INTRODUCTION

Nitric oxide (NO) plays important roles in hepatic physiology and pathophysiology. NO is generated as a by-product of the oxidation of L-arginine to citrulline by the action of three isoforms of NO synthases (NOSs): neuronal NOS (nNOS or NOS1), inducible NOS (iNOS or NOS2), and endothelial NOS (eNOS or NOS3). nNOS and eNOS are constitutively expressed. iNOS expression is absent in resting cells but is induced by immunological stimuli, such as bacterial lipopolysaccharide (LPS) and inflammatory cytokines. In the liver, small amounts of NO generated by eNOS are thought to be important for liver homeostasis and protective against pathological conditions. In contrast, large amounts of NO generated by iNOS are implicated in the etiologies of many liver diseases, including liver fibrosis. The role of nNOS in liver biology is not well known.

This review article will summarize the role of iNOS in liver fibrosis, addressing 1) iNOS biology, 2) iNOS-expressing liver cells, 3) iNOS-related therapeutic strategies, and 4) future directions. (Clin Mol Hepatol 2015;21:319-325)

Keywords: iNOS; NO; Liver sinusoidal endothelial cell; Hepatic stellate cell; Kupffer cell

iNOS BIOLOGY

Mode of action of NO

NO can regulate biological processes by virtue of its highly reac-
tive nature. NO can bind to a wide range of molecules, such as free radicals, metal centers of enzymes [e.g., guanylyl cyclase (GC) and cytochrome C oxidase], tyrosine or cysteine residues of proteins, guanine nucleotides, and polyunsaturated fatty acids. In the most renowned mode of NO action, NO activates GC by binding to its metal center (i.e., ferrous heme iron). This produces cyclic guanosine monophosphate (cGMP), which then binds to phosphodiesterases or cGMP-dependent protein kinases (PKG).

BH4 is an essential cofactor for iNOS activity. iNOS gene
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variation in NO production, and protein stability plays a critical role in iNOS induction.

2. Regulation of iNOS expression by transcription factors

Due to its inducible nature, the regulation of iNOS transcription is considered the most important step in the control of iNOS activity, as opposed to constitutively expressed eNOS and nNOS, the regulation of which is more heavily governed by post-translational modifications and protein–protein interactions than by mRNA expression. The most studied pathway in the liver is the activation of the transcription factor, nuclear factor-kappa β (NF-κβ), which can bind to the iNOS promoter and induce iNOS expression. In addition, various transcription factors have been shown to regulate iNOS expression. Those include signal transducer and activator of transcription-1α (STAT-1α), interferon regulatory factor-1 (IRF-1), activator protein-1 (AP-1), octamer transcription factors, cAMP-dependent transcription factors, T-cell factor 4 (TCF-4), transcription factor 11 (TCF-11), epidermal growth factor receptor, signal transducer and activator of transcription-3 (STAT-3), nuclear receptors, glucocorticoid receptor-α and -β (GR-α and -β), and estrogen receptor-α and -β (ER-α and -β). Furthermore, signaling pathways that stimulate these transcription factors appear to differ, depending on cell type and species. Understanding these differences in iNOS inducers and their related pathways could be a key to the modulation of iNOS induction. In addition to transcriptional regulation, the control of iNOS mRNA stability, mRNA translation, and protein stability plays a critical role in iNOS induction.

3. Regulation of iNOS enzymatic activity

iNOS activity is dependent on the availability of its substrate, arginine, and is influenced by the activity of arginase, which cata-
lyzes the conversion of L-arginine to L-ornithine and urea, thereby decreasing the availability of L-arginine for iNOS activity. Tetrahy-
drobipterin (BH4) is an essential cofactor for iNOS activity. Because homodimerization of iNOS is required for its activity and BH4 is essential for this process, the mechanism regulating BH4 synthesis and consumption can also regulate iNOS activity. GTP cyclohydrolase 1 (GTPCH) is an essential enzyme in the biogenesis of BH4. Deletion of this enzyme in macrophages blocked NO syn-
thesis in response to LPS and IFNγ stimulation, despite the pres-
ence of iNOS protein. In addition, a lack of BH4 biogenesis result-
ed in increased production of reactive oxygen species (ROS) due to the uncoupling of iNOS, which is mediated by BH4. However, these events were reversible because the administration of se-
piapterin, a precursor of BH4, could restore BH4, NO production, and the cellular redox state.

Inhibitory proteins of iNOS activity have been identified. In mu-
rine macrophages, a 110-kDa protein, called NAP110, directly inter-
acted with the amino terminus of iNOS and prevented its di-
merization, thereby inhibiting iNOS activity. iNOS protein
synthesizes NO continuously until the enzyme is degraded.

Therefore, factors affecting iNOS protein degradation could regu-
late iNOS activity and NO production.

INOS AND LIVER FIBROSIS

Liver fibrosis is a consequence of a wide range of liver injuries caused by hepatitis virus, drug, toxin, bile acid accumulation, autoimmunity, obesity/metabolic syndrome, and alcohol. iNOS induction is associated with liver fibrosis of diverse etiology.

The role of iNOS has been studied using iNOS knockout mice and iNOS-specific or NOS inhibitors in vivo and in vitro. iNOS gene deletion resulted in reduced liver fibrosis. iNOS KO mice fed a high-cholesterol diet for 6 weeks exhibited significant reductions in hepatic fibrosis and expression of inflammatory cytokines, including transforming growth factor-beta (TGFβ) and tumor necrosis factor-alpha (TNFα). In addition, iNOS deletion decreased hypoxia-inducible factor-1alpha (HIF1α) levels and HIF1α-associated gene expression, including that of platelet-derived growth factor (PDGF)-A, PDGF-B, fibroblast growth factor-2 (FGF-2), and plasminogen activator inhibitor-1 (PAI-1). A similar reduction in high-cholesterol diet-induced hepatic fibrosis was observed in mice provided with an iNOS-specific inhibitor (PBIT). Taken together, both genetic dele-
tion and pharmacological inhibition of iNOS resulted in decreased hepatic fibrosis, suggesting a role of iNOS in the development of