase, carnitine palmitoyl transferase-1). This alteration results in the deposition of fatty acids and in the development of hepatic steatosis. Ethanol is responsible not only for the generation of ROS but also for altered neutralization of free radicals. Specifically, alcohol reduces the expression of the peroxisome proliferator activated receptor gamma-coactivator 1 alpha. This transcription coactivator induces the activity of various ROS-mediated detoxifying enzymes. Therefore, alcohol in the course of ALD acts dually: it causes an increased level of oxidant agents and simultaneously alters the removal of free radicals[35]. Mitochondria are the key organelles that are susceptible to oxidative stress when present in a high degree. First, mitochondria constitute structures with oxidative energy metabolism. Thus, ROS are normally generated within them as undesirable products. Excess amounts of oxidant agents may be released in liver mitochondria in various cases of liver diseases, including ALD. Clinical research has revealed that mitochondria that are chronically exposed to ethanol display increased production of ROS and undergo several irreversible changes. Alterations include DNA and ribosomes injury that results in impaired and even inhibited protein synthesis[36]. In addition, oxidant particles contribute to disturbances of mitochondrial membrane permeability and transition potential. These disorders cause the release of proapoptotic factors (*e.g.*, cytochrome C and caspase-3) and are accompanied by decreased ATP synthesis. Oxidative stress within the mitochondria is also connected with an inflammatory process in the liver during ALD. Improper metabolism of ROS results in the expression of hypoxia-inducible factor-1 alpha, which increases TNF secretion, leading to an immune response that intensifies the liver injury. Microsomal and lysosomal membranes are susceptible to the harmful activity of ROS. By mean of increased lipid peroxidation, levels of glutathione sulfhydryls and glutathione-S-transferase within the microsomal and lysosomal membranes of the liver decrease considerably. Moreover, several studies have indicated an elevated level of cathepsin B in the cytoplasm of hepatocytes. This alteration indicates lysosomal leakage and correlates with impaired lysosome function. Under proper conditions, particular damaged cellular components may be destroyed within lysosomes though autophagy. ALD and oxidative stress alter this process and cause the accumulation of oxidation-induced damage in hepatocytes[37]. Eventually, oxidative stress leads to cell death. ROS are responsible for the rebuilding of stellate cells and the extracellular matrix in liver. Rebuilding occurs *via* the modification of stellate cells and their transformation into myofibroblasts and the activation of matrix metalloproteinases. The ultimate stage of this impairment is excessive liver fibrosis and cirrhosis. Moreover, various studies (on mice and humans) indicated that oxidative stress inhibits the regenerative capacity of mature hepatocytes. As a result, oval cells become activated (*i.e.*, hepatic progenitors). These phenomena have been described in two models of fatty liver diseases, including chronic alcohol abuse and NAFLD[38].

Nonalcoholic fatty liver disease

NAFLD is the most common chronic hepatic pathology. Its prevalence in developed countries is estimated at 1/3 of the population. The clinical spectrum of NAFLD involves simple hepatic steatosis, NASH, cirrhosis with all the features of portal hypertension and consequently HCC. The pathogenesis of NAFLD is based on the disrupted uptake, synthesis, oxidation and export of fatty acids. This imbalance leads to excessive fat accumulation in the liver[39,40]. NAFLD is accompanied by several predisposing, factors such as obesity, diabetes, dyslipidemia, jejunoileal bypass, drugs and parenteral nutrition. Hepatic stellate cells undergo activation, and progression to advanced fibrosis and cirrhosis is also possible[41,42]. Portal chronic inflammation constitutes the other lesion present in NAFLD; however, it remains insufficiently characterized[43,44]. Several studies have shown that liver injury in the course of NAFLD is mediated by the renin-angiotensin system (RAS) and oxidative stress[45]. RAS is a crucial mechanism in regulating blood pressure and cardiovascular homeostasis. The excess expression of the RAS results in hypertension and cardiovascular disorders. The RAS is present in numerous tissues and organs including the liver. Interestingly, the increased activity of both the systemic and local RAS has been confirmed in cirrhosis, chronic hepatitis HCV and NAFLD. Elevated levels of angiotensin Ⅱ appear to be a starting point of the molecular pathway of ALD pathogenesis. This agent has pro-oxidant, pro-inflammatory and pro-fibrotic effects in the liver and has been detected in Ren2 trnasgenic Ren2 rats with an increased level of endogenous angiotensin Ⅱ. Excessive angiotensin Ⅱ induces significant hepatic ROS generation[46,47]. Oxidative stress is especially harmful to mitochondria, causing damage that results in impaired gene expression, alterations in proteins synthesis, decreased mitochondrial content and impaired mitochondrial beta-oxidation. Moreover, in the course of NAFLD, mitochondrial CYP2E1 expression increases and causes a redox state[48,49]. Disrupted beta-oxidation constitutes an undeniably a crucial component in the pathogenesis of NAFLD[50-52]. It leads to the accumulation of fatty acids within hepatocytes and to the development of the disease. This theory has been proven by research that dem-

