Current concepts on the role of nitric oxide in portal hypertension

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Abstract

Portal hypertension (PHT) is defined as a pathological increase in portal venous pressure and frequently accompanies cirrhosis. Portal pressure can be increased by a rise in portal blood flow, an increase in vascular resistance, or the combination. In cirrhosis, the primary factor leading to PHT is an increase in intra-hepatic resistance to blood flow. Although much of this increase is a mechanical consequence of architectural disturbances, there is a dynamic and reversible component that represents up to a third of the increased vascular resistance in cirrhosis. Many vasoactive substances contribute to the development of PHT. Among these, nitric oxide (NO) is the key mediator that paradoxically regulates the sinusoidal (intra-hepatic) and systemic/splanchnic circulations. NO deficiency in the liver leads to increased intra-hepatic resistance while increased NO in the circulation contributes to the hyperdynamic systemic/splanchnic circulation. NO mediated-angiogenesis also plays a role in splanchnic vasodilation and collateral circulation formation. NO donors reduce PHT in animals models but the key clinical challenge is the development of an NO donor or drug delivery system that selectively targets the liver.

Key words: Nitric oxide; Portal hypertension; Hepatic stellate cell; Liver cirrhosis

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INTRODUCTION

Portal hypertension (PHT) is a common clinical consequence of chronic liver disease that is associated with significant morbidity and mortality. PHT is classified as either pre-hepatic, intra-hepatic or post-hepatic, with intra-hepatic PHT being the form most often caused by cirrhosis, irrespective of etiology[1]. The extent of PHT is quantified in clinical practice by measuring the hepatic portal vein pressure gradient (HPVG)[2], representing the difference between the wedged hepatic vein pressure (a measure of pressure at the level of the hepatic sinusoid), and the free hepatic vein pressure. Thus, HPVG is often used to assess the effects of pharmacological therapy in reducing portal pressure[3].

Based on hydromechanics, fluid pressure in a hollow tube is determined by fluid resistance and flow. In PHT, therefore, the intra-hepatic vascular resistance (IHVR) and splanchnic blood flow are the two main contributors to portal pressure[4]. Under normal circumstances, post-prandial increases in splanchnic blood flow is always associated with an autonomous down-regulation of IHVR, leading to no alteration in portal pressure. In contrast, IHVR is significantly up-regulated by mechanical and hemodynamic factors in the setting of cirrhosis, which is further aggravated by splanchnic vasodilatation[5]. Clinically, this increase in portal pressure is the antecedent to
variceal bleeding with its associated morbidity and high mortality.\textsuperscript{6,7}

IHVR is influenced by both hepatic fibrotic architectural distortion in cirrhosis leading to obstruction to blood flow, as well as by dynamic hepatic stellate cell (HSC) contraction around sinusoidal blood vessels. Angiogenesis, or the formation of new blood vessels, is also an important component of the pathophysiology of PHT. The resulting alterations in vascular contractility and angiogenesis contribute to PHT in both the intrahepatic and splanchnic circulation.

Endothelin 1 (ET-1), angiotensin II, norepinephrine, prostaglandin F\textsubscript{2}, thromboxane A\textsubscript{2}, and thrombin can trigger liver sinusoidal contraction. In contrast, substances such as acetylcholine, vasointestinal peptides, nitric oxide (NO), carbon monoxide, prostaglandin E\textsubscript{2}, and adrenomedullin relax the sinusoidal vasculature.\textsuperscript{8,9} Among these agents, ET-1 and NO are the most important regulators of the sinusoidal microcirculation.\textsuperscript{8,9} In PHT, an insufficient release of vasodilators particularly NO from endothelial cells is critical to the genesis of the dynamic and modifiable component of increased vascular resistance.\textsuperscript{8,9} Consistent with this, improvements in intrahepatic NO availability is beneficial for the treatment of PHT in animals and patients.\textsuperscript{10-14} Hence, this review will focus on an update on the mechanisms whereby NO mediates PHT and on the potential to modulate this system to reduce portal pressure.

