Stress Responses and Cellular Crosstalk in the Pathogenesis of Liver Disease Theme Issue

**REVIEW**

**Oxidative Stress—Induced Liver Damage and Remodeling of the Liver Vasculature**

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As an organ critically important for targeting and clearing viruses, bacteria, and other foreign material, the liver operates via immune-tolerant, anti-inflammatory mechanisms indispensable to the immune response. Stress and stress-induced factors disrupt the homeostatic balance in the liver, inflicting tissue damage, injury, and remodeling. These factors include oxidative stress (OS) induced by viral infections, environmental toxins, drugs, alcohol, and diet. A recurrent theme seen among stressors common to multiple liver diseases is the induction of mitochondrial dysfunction, increased reactive oxygen species expression, and depletion of ATP. Inflammatory signaling additionally exacerbates the condition, generating a proinflammatory, immunosuppressive microenvironment and activation of apoptotic and necrotic mechanisms that disrupt the integrity of liver morphology. These pathways initiate signaling pathways that significantly contribute to the development of liver steatosis, inflammation, fibrosis, cirrhosis, and liver cancers. In addition, hypoxia and OS directly enhance angiogenesis and lymphangiogenesis in chronic liver diseases. Late-stage consequences of these conditions often narrow the outcomes for liver transplantation or result in death. This review provides a detailed perspective on various stress-induced factors and the specific focus on role of OS in different liver diseases with special emphasis on different molecular mechanisms. It also highlights how resultant changes in the liver vasculature correlate with pathogenesis. (Am J Pathol 2023, 193: 1400–1414; https://doi.org/10.1016/j.ajpath.2023.06.002)

Stress-induced health issues are one of the major challenges in modern society. Stress, which is defined by “conditions where an environmental demand exceeds the natural regulatory capacity of an organism, in particular situations that include unpredictability and uncontrollability,” can alter the normal homeostasis of human physiological and psychological status. Both experimental and clinical studies show that stress significantly impacts hepatic physiology and contributes to liver diseases. Furthermore, it can also worsen the conditions of the patients with cardiovascular diseases, cancer, and HIV/AIDS. Liver, the largest solid organ of the body, is the center of metabolism and detoxification, and it is also a site for complex immunologic activity controlled by diverse immune cells populations, as well as nonhematopoietic cells. Liver receives the digested products, microbial agents, and antigens from the body through the portal vein, which can elicit immune cell activation and cause persistent and regulated inflammation. Prolonged exposure to high-fat diet, alcohol, drugs, and stress hormones causes chronic liver inflammation, leading to chronic liver diseases. Episodes of stress conditions such as physiological, psychological, or environmental stressors can have a negative impact with long-term consequences on the pa-

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This article is part of a review series focused on the role of cellular stress in driving molecular crosstalk between hepatic cells that may contribute to the development, progression, or pathogenesis of liver diseases.
tients with pre-existing conditions, such as hepatitis B virus (HBV), hepatitis C virus (HCV), alcoholic hepatitis, nonalcoholic steatohepatitis, oxidative stress (OS), or endoplasmic reticulum (ER) stress in patients with hepatocellular carcinoma (HCC). In these stress-mediated liver damages, both the hepatocytes as well as the non-parenchymal cells, present in the liver vasculature, are impacted. The current review highlights the principal contributing factors for stress-induced damage in liver and the liver vasculature, leading to the progression of liver disease.

Liver Anatomy, Vasculature, and Cellular Distribution

The liver is a highly vascularized organ with a dense network of blood vessels, bile ducts, and lymphatic vessels. It plays a critical role in transforming toxic substances of the body into a form that can be eliminated. Compared with other organs of the body, the liver has a high capacity to receive the cardiac output, and can receive up to 25% of cardiac output at rest. It is also the first organ to receive nutrient-rich blood from the intestines and participates in immune response. It is unique because of the superimposition of two inlet networks (ie, the hepatic artery and the portal vein), running in parallel; and one outlet network composed of central veins, or hepatic veins. The hepatic artery and portal vein play a major role in maintaining the blood supply in the liver. Liver is divided into hexagonal hepatic lobules, which consist of the central vein, portal triads, and liver resident cells. The portal triad is composed of portal vein, hepatic artery, and bile ducts (Figure 1). The portal vein collects blood from digestive tract, pancreas, and spleen, and contributes to approximately 75% of the liver’s blood supply, whereas the remaining 25% is supplied by hepatic artery. The venous blood mixes with oxygenated blood from hepatic artery and flows through the liver sinusoid, the microvascular bed of liver. The sinusoids send the blood to the central vein. The liver sinusoidal endothelial cells (LSECs) are both morphologically and functionally different from the blood endothelial cells, as they form clustered fenestrations on sieve plates, form a discontinuous arrangement, lack the typical basement membrane, and have the attenuated extracellular matrix with fibronectin as the major component. In adult human liver, the length of sinusoids is approximately 250 µm, and their diameter varies between 7 and 15 µm. Under normal physiological conditions, the unique arrangement of the LSECs facilitates exchange of large molecules and pathogens between hepatocytes and blood.