onstrated that treating Ren2 rats with valsartan (an angiotensin type 1 receptor blocker) and tempol (a superoxide dismutase/catalase mimetic) led to decreased oxidative stress. Increased ROS generation in the course of ALD manifests as upregulated activity of NADPH oxidase and decreased activity of cytosolic Cu-ZnSOD. An excess of oxidative agents is involved in lipid peroxidation, which additionally increases mitochondrial permeability and alters their function[53,54]. Moreover, ROS are responsible for the release of reactive aldehydes such as 4-HNE, which inactivate the mitochondrial respiratory chain and hinder electron flow from the mitochondrial respiratory chain. As a result, the production of ROS and oxidative stress in mitochondria increases. Discovering the link between angiotensin Ⅱ, oxidative stress and impaired beta-oxidation in NAFLD has been extremely important[55]. This knowledge offers a new possibility to better understand the pathogenesis of NAFLD and to create the most proper therapeutic approaches. Nevertheless, this mechanism requires further investigation and elucidation.

Hepatic encephalopathy

Hepatic encephalopathy (HE) defines a neuropsychiatric manifestation of acute or chronic liver diseases that involve impaired intellectual, psychomotor and cognitive functions. Ammonia has been defined as a primary toxin in this type of pathology because it induces astrocytic swelling in the brain[56,57]. Furthermore, astrocytes stimulated by ammonia activate N-methyl-D-aspartate (NMDA) receptors. The stimulation of ammonia-induced NMDA receptors lowers antioxidant enzyme activity and increases the production of ROS[58,59]. However, it is exceedingly difficult to differentiate whether oxidative stress induces astrocyte swelling or whether astrocyte swelling itself causes oxidative stress by NMDA receptor- and calcium-dependent processes[60,61]. Cerebral endothelial cells are the key cells involved in astrocyte swelling. They represent the first resident brain cells exposed to harmful substances (*e.g.*, ammonia) and are crucial in triggering an abnormal redox state[62]. Furthermore, in the course of HE, mitochondria are exposed to excessive amounts of glutamine, a state that is responsible for an additional increase in oxidative stress among astrocytes[63]. Furthermore, HE is inseparably connected with local and systemic inflammation/infection and is the reason for neutrophil activation and the enhanced production of ROS[64]. Other studies have established that excessive oxidative agents can oxidize RNA, which results in impaired protein synthesis and molecular disruptions in the brain[65,66]. Cell culture studies and animal models confirmed this theory. The analysis of postmortem cortical brain tissue from patients with HE revealed considerably elevated levels of protein tyrosine-nitrated proteins, heat shock protein-27 and 8-hydroxyguanosine. These proteins constitute markers of RNA oxidation and confirm the importance of redox state in the pathogenesis of HE[65]. The "two-hit" hypothesis is the other theory based on the role of oxidative stress in the course of HE. Liver injury and hyperammonemia represent an "initial hit," with astrocyte swelling and generation of ROS appearing as a consequence. Then, a "second hit," such as an ammonia load is caused by upper gastrointestinal bleeding, dehydration, hyponatremia or infection. Astrocyte damage increases, and the level of ROS rises further. This close connection between astrocyte swelling and oxidative stress results in an auto-amplifying signaling loop, which is reflected by the deterioration of neurocognitive ability[67-69].

Liver fibroproliferative diseases

Hepatic fibrosis is a complex phenomenon that is present in numerous liver disorders. Fibrosis is the wound-healing response to injury of hepatocytes and is characterized by scar accumulation and nodule formation. The overproduction of collagen I plays a direct causative role in liver fibrogenesis. Alcohol consumption, hepatitis B or C virus, cholestasis and iron overload are all largely involved in mechanisms of fibrogenesis, each leading to the transformation of hepatic stellate cells to activated collagen-producing cells[70,71]. Enumerated disorders behave as stimuli for the activation of ROS. Additionally, oxidative agents and lipid peroxidation products contribute to the release of profibrogenic growth factors, cytokines and prostaglandins[72]. Thus, ROS play a critical role in the initiation of fibrosis by integrating various profibrotic factors independently of TGF beta. Nevertheless, TGF beta is a redox-sensitive gene; therefore, oxidative radicals increase TGF beta expression in rat hepatic stellate cells[73]. Moreover, studies have indicated that TGF beta stimulates ROS production in fibroblasts. Different studies have shown that TGF beta-induced ROS generation also occurs by the activation of the membrane-bound enzyme NADPH oxidase and the alteration of complex Ⅳ in the mitochondrial respiratory chain[22,74]. NADPH oxidase activation in hepatic stellate cells is also induced by angiotensin Ⅱ, as shown in experimental models of chronic liver injury.