**SYNTHESIS AND FUNCTION OF NO**

NO is synthesized by nitric oxide synthase (NOS) through a series of redox reactions involving L-arginine (the main substrate), oxygen and nicotinamide adenine dinucleotide phosphate. There are 4 major isoforms of NOS: endothelial nitric oxide synthase (eNOS), inducible nitric oxide synthase (iNOS), neuronal nitric oxide synthase (nNOS) and mitochondrial nitric oxide synthase.\textsuperscript{15} Following synthesis by NOS, the half-life of endogenously generated NO is extremely short, about 1 s. Thus, endogenous NO production is intimately regulated by the activity of NOS.

The generated NO molecule has a large diffusion coefficient and can therefore freely penetrate cellular membranes in an autocrine or paracrine manner. Within the cell, NO stimulates the conversion of guanosine 5'-triphosphate (GTP) to cyclic guanosine 3’-5’-monophosphate (cGMP) by soluble guanylyl cyclase (sGC). The cGMP-dependent protein kinase pathway (Figure 1). This leads to vasodilatation.\textsuperscript{16} The end products of NO metabolism in vivo are nitrate (NO\textsubscript{3}⁻) and nitrite (NO\textsubscript{2}⁻) that are an indirect measure of the total NO concentration.\textsuperscript{17}

NO is also highly reactive with other molecules including superoxide anion (O\textsubscript{2}⁻), oxygen (O\textsubscript{2}) and hemoproteins such as hemoglobin and myoglobin. The intermediate products of these reactions are known as reactive nitrogen species, which promotes many pathophysiologically damaging reactions including lipid peroxidation, DNA strand breaks, and the generation of nitrosoamines, nitrotyrosine and nitro guanosine.

**MOLECULAR MECHANISMS REGULATING NOS**

eNOS serves a key role in maintaining circulatory homeostasis and is expressed mainly in endothelial cells and to a lesser extent in cardiac myocytes and platelets.\textsuperscript{18} The enzyme localizes to small invaginations of the plasma membrane named caveolae in quiescent cells. eNOS protein is constitutively expressed in the cell and activation mostly comprises post-translational regulation and modifications in its subcellular localization.\textsuperscript{19}

Within cells, eNOS closely associates with several proteins that impact on its function, including caveolin. Caveolin negatively regulates eNOS by directly abrogating the enzyme’s activation and blocking the binding site for calmodulin.\textsuperscript{20} In contrast, calmodulin acts as an indispensable protein competing with caveolin for binding with, and activating eNOS.\textsuperscript{21,22} Other relevant proteins in relation to NO production include heat shock protein 90 and tetrahydrobiopterin (BH4) that are positive regulators of eNOS\textsuperscript{23,24}. Finally, eNOS interacting protein and eNOS trafficking inducer protein participate in the subcellular trafficking of eNOS when eNOS translocates from caveolae into the cytoplasm.\textsuperscript{25}

Phosphorylation at key serine residues is the major post-translational modification that is required for eNOS...
function. Phosphorylation of Ser 1177, Ser 635 and Ser 617 activates eNOS whereas phosphorylation of Thr 495 and Ser 116 inhibits eNOS activity. Phosphorylation at Ser 1177 can be initiated by activation of several intracellular pathways including phosphatidylinositol-3-kinase (PI3K/AKT), cAMP-dependent protein kinase A (PKA), adenosine monophosphate-activated protein kinase (AMPK), protein kinase B (AKT) and protein kinase A (PKA) pathways, whereas protein phosphatase 2 (PP2A) de-phosphorylates eNOS. In addition, S-nitrosylation (SNOs) by eNOS-derived nitric oxide (NO) inhibits eNOS activity. Endothelial nitric oxide synthase interacting protein (NOSIP) and endothelial nitric oxide synthase trafficking inducer protein (NOSTRIN) regulate the sub-cellular location of eNOS protein between the caveolae and cytoplasm. The principal location of eNOS is in caveolae where its function is inhibited by binding to caveolin (Cav). HSP90, calmodulin (Calm) and tetrahydrobiopterin (BH4) are indispensable proteins and cofactors for catalyzing NO production. PI3K: Phosphatidylinositol-3-kinase; GPCR: G protein-coupled receptor.