Bile ducts carry bile from the liver to the gallbladder in a direction opposite to the liver sinusoids. Along with the blood vasculature, the liver has dense lymphatic vasculature, and a lymphatic system critical for the maintenance of body fluid homeostasis, inflammation, and immune response. Recent studies in murine models show that the liver lymphatics are organized segmentally, and intrahepatic lymphatic vessels are populated around the portal triad. Lymphatic capillaries, the thin-walled vessels composed of...
single layered lymphatic endothelial cells (LECs), collect the lymph, which is rich in cellular proteins, lipoproteins, and lymphocytes, and drain it into the collecting lymphatic vessels. Collecting lymphatic vessels, unlike the lymphatic capillaries, are covered by lymphatic muscle cells, which can pump the lymph from the liver to the lymph nodes.21,24

Hepatocytes

About 70% to 80% of adult human liver is composed of parenchymal hepatocytes. Hepatocytes play crucial roles in metabolism, detoxification, innate immune regulation, protein synthesis, and protein secretion in the circulation.25,26 The main proteins secreted by the hepatocytes include α-fetoprotein, serum albumin, transferrin, plasminogen, fibrinogen, α-1-antitrypsin, C-reactive protein, and blood clotting factors (except for factor VIII).25 Hepatocytes are the first site for initiation of bile acid (BA) synthesis, which is then transported to the bile canaliculi. BAs take part in the emulsification, digestion of dietary fats, and removal of metabolic wastes.25 Bile is transported through a series of bile ductules to bile ducts and eventually transferred to the gallbladder and stored there until being hormonally stimulated and released into intestines. However, BAs retained in the liver because of impaired secretion cause hepatocellular toxicity.27 Under pathologic conditions, enhanced level of BAs, deoxycholic acids, and taurodeoxycholic acids in the liver induce reactive oxygen species (ROS) production, which, in turn, activates the downstream signaling of extracellular signal-regulated kinase 1/2 and AKT pathways.27 Hepatocytes are the major targets of ROS-mediated damage. High level of ROS stimulates mitochondrial hypertonicity, leading to the release of cytochrome c and hepatocyte apoptosis.28

Cholangiocytes

The three-dimensional network of bile duct, or biliary tree, is lined with the heterogeneous, highly dynamic population of epithelial cells or cholangiocytes, which represent only 3% to 5% of total liver cell population.29 However, cholangiocytes are responsible for up to 30% of total bile flow in humans, and the remaining 70% is produced from hepatocytes.29 Cholangiocytes are activated by several stimuli, which include xenobiotic stimuli, cholestasis, ischemia, infections, and OS. Activated cholangiocytes have altered proliferation capacity and secrete a variety of profibrotic and proinflammatory factors.29,30

Hepatic Stellate Cells

Hepatic stellate cells (HSCs), which represent approximately 10% of the liver cells, are the liver-specific fibroblasts that reside in the space of Disse, the space between the LSECs and hepatocytes (Figure 1). In healthy liver, HSCs are the primary storage site and metabolism sites of vitamin A.15 In case of liver injury, the HSCs get activated, differentiate into myofibroblasts, produce extracellular matrix components, and cause fibrosis in the liver. Interestingly, activated HSCs also produce a plethora of cytokines and chemokines, which include monocyte chemoattractant protein 1, IL-6, and transforming growth factor-β, which enable leukocyte recruitment into the liver. Recruited leukocytes secrete the proinflammatory factors and elicit the immune response as well as contribute to further activation of HSCs.31 Under OS, ROS activate the quiescent HSCs and cause extracellular matrix production, which, in the long run, contribute to fibrosis, cirrhosis, and HCC.32

Kupffer Cells

Kupffer cells (KCs) are the major liver resident macrophages and are present in the liver sinusoid and adherent to the LSECs of the sinusoidal wall (Figure 1). The KCs serve as the first line of defense against bacterial infection and play important roles in host immune response.33-35 Under pathologic conditions, KCs are differentiated into M1-like (classic) or M2-like (alternative) macrophages. KCs are major sources of proinflammatory cytokines, which can cause liver damage.36,37 In response to stimuli, oxidized low-density lipoprotein, and lipopolysaccharide, the KCs and infiltrating macrophages in the liver produce ROS through the activation of NADPH oxidase 2 (Nox2), which eventually stimulate the KCs to secrete proinflammatory cytokines [eg, tumor necrosis factor (TNF)-α, IL-6, and IL-1β].38