Various studies have demonstrated that the inhibition of angiotensin Ⅱ synthesis lowers hepatic fibrosis[75]. Oxidative stress among patients suffering from cirrhosis has also been carefully investigated. These patients exhibit elevated levels of pro-oxidant markers (*e.g.*, serum MDA) and reduced levels of antioxidant factors (*e.g.*, RBC catalase, SOD, and blood reduced GSH. Oxidative stress influences red blood cells. Patients with liver cirrhosis display alterations in their erythrocyte membranes caused by the redox state. This phenomenon is reflected by elevated levels of nitric oxide in these patients[76]. The presented alterations correspond with worsening Child-Pugh scores.

Hepatitis C virus

Hepatitis C virus (HCV) accounts for chronic liver disease in approximately 2%-3% of the population worldwide. HCV infection often results in liver fibrosis and cirrhosis; various metabolic alterations including steatosis,

insulin and interferon resistance or iron overload; and the development of HCC or non-Hodgkin lymphoma. Various molecular mechanisms cause the development of the above-mentioned disorders. Subsequently, the participation of ROS in these pathologies has been investigated. The correlation between chronic HCV and oxidative stress was established in the mid-1990s. Liver biopsies of patients revealed the presence of oxidative stress within the diseased liver. Direct measurement of the level of oxidative agents revealed many disruptions. The level of glutathione (one of the most important antioxidants) was decreased, and the ratio between the oxidized and reduced forms of glutathione increased and the glutathione turnover was enhanced during the course of chronic hepatitis HCV-induced oxidative stress. Additionally, the activity of antioxidant enzymes (*e.g.*, SOD, glutathione reductase, glutathione peroxidase) was also significantly reduced[77,78]. Additional proof of a prevailing redox state during chronic hepatitis HCV includes advanced oxidation of lipids and proteins, with the production of 8-hydroxydeoxyguanosine found in peripheral mononuclear cells. Identifying the mechanism of chronic hepatitis HCV-mediated oxidative stress became a matter of utmost importance, and almost all HCV proteins (including the core, E1, E2, NS3/4A, NS4B and NS5A) were demonstrated to be involved in this process, with the HCV core protein having the highest participation. HCV replication, or the expression of its core protein, contributes to mitochondrial alterations often followed by apoptosis. This mitochondrial deregulation is connected with excessive ROS production due to the inhibition of electron transport complex I activity. Mitochondrial dysfunctions are also ascribed to core-induced increased expression of the mitochondrial chaperone prohibitin, which interacts with and regulates the expression of mitochondrial respiratory complex Ⅳ and possibly electron transport complex Ⅰ. HCV induces the generation of ROS through calcium redistribution among the ER, cytoplasm and mitochondria[79,80]. Special chelators of intracellular calcium inhibit the release of oxidative agents in cells expressing the HCV polyprotein, NS4B or the core proteins. In cells with the HCV core and NS5A expression, two different molecular pathways that explain the increase of mitochondrial calcium concentrations have been identified. The mitochondrial Ca^{2+} uniporter may be stimulated by the HCV core protein[81-84]. Additionally, both NS5A and core protein can deplete ER Ca^{2+} stores, resulting in excessive cytoplasmic Ca^{2+} concentration by inducing a passive leak of calcium ions and inhibiting SR $Ca(^{2+})$ ATPase[85]. In response to HCV infection, the activity of NADPH oxidases is increased. The NADPH oxidase family of enzymes also triggers oxidative stress, with several such enzymes induced by calcium signaling. Furthermore, HCV-infected cells may present elevated ROS production *via* ER-residing CYP2E1, which is involved in ethanol catabolism. Alcohol abuse during the course of chronic hepatitis HCV leads both to more escalated oxidative stress and to severe disease progression. Additionally, HCV infection increases the expression of CYP2E1 in the liver in several cases of hepatitis HCV. Finally, ER stress may also result is extra ROS and is related to the unfolded protein response. ER stress may be caused by a number of chemicals and various viral infections, which hamper protein folding or cause ER overload. Oxidative stress mediated by HCV infection may consequently lead to the development of HCC. Studies conducted on HCV core-transgenic mice have presented indisputable evidence of the carcinogenic potential of an HCV-induced redox state. The mice exhibited increased oxidative stress markers and developed HCC even in the absence of an inflammatory state. Several mechanisms that may participate in this phenomenon have been proposed. ROS-mediated apoptosis is the cause of DNA damage and leads to the accumulation of various mutations[86,87]. Subsequently, viral NS5A protein behaves as an inhibitor of the Kv2.1 potassium channel. Under normal circumstance, this channel is responsible for the induction of apoptosis in cases of chemically induced oxidative stress through the amplification of an outward K(+) current. Hence, the NS5A-induced alteration of the Kv2.1 channel prevents apoptosis and stimulates proliferation. Currently, molecular markers of particular disorders need to be introduced into diagnostic practice. Thus, indicators of oxidative stress are essential. Advanced oxidation protein products (AOPP) constitute a new redox state indicator; it presents the oxidation-mediated protein damage and functions as an inflammatory mediator[88]. Recently, the role of AOPP in uremia, coronary artery disease and diabetes mellitus has received substantial concern. AOPP is the result of neutrophil myeloperoxidase enzyme activity during the course of oxidative stress. Chloramine oxidants, which are the byproducts of this process, cause the production of AOPP. These products are classified as cross-bonded proteins, including di-tyrosine, and are perceived as reliable markers of protein oxidative modifications. Serum AOPP levels tend to be significantly elevated in chronic hepatitis HCV patients as compared with healthy controls[89,90]. Not only does protein alteration occur during oxidative stress, but lipid peroxidation also occurs. For example, MDA is the end-product of lipid peroxidation and is formed by degradation of the polyunsaturated lipids by ROS. This marker is also increased in chronic hepatitis HCV patients, confirming the crucial role of oxidative agents in HCV infection.