Figure 2 The molecular regulation of endothelial nitric oxide synthase activity. Endothelial nitric oxide synthase (eNOS) phosphorylation can be triggered by shear stress, vascular endothelial growth factor (VEGF), endothelin 1 (ET-1) and other factors though adenosine monophosphate-activated protein kinase (AMPK), protein kinase B (AKT) and protein kinase A (PKA) pathways, whereas protein phosphatase 2 (PP2A) de-phosphorylates eNOS. In addition, S-nitrosylation (SNOs) by eNOS-derived nitric oxide (NO) inhibits eNOS activity. Endothelial nitric oxide synthase interacting protein (NOSIP) and endothelial nitric oxide synthase trafficking inducer protein (NOSTRIN) regulate the sub-cellular location of eNOS protein between the caveolae and cytoplasm. The principal location of eNOS is in caveolae where its function is inhibited by binding to caveolin (Cav). HSP90, calmodulin (Calm) and tetrahydrobiopterin (BH4) are indispensable proteins and cofactors for catalyzing NO production. PI3K: Phosphatidylinositol-3-kinase; GPCR: G protein-coupled receptor.

MOLECULAR REGULATION OF NOS IN LIVER CIRRHOSIS AND PHT

Regulation of intra-hepatic eNOS

In cirrhosis and PHT, there is reduced NO production
by hepatic endothelial cells that is attributed to dysfunction of the eNOS system. Many factors contribute to intra-hepatic eNOS dysfunction/reduced eNOS activity. These include increases in oxidative stress, caveolin-1, RhoA, thromboxane A2 (TXA2), G-protein-coupled receptor kinase-2 (GRK2) and asymmetric dimethylarginine (ADMA) as well as decreased AKT and BH4 activity.

Reduced AKT activity and increased binding ability of caveolin-1 to eNOS in cirrhosis attenuates eNOS expression. Liu et al. reported that ET-1 activates G-protein-coupled receptor kinase-2 (GRK2) which directly interacts with and inhibits AKT phosphorylation. They also noted that the IHVR was significantly reduced in bile duct ligation (BDL) mice genetically deficient in GRK2. In another study of eNOS expression during BDL, Morvarid et al. noted that total eNOS protein was unchanged, but that functional, phosphorylated eNOS protein was decreased. Similarly, AKT expression was down-regulated in a time dependent manner. In contrast, caveolin-1 was increased.

Intrahepatic oxidative stress is a key mediator of sinusoidal endothelial dysfunction and impairment of eNOS/NO expression. For example, Gracia et al. noted that increased intrahepatic oxidative stress (increased ROS and O2·−) was associated with reduced NO production and NO bioavailability. The authors went on to demonstrate that cyclooxygenase (COX) attenuated eNOS activation by stimulating TXA2 which inhibits AKT phosphorylation in endothelial cells. A superoxide dismutase mimetic, Tempol significantly decreased superoxide, and increased NO in cultured hepatic endothelial cells. As expected, Tempol administration also resulted in a decline of portal pressure.

ADMA, an endogenous inhibitor of NO, causes uncoupling of NO leading to generation of RNs, such as peroxynitrite. In BDL rats, a higher serum ADMA level was observed. Further, impaired endothelial cell-mediated relaxation in perfused livers of BDL rats was exacerbated by ADMA and was associated with a decreased rate of ADMA removal.

BH4, a cofactor of eNOS, has been reported to be associated with dysfunction of the NO system. BH4 expression is down-regulated in liver cirrhosis and can further be oxidized and inactivated by O2·−. In the absence of BH4, eNOS cannot generate NO but instead produces O2·−, thereby leading to further decreases in NO production. In an in vivo study, Matei et al. observed that in rats rendered cirrhotic after the administration of carbon tetrachloride (CCL4), exogenous BH4 resulted in hepatic NO and cGMP activation and a reduction in portal pressure.

Rho-associated protein kinase (ROCK) is a kinase belonging to the AGC (PKA/PKG/PKC) family of serine/threonine kinases. It is mainly involved in regulating the shape and movement of cells by acting on the cytoskeleton. Rho-kinase is substantially involved in the contraction of activated HSCs. In BDL rats, fasudil (a potent Rho-kinase inhibitor) significantly suppressed liver Rho-kinase activity and increased eNOS phosphorylation compared with controls. Fasudil also reduced the binding of the serine/threonine AKT to Rho-kinase and increased the binding of AKT to eNOS.