Liver Sinusoidal Endothelial Cells

The highly specialized LSECs are specialized endothelial cells in the liver that form the interface between the lumen of sinusoid and the liver hepatocytes and hepatic stellate cells. In contrast to the other endothelial cells, LSECs display clusters of fenestrations that form the sieve plates.39 Because of the lack of organized basement membrane, the LSECs form highly permeable membrane.40 In normal liver, LSECs selectively control the diffusion of substrate between the blood and the space of Disse.31,42 The phenotypes of the LSECs are maintained by the vascular endothelial growth factor-A (VEGF-A) stimulation of nitric oxide—dependent or nitric oxide—independent signaling.43 In healthy liver, LSECs keep the HSCs quiescent. HSCs are the major source of fibrillar collagens and other components of the liver scar.44,45 In diseased condition, the damaged LSECs secrete proinflammatory cytokines and transforming growth factor-β and stimulate the HSCs and liver hepatocytes, which, in turn, causes further liver damage.7,44

Liver LECs

Liver is the largest lymph-producing organ of the body, and it has a highly dense lymphatic system.46 The plasma
component of the blood is filtered through the fenestrae of the LSECs and transferred to the space of Disse. This fluid flows through the space of mall, enters the interstitial space of the portal triad, and finally enters the lymphatic capillaries. Lymphatic vessels are primarily located around the portal triad and drain the lymph to nearby draining lymph nodes outside the liver. Inside the liver, the lymphatic vessels are composed of LECs, which are identified by specific markers lymphatic vessel endothelial hyaluronan receptor 1 (Lyve-1), prospero homeobox protein 1 (Prox1), and podoplanin, and absence of α-smooth muscle actin—positive cells (smooth muscle cells/pericytes). LSECs also express Lyve-1, and hepatocytes express Prox1. Thus, the co-existence of the above three markers identifies LECs. In normal healthy livers, LECs are required to maintain the homeostasis of fat metabolism as they can uptake the cholesterol through the scavenger receptor class B type 1 (SR-B1) and can maintain the homeostasis of fat metabolism as they can uptake the cholesterol through the scavenger receptor class B type 1 (SR-B1). Several studies report that, under pathologic conditions, enhanced level of OS, which is associated with inflammation, is detrimental to the surrounding vasculature. High concentrations of ROS over a long period are harmful to tissues, whereas short periods or low concentrations of ROS promote neovascularization and activate different signaling mechanisms. The mitochondrial transport chain transfers electrons and hydrogen through NADH dehydrogenase and ubiquinone cytochrome c reductase to produce water as a nontoxic by-product. Instead of accepting two electrons, O2 only accepts one electron, reducing O2 to O2−, the superoxide form. Superoxide is then converted to hydrogen peroxide, H2O2, by superoxide dismutase. H2O2 is then converted to hydroxyl radicals (·OH) via the Fenton reaction. In addition, O2− reacts with nitric oxide, which generates another ROS known as peroxynitrite (ONOO−). The highly reactive superoxide, O2−, and ·OH radicals react with other molecules to gain or lose an electron to become stable. The affected molecule becomes a free radical and generates a cascade of free radicals that damage the hepatocyte. The nonradical ROS, such as hydrogen peroxide, H2O2, also lead to cascades of free radicals. By generating radical and nonradical ROS, damage in hepatocytes occurs in the form of oxidative DNA damage, abnormal protein expression, and oxidative degeneration of lipids. In Figure 2, different stimuli that stimulate mitochondrial dysfunction and hepatocyte damage have been described (Figure 2).

### Sources of ROS

The mitochondria and ER are the main sites of ROS formation within the hepatocytes. Several extrinsic (alcohol consumption, drug overuse, environmental toxins, viruses, and smoking) and intrinsic (obesity and insulin resistance) sources can promote ROS production in the liver. Under pathologic conditions, liver inflammations, or injury, multiple cells contribute to the production of ROS (Table 1). ROS are a double-edged sword, and different concentrations can have different physiological impact on the surrounding vasculature. High concentrations of ROS over a long period are harmful to tissues, whereas short periods or low concentrations of ROS promote neovascularization and activate different signaling mechanisms. The mitochondrial transport chain transfers electrons and hydrogen through NADH dehydrogenase and ubiquinone cytochrome c reductase to produce water as a nontoxic by-product. Instead of accepting two electrons, O2 only accepts one electron, reducing O2 to O2−, the superoxide form. Superoxide is then converted to hydrogen peroxide, H2O2, by superoxide dismutase. H2O2 is then converted to hydroxyl radicals (·OH) via the Fenton reaction. In addition, O2− reacts with nitric oxide, which generates another ROS known as peroxynitrite (ONOO−). The highly reactive superoxide, O2−, and ·OH radicals react with other molecules to gain or lose an electron to become stable. The affected molecule becomes a free radical and generates a cascade of free radicals that damage the hepatocyte. The nonradical ROS, such as hydrogen peroxide, H2O2, also lead to cascades of free radicals. By generating radical and nonradical ROS, damage in hepatocytes occurs in the form of oxidative DNA damage, abnormal protein expression, and oxidative degeneration of lipids. In Figure 2, different stimuli that stimulate mitochondrial dysfunction and hepatocyte damage have been described (Figure 2).