Hepatocyte injury during hypoxia/reoxygenation

I/R constitutes a major mechanism of liver injury following hepatic surgery (*e.g.*, the resection of large hepatic tumors or vascular reconstructions) or transplantation. It may also be associated with chronic hepatic inflammation or infection. Oxidative agents that are produced very soon after reperfusion of ischemic tissue lead to a redox state, which is the critical reason for cellular injury. Hypoxia alters the mitochondrial electron transport chain and contributes to an increase in ROS[91,92]. Moreover, ischemia converts the physiological form of xanthine de-

hydrogenase into xanthine oxidase. This modified enzyme reacts with molecular oxygen and produces ROS as a result. The extracellular space of the liver may be the area of the highest level of oxidative stress in I/R. Kupffer cells are responsible for this redox state[93,94]. Neutrophils constitute the other cells involved in the generation of ROS after reperfusion. NADPH oxidase causes neutrophils to release oxidative stress markers primarily at 6-24 hours from the beginning of I/R. Mac-1 (CD11b/CD18) is the most important receptor for neutrophil activation and redox state. The inhibition of this receptor significantly reduces liver injury during reperfusion[6].

Antioxidants as therapeutic agents for liver disease

In light of the crucial role of oxidative stress in liver diseases, antioxidants are understandably considered as a good therapeutic strategy for the treatment of liver disorders. To date, the study outcomes remain inconclusive and controversial; however, the therapeutic efficacy of particular antioxidants has been proven. Several studies have indicated that curcuminoids protect DNA against ROS and support hepatocytes during the course of injury and cirrhosis[95]. Research in the field of chronic HCV has revealed improvement after antiviral therapy supported with silymarin (extract from milk thistle), which conserves GSH within hepatocytes. Ascorbic acid, lipoic acid, quercetin (a flavonoid antioxidant) and mitoquinone (a mitochondria-targeted antioxidant agent) also exert beneficial effects in patients with chronic HCV infection. The antioxidant properties of resveratrol lower hepatic lipid peroxidation, increase the amount of GSH in liver and scavenge ROS. Its functions are exerted during liver damage caused by hepatotoxins, *e.g.*, ethanol. In addition, ebselen (an analog of glutathione peroxidase) constitutes a therapeutic agent in early alcohol-induced liver injury. Studies have indicated that vitamin E suppresses HBV replication[96,97] and inhibits TGF beta gene expression in rat models of NASH. Further research has revealed that vitamin E can also prevent the progress of NAFLD. Currently, the best clinical evidence of successful antioxidant therapy in liver diseases is the use of vitamin E for NASH[98]. However, despite numerous studies on humans and animal models, it is extremely difficult to understand and describe the efficacy of antioxidative agents in hepatology[99,100].

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