**Regulation of extra-hepatic vascular eNOS, iNOS and nNOS in cirrhosis**

In contrast to the hypoactive SECs in the intrahepatic microcirculation, hyperactive endothelial cells with increased NO production play a critical role in modulating the vascular changes observed in the splanchic and systemic circulation. For example, increased activity of peripheral vascular AKT signaling is noted, while constitutive AKT inhibition by an inactive mutant decreases aortic eNOS and improves systemic hemodynamics, splanchic perfusion pressure and renal excretory function without affecting portal pressure. Other studies reported that VEGF induces NO production by activation of eNOS protein expression and activity. Likewise, in portal hypertensive rats, NO production is increased in response to shear stress. LPS detoxification is limited in liver with PHT thereby increasing plasma LPS. Resident macrophages in the splanchic circulation respond to this circulating LPS with the production of proinflammatory cytokines, such as TNF-α that then induces iNOS in extrahepatic vasculature. Bacteria-derived TNF-α also triggers the expression and activity of the key enzyme involved in the regulation of BH4, GTP-cyclohydrolase I, thereby increasing eNOS-derived NO in the mesenteric vasculature. Finally, nNOS expression is augmented in mesenteric nerves in portal hypertensive rats (portal vein ligation), an effect mediated by HSP-90.

**The role of NO/NOS in the regulation of IHVR**

An increase in IHVR can be induced by reversible hemodynamic modifications to vascular tone which may represent 28%–40% of the increase in portal pressure in cirrhosis. Anatomical structures leading to this change include vascular smooth muscle cells surrounding branches of the portal vein, and HSCs located in the space of Disse. Both cells types have contractile properties and thus modulate IHVR.

The role of NO in the modulation of IHVR has been well documented. eNOS dysfunction in sinusoidal endothelial cells and consequent reduction in NO production (or bioavailability) plays an essential role. This results in reduced vasodilatation and a decreased capacity for antagonizing contractile factors such as ET-1, angiotensin II, norepinephrine, prostaglandin F2, and thromboxane A2.

Recently, gene delivery techniques have been used to increase NO (eNOS or nNOS) delivery to the liver of CCL4 treated mice. In one study, a plasmid eukaryotic expression vector (liposome-pcDNA3/eNOS) or control vector was injected into rat portal vein, leading to increased eNOS mRNA and protein in liver. Hepatic
NO production was enhanced and IHVR and portal vein pressure (PVP) reduced\textsuperscript{[89]}. In another study, recombinant adenovirus carrying the nNOS gene (Ad.nNOS) or control vector was administered \textit{via} the femoral vein to rats. Again, Ad.nNOS reduced IHVR and portal pressure\textsuperscript{[89]}. These data indicate that NO deficiency in cirrhotic liver contributes to the elevation in IHVR and conversely that NO delivery may play a therapeutic role\textsuperscript{[89-92]}

Activation and contraction of HSCs also contributes significantly to the dynamic and reversible component of IHVR. Indeed, activated HSCs are more susceptible to vasoconstrictor substances than quiescent cells\textsuperscript{[83,93]}. Under physiological conditions, NO produced by hepatic endothelial cells inhibits the growth, migration and contraction of HSCs through paracrine pathways\textsuperscript{[94,95]}. However, reduced NO production and/or impaired NO bioavailability in cirrhosis promotes HSCs activation and contraction, leading to sinusoidal remodeling and elevation of the IHVR.

iNOS has also been suggested to contribute to the hyperdynamic status seen in PHT. However, its role in mediating IHVR is unclear. In one study, liver iNOS was increased in BDL rats and reduction of portal pressure by ursodeoxycholic acid was associated with iNOS down-regulation\textsuperscript{[96,71]}

**ROLE OF NO/NOS IN THE REGULATION OF SPLANCHNIC BLOOD FLOW**

A hyperdynamic splanchnic circulatory state is a major accompaniment of PHT. The increase in splanchnic blood flow and the subsequent increase in portal venous inflow aggravates and perpetuates PHT. The mechanisms underlying this phenomenon are not fully understood, but overproduction of endogenous vasodilators and decreased vascular reactivity to vasoconstrictors has been suggested\textsuperscript{[99]}