### Table 1  Cellular Sources of ROS

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CCL4, carbon tetrachloride; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; ROS, reactive oxygen species; TNF-α, tumor necrosis factor-α.

### Contribution of Stress and Stress-Induced Factors to Liver Injury

This current review primarily focuses on the various aspects of OS and reactive oxygen species on liver diseases and their impact on liver vasculature.

### Oxidative Stress

The liver is the primary detoxifying organ that metabolizes various compounds that produce free radicals. Liver homeostasis is disrupted when the number of free radicals, including ROS, exceeds the endogenous antioxidant components. Excessive ROS within hepatocytes can cause damage to the proteins, lipids, and DNA, which results in structural and functional abnormalities in the liver that develop into various diseases, such as HCC, chronic hepatitis, and fibrosis. The sections below provide a comprehensive review of the sources, consequences, and treatments of OS on liver disease and liver vasculature.
OS in Inflammatory Liver Diseases

Liver diseases with OS response, including viral hepatitis, liver cirrhosis, HCC, cholangiocarcinoma (CCA), alcoholic liver diseases (ALDs), nonalcoholic fatty liver diseases (NAFLDs), and drug-induced liver injury (DILI), are discussed in the following section.

OS in Viral Hepatitis

One of the most common causes of chronic liver injury is the chronic infection with HBV and HCV.70–72 The main viral hepatitis is hepatitis A-E, along with which hepatitis B, C, D, and E are known to cause chronic liver diseases. Eighty percent of HCC cases are associated with chronic infection with HBV and HCV.73 HCV, among all viral hepatitis, is responsible for liver fibrosis, HCC, steatosis, and liver failure.74 An elevated level of oxidative stress and high levels of ROS and reactive nitrogen species have been reported in patients with HCV. The nonstructural protein 3 (NS3) protein of HCV activates Nox2 protein (the major component of oxidation-reduction system), on the phagocytes, and causes apoptosis and dysfunction of T cells and natural killer cells, thereby increasing ROS production by the surrounding neighboring cells.75 The HCV core protein plays a critical role in the development of chronic HCV and HCC by activating tumor necrosis factor receptor (TNFR)-, protein kinase R (PKR)-, and STAT3-mediated pathways.76–78 Higher level of ROS causes the damage of liver cells and vasculature.79 HBV also infects KCs that signal macrophages to produce proinflammatory cytokines, such as IL-1, IL-6, CXCL-8, and TNF-α.80,81 These cytokines damage mitochondrial cytochrome oxidase, inhibiting the electron transport chain, increasing ROS levels, and inducing HCC. Furthermore, patients with HCV-related HCC have more markers of OS, such as 8'-hydroxy-2'-deoxy guanosine, and reactive oxygen metabolites in their serum than patients with HBV-related HCC, indicating...
more significant levels of OS in HCV infection. Chronic HBV and HCV infection are also associated with an elevated level of bivalent iron accumulation in the liver, which also cause the production of ROS and hydroxyl free radicals. ROS also act as inducers of VEGF production. In patients with chronic HCV, there is a high level of VEGF, which is one of the major angiogenic factors. HBV infection induces the mitochondrial ROS accumulation in hepatocytes, which causes the sustained activation of IL-6/STAT3 pathway and increase in production of downstream factor VEGF. Altogether, the HBV-induced OS promotes the hepatocarcinogenesis. The hepatitis B virus X protein (HBx), a regulatory protein produced by HBV, promotes activation and stabilization of hypoxia-inducible factor-α (HIF-1α). Activated HIF-1α, which is the upstream regulator of VEGF, enhances the transcription of VEGF and finally promotes angiogenesis.

**OS in Liver Cirrhosis and Fibrosis**

HSCs and KCs are related to the development of liver cirrhosis and fibrosis. OS promotes the activation of HSCs, which can produce extracellular matrix and contribute to the development of liver fibrosis, a significant risk factor for HCC. ROS and O2− activate HSC and damage hepatocytes while also triggering the activation of NF-κB, which regulates genes involved in cell transformation, proliferation, and angiogenesis. NF-κB generates a positive feedback loop, further increasing nitrogen monoxide and ROS production and forming oxidized low-density lipoprotein, exacerbating hepatocyte damage. In addition, KCs are constantly activated in liver fibrosis and cirrhosis, producing large amounts of extracellular ROS that cause hepatocyte necrosis.

