Review

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Nitric oxide in liver diseases

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Nitric oxide (NO) and its derivatives play important roles in the physiology and pathophysiology of the liver. Despite its diverse and complicated roles, certain patterns of the effect of NO on the pathogenesis and progression of liver diseases are observed. In general, NO derived from endothelial NO synthase (eNOS) in liver sinusoidal endothelial cells (LSECs) is protective against disease development, while inducible NOS (iNOS)-derived NO contributes to pathological processes. This review addresses the roles of NO in the development of various liver diseases with a focus on recently published articles. We present here two recent advances in understanding NO-mediated signaling – nitrated fatty acids (NO_2-FAs) and S-guanylation – and conclude with suggestions for future directions in NO-related studies on the liver.

NO in liver physiology and pathophysiology

NO is an important mediator of liver physiology and pathophysiology. NO is generated by three isoforms of nitric oxide synthase (NOS): neuronal NOS (nNOS) (NOS1), iNOS (NOS2), and eNOS (NOS3) [1]. NOS catalyzes the oxidation of L-arginine to NO and citrulline [2]. In the cell, nNOS and iNOS are predominantly found in the cytosol, while eNOS binds to the membrane via palmitoylation and myristoylation [3].

In liver biology, eNOS and iNOS are major players, whereas the role of nNOS is little known. eNOS is mainly expressed in LSECs and endothelial cells of the hepatic artery, portal vein, central vein, and lymphatic vessels. eNOS is constitutively expressed and produces small amounts of NO in response to stimuli such as flow shear stress and vascular endothelial growth factor (VEGF) (Figure 1A). eNOS-derived NO maintains liver homeostasis and inhibits pathological conditions in the liver. By contrast, iNOS is induced in various liver cells, including LSECs, hepatocytes, Kupffer cells (liver resident macrophages), hepatic stellate cells (HSCs), smooth muscle cells, cholangiocytes, and other immune cells [4–6]. Under many pathological conditions, iNOS produces large amounts of NO, which is a major source of reactive nitrogen species (RNS) (Figure 1B). Particularly, peroxynitrite ($ONOO^-$) can damage a wide range of cellular molecules including DNA, lipids, and proteins and can also facilitate protein nitration, affecting the structure and function of many target proteins [7] (Figure 1C).

This review summarizes current understanding of NO biology including two new NO-mediated protein modifications – NO_2-FAs and guanine nucleotides (8-nitroguanosine) – and the roles of NO in liver diseases, concluding with suggestions for interesting areas of study to be explored in the context of NO and liver biology.

Mode of action of NO

The most recognized action of NO is through cGMP as the second messenger. The cGMP-dependent action is initiated by the binding of NO to a metal center of guanylyl cyclase (GC), which activates this enzyme, leading to increased production of cGMP. However, cGMP-independent actions of NO, such as protein tyrosine nitration and S-nitrosylation, have also been implicated in various diseases. For example, proteomic analysis of the liver has indicated the importance of S-nitrosylated proteins in the regulation of liver function [8,9]. In pathological conditions, these protein modifications are generally enhanced by increased iNOS-derived NO in conjunction with increased free radicals. In addition to these conventional modes, this section also addresses novel mediators of NO-related action: NO_2-FAs and nucleotides.

Metal ions

NO acts as a signaling molecule by binding to metal ions, proteins, lipids, and guanine nucleotides (Figure 2). Among metal ions, a primary target is ferrous heme iron [10], which forms highly stable NO complexes. A classic example is NO binding to the ferrous heme iron of soluble GC (sGC) to produce cGMP as the second messenger [11,12]. Elevated cGMP directly modulates the activity of phosphodiesterases (PDEs), ion-gated channels, or cGMP-dependent protein kinases to regulate a wide range of physiological functions, including vasodilation, platelet aggregation, and neurotransmission [13].

NO also binds to a heme/copper center of cytochrome c oxidase, also known as Complex IV, the terminal enzyme in the mitochondrial respiratory chain [14,15] and irreversibly inhibits its activity. This inhibition facilitates the reduced state of electron carriers in the respiratory chain, increasing O_2^- generation as well as reducing ATP production [14,15] and thereby influencing energy metabolism.

Another important example is the binding of NO to the heme of NOS, which inhibits NOS enzyme activity [16]. The binding of NO to Fe(II) or Fe(III) heme attenuates the oxygenase activity of NOS. This may serve as a negative feedback system of NOS activity. It is also possible that

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the increased iNOS-derived NO observed in many types of liver disease may inhibit eNOS activity through this mechanism, contributing to decreased eNOS-derived NO in LSECs and thus LSEC dysfunction.

Free radicals

NO reacts with reactive oxygen species (ROS) to form RNS such as $\bullet NO_2$, $ONOO^-$, HNO_2, and NO_2^+ (Figure 1C). Perhaps the most studied RNS-forming reaction is the reaction between •NO and the superoxide radical $O_2\bullet^-$, which generates peroxynitrite ($ONOO^-$) [17], a strong biological oxidant generally implicated in toxic effects on cells and tissues. Mitochondria have been considered the main source of ROS, which mostly originate from the mitochondrial respiratory chain [18]. Excess mitochondrial $ONOO^-$ can impair oxidative phosphorylation by inhibiting the respiratory chain complexes (Complex I, Complex IV, and ATP synthase) and manganese superoxide dismutase (MnSOD) activity [19]. In addition, $ONOO^-$ can oxidize DNA bases, tyrosine residues of proteins, and thiol groups [i.e., cysteine thiol and glutathione (GSH)], causing hepatocyte death [20,21].