Overproduction of NO in the splanchnic and systemic circulation contributes to this phenomenon as NO inhibition effectively ameliorates splanchnic hyperemia\textsuperscript{[99,100]}. eNOS up-regulation and increased NO release by the superior mesenteric arteries endothelium occur before the development of the hyperdynamic splanchnic circulation\textsuperscript{[101]}. Juan \textit{et al}\textsuperscript{[70]}, noted increased eNOS expression in portal-hypertensive rats with even mild increases in portal pressure. In another study, phosphorylated eNOS protein was increased, whereas caveolin-1 was decreased in the aorta of BDL rats\textsuperscript{[52]}. In contrast, in eNOS knockout mice injected with CCLA, attenuated splanchnic blood flow was observed. However, this was associated with an increase in IHVR, presumably due to the reduced NO within the liver\textsuperscript{[102]}. Taken together, these results suggest up-regulated eNOS expression during splanchnic hyperemia, contrasts with the relative eNOS deficiency in liver.

There are also several studies demonstrating the importance of iNOS in the hyperdynamic circulation of cirrhosis\textsuperscript{[84,72,73,103,104]}. In cirrhosis, endotoxins, cytokines and bacterial infection promote iNOS formation and overproduction of NO\textsuperscript{[84,105-107]}. The increased splanchnic iNOS appears to reside in resident macrophages of the superior mesenteric artery\textsuperscript{[73,108]}. Supporting this concept, Ferguson \textit{et al.}\textsuperscript{[64]}, observed that a selective iNOS inhibitor, N-[3-(aminomethyl) benzyl]acetamidine, caused peripheral vasoconstriction in patients with cirrhosis. It is interesting to note that there also exists an interaction between eNOS and iNOS in the vasculature. For example, in cirrhosis, increased and dominant expression of eNOS in large arteries results in systemic hypotension and increased blood flow. These effects could be abrogated by activated iNOS in the small vessels of the splanchnic circulation as iNOS activation inhibited eNOS expression in the small vessels\textsuperscript{[109]}. nNOS may likewise promote vasodilation of the splanchnic circulation, though its contribution is overall less significant\textsuperscript{[110,111]}

**NO AND ANGIOGENESIS IN PHT**

It is now established that angiogenesis is associated with the progression of PHT\textsuperscript{[112,113]}. Angiogenic factors stimulate collateral vessel formation both in the liver and in extrahepatic locations, manifesting as the reopening of pre-existing shunts\textsuperscript{[114,115]}. This pathological angiogenesis may directly participate in the development of liver fibrosis\textsuperscript{[6,116,117]}

Again, NO is an important mediator of intrahepatic microcirculatory remodeling\textsuperscript{[114,115]}. Thus, NO inhibition prevents angiogenesis and diminishes mesenteric vascular proliferation in animals with PHT\textsuperscript{[112,116,119]}. Shaki \textit{et al.}\textsuperscript{[120]} found that NO-mediated angiogenesis was mediated by endothelial VEGF and VEGF receptor-1. Most recently, Huang \textit{et al}\textsuperscript{[121]}, reported that through mesenteric eNOS and COX1 down-regulation, the cannabinoid receptor 2 agonist JWH 015, could alleviate mesenteric and intrahepatic angiogenesis, PHT, the severity of portosystemic collaterals and the extent of fibrosis in BDL cirrhotic rats.

**NO-BASED PHARMACOTHERAPY**

As discussed, NO is paradoxically regulated in PHT. There is excessive production of NO in the splanchnic circulation (thereby leading to vasodilation), while in the intra-hepatic microcirculation, a deficit of NO production is associated with increased IHVR. These paradoxical roles of NO initially raised concerns about the use of NO inhibitors or donors as therapy for PHT. However, inhibition of NO release has been shown in animals and humans to attenuate the hyperdynamic circulation of cirrhosis\textsuperscript{[122,123]}. No significant reduction in portal pressure was achieved\textsuperscript{[122-124]}. This is likely a consequence of reductions in portal venous inflow induced by the NO inhibitors being offset by an increase in intra-hepatic resistance.

In recent years, many animal and clinical studies have
demonstrated that NO donors result in a substantial reduction in portal pressure. These agents could theoretically aggravate the cirrhotic vasodilatory syndrome leading to harmful effects such as systemic hypotension and renal dysfunction. For these reasons, the ideal NO drug for the treatment of PHT should act to decrease HVVR without worsening splanchic/systemic vasodilatation.