**OS in Liver Cancers: HCC and CCA**

In patients with chronic hepatitis, an increased amount of ROS-mediated DNA damage, accumulation of mutations, chromosomal rearrangements, and genomic instability in hepatocytes can cause extensive cellular and molecular alterations in the hepatocytes and their microenvironment, which can lead to cirrhosis and eventually HCC. In addition to DNA damage, OS can activate various signaling pathways that promote the growth and survival of cancer cells. ROS can activate the NF-κB pathway, enabling the expression of genes involved in cell proliferation, angiogenesis, and apoptosis resistance. ROS can also activate the mitogen-activated protein kinase/extracellular signal-regulated kinase pathway, which is involved in cell growth and survival. OS can also stimulate the production of proinflammatory cytokines and chemokines, attracting immune cells and promoting inflammation in the liver. Chronic inflammation can also contribute to the development of HCC by promoting the proliferation and survival of cancer cells.

CCA is a heterogeneous group of malignancies in the biliary tree with a dismal 5-year survival rate after metastasis to distal organs. In fact, the CCA metastasis to the draining lymph nodes with enhanced level of both intratumoral and peritumoral lymphangiogenesis is the primary prognostic factor of the aggressiveness of this disease. In both human tissues and murine models of intrahepatic CCA, mitochondrial dysfunction and oxidative stress in the tumor micro-environment promote the accumulation of ROS and infiltration of TNF-α—producing macrophages, which, in turn, generates a favorable environment for the proliferation of biliary cells. In cholestatic liver diseases, a high level of BA, through their cognate receptor Takeda G-protein-coupled receptor 5 on cholangiocytes, induces increased production of ROS, activates cellular Src-mediated epidermal growth factor receptor transactivation, and activates subsequent Erk1/2 phosphorylation, increasing the cellular proliferation. Under inflammatory conditions, inflamed LECs secrete high levels of chemokine CXCL5, which binds to its receptor C-X-C chemokine receptor (CXCR2) on CCA cells, alters cellular metabolism, enhances production of mitochondrial ROS, and promotes lymphangiogenesis and tumor-inducing pathways in CCA.

**OS in ALD and NAFLD**

Globally, ALD is a predominant form of chronic liver disease, inflicted by varying quantities of excessive alcohol consumption. ALD initiation is marked by alcoholic fatty liver, defined by the accumulation of triglycerides in hepatocytes known as hepatic steatosis. The condition can then progress to inflammation and fibrosis, driving the eventual development of cirrhosis, liver failure, and HCC. Oxidation of ethanol through the alcohol dehydrogenase pathway leads to the production of the toxic and carcinogenic acetaldehyde. Cytochrome P450 2E1, an enzyme in the ER and mitochondria of hepatocytes, additionally bolsters these products by metabolizing alcohol to acetaldehyde in the presence of oxygen and NADPH. Stimulated by chronic alcohol consumption, cytochrome P450 2E1—led alcohol metabolism is another pathway for alcohol oxidation. Acetaldehyde promotes the production of neoantigens, subsequently causing cirrhosis of the liver, increase in oxidative stress, altered DNA methylation, and a reduction of retinoic acid, thereby instigating hepatocarcinogenesis. Ethanol and acetaldehyde thus have a deleterious effect on hepatocytes by generating ROS and damaging intestinal mucosal barrier. Cellular oxidative stress caused by an excess of free radicals and the deficiency of glutathione, vitamin E, and phosphatidylcholine may be the primary factors behind the progression of ALD. Neoantigens from acetaldehyde may also propagate inflammation by cytokine and chemokine induction that is reported in individuals with acute alcoholic hepatitis. TNF-α and IL-6 are increased after chronic alcohol consumption.
following NF-κB activation, instigated by toll-like receptor 4—sensing lipopolysaccharides.\textsuperscript{102} OS also plays a predominant role in exacerbation of NAFLD, one of the leading causes of chronic liver disease worldwide, with a prevalence of 25% globally.\textsuperscript{103} Without alcohol or drugs implicated as causal factors, primary risk factors for disease progression include age, increasing body mass index, high-lipid diets, and diabetes.\textsuperscript{104} The presence and stage of fibrosis are the foremost predictors of overall and disease-specific mortality, with liver-related mortality increasing exponentially with each progressive fibrosis stage.\textsuperscript{105} The chief cause of death for patients with NAFLD is cardiovascular disease (40%), with recent studies suggesting NAFLD may directly heighten risk of heart disease.\textsuperscript{106} Polyunsaturated fats play important roles in NAFLD through proinflammatory and anti-inflammatory effects, dependent on their structure.\textsuperscript{107} A high ratio of proinflammatory-promoting n-6 polyunsaturated fats/anti-inflammatory n-3 polyunsaturated fats is correlated with NAFLD and nonalcoholic steatohepatitis, yet another example of inflammatory microenvironments promoting liver disease progression.\textsuperscript{108} Innate immunity, determined by toll-like receptor signaling manipulation, in\textsuperscript{fl}ammasome activation, macrophage activation, increase in proinflammatory cytokines and chemokines, and decreased immune cell recruitment (natural killer T cells), can have major involvement in the progression to severe liver diseases, like HCC.\textsuperscript{109} Additional causal factors include the development of lipotoxic lipids from saturated free fatty acid accumulation, which induces mitochondrial dysfunction, oxidative stress, and ROS.\textsuperscript{113} Hepatocellular