The depletion of the GSH pool and an increase in an oxidized form of GSH (GSSG) are closely related to the action of ROS and RNS on cell and liver function [22]. GSH is a triple peptide (γ-L-glutamyl–L-cysteinyl–glycine) synthesized in the cytosol and transported to cellular organelles including the endoplasmic reticulum (ER), nucleus, and mitochondria. With cysteine in its backbone, GSH plays an important role in the reduction of electrophiles and oxidants [23]. GSH becomes oxidized to GSSG when it reduces target molecules. Thus, the ratio of GSH to GSSG is a good indicator of oxidative stress and redox balance [24]. Depletion of the GSH pool is associated with the pathogenesis of liver diseases such as drug-induced liver toxicity (i.e., acetaminophen overdose) [25], chronic alcohol intake [26], nonalcoholic fatty liver disease (NAFLD) [27], and biliary cirrhosis [28]. Chronic alcohol intake, NAFLD, and biliary cirrhosis deplete the mitochondrial GSH pool by impairing GSH transport to the mitochondrial matrix, which is largely caused by alterations of the lipid composition (i.e., increased cholesterol content) in mitochondrial membranes [22].

NOS generates the superoxide anion (O^{2-}) instead of NO [29,30] in a condition known as 'NOS uncoupling'. NOS uncoupling occurs when tetrahydrobiopterin (BH4), an essential cofactor, is depleted [31]. NOS can convert L-arginine to NO and L-citrulline only as a dimer because the BH4-binding site is located at the dimer interface and monomeric NOS cannot bind BH4 or L-arginine [32]. Oxidative stress plays a major role in depleting the cellular BH4 pool, causing NOS uncoupling and further exacerbating oxidative stress [33]. Reduced bioavailability of NO as a result of NOS uncoupling has also been implicated in endothelial cell dysfunction [34].

Protein modification (tyrosine nitration and S-nitrosylation)

Nitration of protein tyrosine residues creates irreversible and stable adducts and has been considered an indicator of nitrosative stress (Figure 2). Tyrosine nitration of proteins often occurs in inflammatory conditions and mediates peroxynitrite-induced cell death signaling [35].

Unlike tyrosine nitration, S-nitrosylation is a reversible reaction (Figure 2), in which oxidized NO binds to a specific cysteine thiol anion in proteins and peptides [36]. Protein S-nitrosylation is enhanced by NOS activity but decreased by S-nitrosoglutathione (GSNO) reductase (GSNOR), a ubiquitous and highly conserved denitrosylase, thus serving as a key regulator of protein S-nitrosylation [37]. eNOS facilitates S-nitrosylation in endothelial cells by specifically localizing at the Golgi apparatus and generating a high concentration of the NO pool locally [38,39].

Several potential targets, such as CD147 and various mitochondrial proteins, for protein S-nitrosylation have been identified in the liver [8,9]. It has been suggested that the dysregulation of protein S-nitrosylation, mostly mediated by iNOS, is associated with the pathogenesis of various liver diseases, including hepatocellular carcinoma (HCC) [40,41], hepatic steatosis [9], and cholestasis [42,43].

For example, lack of GSNOR resulted in S-nitrosylation, ubiquitination, and proteosomal degradation of O^6-alkylguanine-DNA-alkyltransferase (AGT). AGT is a key DNA repair enzyme and was reported to provide protection against dialkylnitrosamine-induced HCC [44]. Both pharmacological inactivation and genetic deletion of iNOS prevented S-nitrosylation and inactivation of AGT in GSNOR knockout (KO) mice, suggesting a role of iNOS in S-nitrosylation and liver carcinogenesis in the absence of the GSNOR gene [40]. Similarly, decreased expression and activity of GSNOR as well as increased S-nitrosylated proteins were observed in cholestatic livers isolated from mice following bile duct ligation (BDL) surgery (an experimental model of cholestasis). Treatment with an iNOS inhibitor, S-methylisothiourea, ameliorated hepatocellular injury with restoration of GSNOR activity and decreased S-nitrosylated proteins, suggesting a contribution of increased S-nitrosylated proteins generated by increased iNOS-derived NO to cholestatic liver disease [43].

Another S-nitrosylated protein was also reported for its possible contribution to cholestasis. Taurocholic acid, a form of bile acid, is involved in the emulsification of fat. Its uptake by hepatocytes is mediated by the transporter NTCP/Ntcp, a Na^+-dependent taurocholate cotransporting polypeptide. NO derived from iNOS S-nitrosylated Ntcp at Cys96 and inhibited taurocholic acid uptake in hepatocytes *in vitro*. This result implies that S-nitrosylation of Ntcp causes sepsis-associated cholestasis by inhibiting taurocholic acid uptake and thus decreasing bile flow [42].

NO signaling also appears to be an important regulator of the bile salt pool in hepatocytes [45]. Excess bile salts stimulated NO production in hepatocytes, leading to S-nitrosylation and nuclear translocation of GAPDH. Interestingly, S-nitrosylated GAPDH (SNO-GAPDH) transnitrosylated HDAC2 and SIRT1 in the nuclei of hepatocytes. S-nitrosylated HDAC2 formed a complex with SHP that repressed gene expression of CYP7A1, an enzyme that converts cholesterol to bile acids. Thus, this mechanism could work as a negative feedback by decreasing bile acid production.