NCX-1000 is a drug synthesized by adding an NO-releasing moiety to ursodeoxycholic acid. The compound is selectively metabolized by hepatocytes to release NO in the liver. Animal studies demonstrate that this drug alleviates HVVR and portal pressure without changes in systemic hemodynamics. However, human clinical trials were disappointing as NCX-1000 failed to decrease HVPG, there were postprandial increases in portal pressure and systolic blood pressure was reduced in a dose-dependent manner.

O2-vinyl(4-pyrrolidin-1-yl)diazene-1-ium-1,2-diolate (V-PYRRO/NO) was designed as a liver-selective NO-producing prodrug activated by hepatic P450. The drug has a short half-life and may additionally alleviate liver injury by NO-mediated protection of hepatocytes. Continuous administration of V-PYRRO/NO to BDL rats was shown to improve liver fibrosis and splanchic hemodynamics without adverse systemic effects. In another study in mice using the CCl4 model, V-PYRRO/NO significantly lowered mean arterial pressure making it less suitable for use in humans.

AVE-9488 (4-fluoro-N-indan-2-yl-benzamide) is a novel agent that up-regulates eNOS expression. Biecker et al. reported that oral application of AVE 9488 ameliorated portal pressure by 24% in BDL rats, without any impact on the mean arterial pressure. Additional experiments confirmed that AVE 9488 increased hepatic eNOS protein synthesis, but not in the aortic and superior mesenteric artery. However, following 3-d use, AVE 9488 increased blood flow in the collateral circulation.

Recently, an inorganic gold and silica nanoparticle mediated drug delivery system using SNAP (S-nitroso-N-acetyl-DL-penicillamine), an NO donor was reported. This system inhibited HSC proliferation and HSC tube formation, though the relevance of the latter to the situation in vivo is unclear. The methodology described however, does provide a novel approach to deliver NO into specific liver cell types. Whether this drug modulates PHT in vivo is unclear. Taken together, the data presented indicates that there are no liver-selective NO donors/drugs with demonstrated efficacy for the treatment of PHT.

CONCLUSION
NO plays a pivotal role in the pathogenesis of PHT. NO levels are differentially altered in cirrhosis, with reduced production in the intrahepatic circulation and increased NO production in the splanchic bed. Ideally, a NO donor or drug delivery system that selectively targets liver cells (HSCs or SECs) without actions on the systemic circulation is required to reduce PHT without adverse systemic effects.