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CYP3A4, cytochrome P450 family 3 subfamily A member 4; CYP3A7, cytochrome P450 family 3 subfamily A member 7; CYP2C9, cytochrome P450 family 2 subfamily C member 9; HLA, human leukocyte antigen; iNOS, inducible nitric oxide synthase; JNK, c-Jun N-terminal kinase; Nrf2, nuclear factor E2-related factor 2; OXPHOS, oxidative phosphorylation; ROS, reactive oxygen species; SLD, sulindac; TNF-α, tumor necrosis factor-α.
injury and death, triggered by autophagy, apoptosis, necroptosis, and pyroptosis, are also pivotal to NAFLD and supplement the progression toward chronic liver disease.114–116 Beginning with nonalcoholic fatty liver, followed by nonalcoholic steatohepatitis, fibrosis, cirrhosis, and, finally, liver cancers, like hepatocellular carcinoma, the general progression from a healthy liver to chronic liver diseases can primarily be combated by weight loss, with 10% weight loss accounting for nearly 50% of patients experiencing fibrosis regression.117 Other management methods include monitoring blood pressure, controlling lipid intake, and controlling diabetes.

**DILI and OS**

DILI is the foremost instigator of acute liver failure, with causal factors, such as antimicrobials, herbal agents, dietary supplements, cardiovascular drugs, central nervous system agents, antineoplastic drugs, and analogs.118,119 Intrinsic DILI categorizes the predictable dose-dependent sources of DILI, often associated with the drug’s toxicity and a quick onset of symptoms.120 The analgesic acetaminophen is often a cause of intrinsic hepatotoxicity, generation of oxidative stress, mitochondrial dysfunction, and ER stress, which can eventually cause cell death.119 Table 2121–157 compiles an array of drugs known to induce stress and lead to liver injury. Prediction, prevention, and mitigation of liver injury can be supplemented by keeping track of common drugs with hepatotoxic properties. Effective clinical management of liver damage and disease involves continued investigation into the hepatotoxic properties of drugs still in development.

The principal drug or its associated metabolites, metabolized by the cytochrome P450 family, that perform oxidative phase 1 drug metabolism initiate DILI by causing direct cell stress, targeting mitochondrial function, or catalyzing certain immune functions.158 Metabolites from phase 1 and conjugative phase 2 metabolism induce cellular stress through direct binding of cell structures, such as enzymes, lipids, and nucleic acids, or by glutathione depletion. Mitochondrial malfunction occurs by the metabolite-navigated uncoupling of the mitochondrial electron transport chain, depriving cells of major portions of ATP, increasing ROS, inhibiting β-oxidation, altering mitochondrial DNA composition and replication, or inducing the mitochondrial permeability transition pore to open on the inner mitochondrial membrane.159–162 Mitochondrial dysfunction can be propelled intrinsically, during which severe cellular stress activates mitochondrial permeability transition by activation of pro-apoptotic and inhibition of anti-apoptotic proteins of the Bcl-2 family following stimulation of lysosomal permeabilization, the ER pathway, or c-Jun N-terminal kinase.163,164 Mitochondrial dysfunction also follows extrinsic inflammatory responses instigated by the innate immune system, which predispose the liver to be more vulnerable to the lethality of TNF-α, FasL, and interferon-γ.165 The consequent disruption of the proton gradient driving most ATP production in the mitochondria leads to necrotic or apoptotic cell death in the liver.

**Impact of OS on Liver Vasculature**

**Oxidative Stress and Angiogenesis in the Liver**

Along with the damaging effect on liver cells, oxidative stress also positively regulates angiogenesis and lymphangiogenesis in several liver pathologies, including HCC and CCA. In case of pathologic angiogenesis, the demand for excess oxygen in surrounding tissues results in hypoxia/re-oxygenation cycle. Under hypoxic conditions, the insufficiency of oxygen supply to the hepatocytes and other liver tissue causes pathologic changes, leading to liver damage.166 The liver is susceptible to hypoxic injury because of its central location in the circulatory system and high metabolic demand. During hypoxia, the decreased oxygen supply leads to a disruption of oxidative phosphorylation, which results in decreased ATP production and an increase in ROS production.3 These changes can lead to mitochondrial dysfunction, OS, and inflammation, ultimately resulting in cell damage and death.3 Hypoxia-induced liver damage has been linked to the activation of HIFs, a family of transcription factors that play a critical role in the cellular response to hypoxia. HIFs promote the expression of genes involved in angiogenesis, such as VEGF; metabolism; and cell survival, which contribute to the development and progression of liver disease. HIFs have been implicated in the development and progression of HCC, as they promote angiogenesis and tumor growth.167 In particular, the expression of VEGF is vital in liver angiogenesis, as it promotes the formation of new blood vessels from pre-existing vessels.167 VEGF stimulates endothelial cell proliferation, migration, and survival, leading to new blood vessel formation. By promoting angiogenic genes such as VEGF, HIFs cause the development of liver fibrosis, cirrhosis, and metastasis of tumors in the liver.167 HCC is one of the most hypoxic malignancies. HCCs, like many other cancer cells, can grow in low oxygen levels or hypoxic environments.168 To survive and proliferate, HCC cells adapt to hypoxia through a concerted transcriptional response that HIFs regulate. Hypoxia-induced liver damage can have various consequences, depending on the severity and duration of the hypoxia. In mild cases, hypoxia may lead to a transient increase in liver enzymes, such as alanine aminotransferase and aspartate aminotransferase, indicating liver injury.169 In more severe cases, hypoxia can lead to acute liver failure, characterized by a rapid onset of liver dysfunction, which can be life threatening. Hypoxia can also contribute to the development and progression of liver diseases, such as NAFLD and cirrhosis.170 Chronic hypoxia can result in
the accumulation of fat in the liver, which can progress to inflammation and fibrosis, ultimately leading to HCC.170

Oxidative Stress and Lymphangiogenesis

Several lines of evidence suggest that dysfunctional lymphatics are associated with inflammatory liver diseases.21,22,171–173 In the postnatal liver, the lymphatic system remains quiescent. However, under pathologic conditions, like chronic hepatitis, liver fibrosis, and CCA, a higher level of lymphangiogenesis has been reported.22,24 Although LECs are the cornerstone of new lymphatic vessel formations, other cells such as macrophages, dendritic cells, Kupffer cells, T cells, and B cells, contribute to lymphangiogenesis.24 One major cause of an enhanced level of lymphangiogenesis is tissue inflammation.174 Under inflammatory conditions, surrounding cells in lymphatic vasculature produce VEGF, including VEGF-C and VEGF-D, the major growth factors for growth of lymphatic vasculature.175,176 In inflamed skin, a profound infiltration of CD11b+/Gr-1+ in the draining lymph nodes and the inflamed skin has been reported, which, in turn, secrete VEGF-C, VEGF-D, and VEGF-A and contribute to the increase in lymphangiogenesis.174 In cholestatic liver diseases, primary sclerosing cholangitis, a progressive biliary inflammation, is associated with an increased level of BAs in the liver.177,178 In multidrug resistance protein 2 (MDR2)179–181 knockout mice, an in vivo model of sclerosing cholangitis,179 elevated levels of conjugated BAs are associated with increased lymphangiogenesis.51 Conjugated BAs, taurocholic acid, and chenodeoxycholic acids induce oxidative stress with enhanced levels of ROS in LECs. The heightened level of ROS-mediated oxidative stress in LECs activates the oxidation-reduction—sensitive kinase 90 kDa ribosomal s6 kinases (p90RSK), which, via the post-translational modification of the transcription factor Prox1, causes the transcriptional activation of VEGF receptor 3 and promotes lymphangiogenesis.51 Thus, inflammation-, ROS-, and oxidative stress—induced LEC proliferation and lymphangiogenesis are closely associated with progression of liver disease.

Endogenous and Dietary Antioxidants: Tools for Rescuing from the OS in Liver Diseases

The impact of OS on the development and progress of stress-related liver pathologies highlights the importance of promoting endogenous antioxidant levels and/or supplementation with dietary antioxidants. The cells in the liver have their own defense mechanism to produce antioxidants enzymatically or nonenzymatically to neutralize the cellular ROS.55 The important enzymatic systems involved in this oxidation-reduction control mechanism are superoxide dismutase (SOD), catalase, reductase [glutathione-peroxiredoxin (GSH-Prx) or glutathione peroxidase (GPxs)], and glutathione peroxidase. The antioxidant enzymes are in different cellular and subcellular locations. For example, SOD has two isoforms: SOD-1, which is a cytosolic (Cu/Zn-SOD) isofrom; and SOD-2, the mitochondrial (Mn-SOD) isoform. SODs act as the first line of defense in the antioxidant mechanism in hepatocytes by reducing the superoxide radicals to H2O2 and O2. The H2O2 is then degraded by other detoxifying enzymes, catalase, mitochondrial glutathione peroxidases (Gpx1,4), and hydroperoxides (PrxIII and Trx2).180

Along with the enzymatic antioxidant systems, the nonenzymatic molecules, like glutathione, retinol (vitamin A), ascorbic acid (vitamin C), and tocopherol (vitamin E), scavenge the free radicals produced by the cells. Another regulatory mechanism in the antioxidant defense is the transcriptional regulation of the antioxidant genes, regulated by nuclear factor E2-related factor 2. The nuclear factor E2-related factor 2 controls an array of antioxidant genes.181 Thus, boosting the nuclear factor E2-related factor 2 machinery with exogenous agents is an important strategy to control the oxidation-reduction balance in the cells.181,182 Dietary supplements with anti-oxidative properties are used in oxidative stress—induced liver diseases.55 Dietary cocoa in chocolate, rich in antioxidants, is effectively used for the amelioration of NAFLD.183,184 Ginger is another commonly used dietary antioxidant, which is effective in the prevention of stress-induced liver diseases.55,185,186 Lemon juice,186 green tea extracts,55 quercetin, and resveratrol from

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**Table 3** List of Potential Antioxidants for Liver Diseases in Human or Rodents

<table>
<thead>
<tr>
<th>Antioxidants</th>
<th>Potential mechanisms and effect on liver diseases</th>
<th>References</th>
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<tbody>
<tr>
<td>Vitamin E</td>
<td>Improved the lipid metabolism and reduced the activation of HSCs in NAFLD</td>
<td>187</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>Altered the PARPα-dependent fatty acid β-oxidation, reduced the high-fat diet—induced obesity in NAFLD, and improved the glucose metabolism</td>
<td>187,188</td>
</tr>
<tr>
<td>Caffeine</td>
<td>Increased Nrf2 levels, reduced the NF-κB activation and inflammatory cytokine level, and reduced the oxidative stress in NASH rat model</td>
<td>189</td>
</tr>
<tr>
<td>Metformin</td>
<td>Increased the anti-oxidative mechanism by Nrf2, increased the expression of heme-oxygenase-1 gene, and reduced the oxidative stress in NAFLD, NASH, and HCC</td>
<td>190</td>
</tr>
<tr>
<td>Hesperetin</td>
<td>Reduced hepatic oxidative stress and inflammation via PI3K/AKT-Nrf2-ARE pathway in NAFLD rat model</td>
<td>191</td>
</tr>
</tbody>
</table>

ARE, antioxidant response element; HCC, hepatocellular carcinoma; HSC, hepatic stellate cell; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; Nrf2, nuclear factor E2-related factor 2; PARP, poly (ADP-ribose) polymerase; PI3K, phosphatidylinositol 3-kinase.
plant extracts have antioxidant properties and are used as potential compounds for the treatment of stress-induced liver diseases. Table 3 lists potential antioxidants in the context of OS-induced liver injury.

Conclusion and Future Approach

The dense vascularization of the hepatic lobules by blood vessels, bile ducts, and lymphatic vessels commits the liver to a multitude of tasks, ranging from fluid homeostasis, immune surveillance, detoxification, bile production, and other functions proffered by its morphology. Maintaining a healthy liver anatomy is crucial for a high quality of life. Because OS and ROS play critical roles in liver pathologies and liver vasculature, in this current review, the role of oxidative stress—induced liver and liver vasculature damage was discussed in detail to elucidate their source and mechanisms that impair these requisite functions. The diverse pathways discussed that lead to liver damage, including oxidative stress, viruses, environmental toxins, drugs, alcohol, and high-fat diet, all converge at one point—increased mitochondrial ROS output. The molecular pathways induced by ROS include NF-κB, mitogen-activated protein/extracellular signal-regulated kinase, and c-Jun N-terminal kinase/c-Jun, which alter the complex microarchitecture constructed by parenchymal hepatocytes, cholangiocytes, LSECs, HSCs, and LECs of the liver, inflicting cell death and altered cellular secretions that further catalyze liver damage. Subsequently, angiogenesis, lymphangiogenesis, inflammation, and increased tumor cell invasion occur. At this point of morphologic disturbance, they often develop into liver fibrosis, cirrhosis, and liver cancers. Further research delving into the molecular pathways stimulated by ROS and pharmaceutical inhibitors that may mitigate the liver and liver vasculature damage caused by these pathways warrants attention. Additional investigation of drugs that are focused on alleviation of oxidative stress—mediated damage is thus of profound importance. Through these efforts, the dismal outcomes associated with accumulated liver damage over years may be alleviated.

Author Contributions

S.C., P.B., and N.G. conceptualized the article; P.B., N.G., and V.C. performed literature review and wrote the article; and S.C. provided intellectual input and supervision. All authors have critically reviewed, edited, read, and agreed to the published version of the manuscript.

References

Banerjee et al


142. Banerjee et al


Nrf2 enhances functional liver regeneration. Hepatology 2021, 74:973–986


Munteanu C, Schwartz B: The effect of bioactive aliment compounds and micronutrients on non-alcoholic fatty liver disease. Antioxidants (Basel) 2023, 12:903


Arroyave-Ospina JC, Wu Z, Geng Y, Moshage H: Role of oxidative stress in the pathogenesis of non-alcoholic fatty liver disease: implications for prevention and therapy. Antioxidants (Basel) 2021, 10:174