REFERENCES
1 Buob S, Johnston AN, Webster CR. Portal hypertension: pathophysiology, diagnosis, and treatment. J Vet Intern Med 2011; 25: 169-186 [PMID: 21382073 DOI: 10.1111/j.1939-1676.2011.00691.x]
2 Burroughs AK, Thalheimer U. Hepatic venous pressure gradient in 2010: optimal measurement is key. Hepatology 2010; 51: 1894-1896 [PMID: 20512984 DOI: 10.1002/hep.23710]
3 García-Tsao G, Bosch J, Grossmann RJ. Portal hypertension and varical bleeding–unresolved issues. Summary of an American Association for the study of liver diseases and European Association for the study of the liver single-topic conference. Hepatology 2008; 47: 1764-1772 [PMID: 18435460 DOI: 10.1002/hep.22273]
4 Cichoł-Lach H, Celinski K, Słomka M, Kasztelan-Szczepińska B. Pathophysiology of portal hypertension. J Physiol Pharmacol 2008; 59 Suppl 2: 251-258 [PMID: 18812641]
5 Moneta GL, Taylor DC, Helton WS, Mulholand MW, Strandness DE. Duplex ultrasound measurement of post-prandial intestinal blood flow: effect of meal composition. Gastroenterology 1988; 95: 1294-1301 [PMID: 3049214]
6 Albillos A, Bañares R, González M, Catalina MV, Pastor O, González R, Ripoll C, Bosch J. The extent of the collateral circulation influences the postprandial increase in portal pressure in patients with cirrhosis. Gut 2007; 56: 259-264 [PMID: 16837532 DOI: 10.1136/gut.2006.095240]
7 Bellis L, Berzigotti A, Albraldes JG, Moitinho E, Garcia-Pagán JC, Bosch J, Rodés J. Low doses of isosorbide mononitrate attenuate the postprandial increase in portal pressure in patients with cirrhosis. Hepatology 2003; 37: 378-384 [PMID: 12540788 DOI: 10.1053/jhep.2003.50053]
8 Rockey DC. Hepatic fibrosis, stellate cells, and portal hypertension. Clin Liver Dis 2006; 10: 459-79, vi-vii [PMID: 17162233 DOI: 10.1016/j.cld.2006.08.017]
9 Ikewaki Y, Grossmann RJ. Vascular endothelial dysfunction in cirrhosis. J Hepatol 2007; 46: 927-934 [PMID: 17391799 DOI: 10.1016/j.jhep.2007.02.006]
10 Laleman W, Van Landeghem L, Van der Elst I, Zeegers M, Feyer J, Nevens F. Nitroflurbiprofen, a nitric oxide-releasing cyclooxygenase inhibitor, improves cirrhotic portal hypertension in rats. Gastroenterology 2007; 132: 709-719 [PMID: 17258737 DOI: 10.1053/j.gastro.2006.12.041]
11 Albraldes JG, Rodríguez-Vilarrulpa A, Grauper A, Zafra C, García-Calderó H, García-Pagán JC, Bosch J. Simvastatin treatment improves liver sinusoidal endothelial dysfunction in CCl4 cirrhotic rats. J Hepatol 2007; 46: 1040-1046 [PMID: 17335931 DOI: 10.1016/j.jhep.2007.01.020]
12 Trebicka J, Hennenberg M, Laleman W, Shelest N, Beicker E, Schepeke M, Nevens F, Sauverbruch T, Heller J. Atorvastatin lowers portal pressure in cirrhotic rats by inhibition of RhoA/Rho-kinase and activation of endothelial nitric oxide synthase. Hepatology 2007; 46: 242-253 [PMID: 17596891 DOI: 10.1002/hep.21673]
13 Albraldes JG, Albillos A, Bañares R, Turnes J, González R, García-Pagán JC, Bosch J. Simvastatin lowers portal pressure in patients with cirrhosis and portal hypertension: a randomized controlled trial. Gastroenterology 2009, 136: 1651-1658 [PMID: 19288350 DOI: 10.1053/j.gastro.2009.01.043]
14 Zafra C, Albraldes JG, Turnes J, Berzigotti A, Fernández M, García-Pagán JC, Rodés J, Bosch J. Simvastatin enhances hepatic nitric oxide production and decreases the hepatic vascular tone in patients with cirrhosis. Gastroenterology 2004; 126:
Hu LS et al. Nitric oxide and portal hypertension

749-755 [PMID: 14988829 DOI: 10.1053/j.gastro.2003.12.007]

Dudzinski DM, Igarashi J, Greif D, Michel T. The regulation and pharmacology of endothelial nitric oxide synthase. *Anna Rev Pharmacol Toxicol* 2006; 46: 235-276 [PMID: 16402905 DOI: 10.1146/annurev.pharm.tox.44.101802.121044]

Wiest R, Groszmann RJ. The paradox of nitric oxide in cirrhosis and portal hypertension: too much, not enough. *Hepatology* 2002; 35: 478-491 [PMID: 11826425]

Miles AM, Chen Y, Owens MW, Grisham MB. Fluorometric determination of nitric oxide. *Methods* 1995; 7: 40-47 [DOI: 10.1016/meth.1995.1006]

Dudzinski DM, Michel T. Life history of eNOS: partners and pathways. *Cardiosorces* 2007; 75: 247-260 [PMID: 17466957 DOI: 10.1016/j.cardiores.2007.03.023]

Minshall RD, Sessa WC, Stan RV, Anderson RG, Malik AB. Caveolin regulation of endothelial function. *Am J Physiol Lung Cell Mol Physiol* 2003; 285: L1179-L1183 [PMID: 14608487]

Shah V, Cao S, Hendrickson H, Yao J, Katusic ZS. Regulation of hepatic eNOS by caveolin and calmodulin after bile duct ligiation in rats. *Am J Physiol Gastrointest Liver Physiol* 2001; 280: G1209-G1216 [PMID: 11352814]

Chen ZP, 17625-17628 [PMID: 11292821 DOI: 10.1074/jbc.C100122200]

Shaul PW. Regulation of endothelial nitric oxide synthase: location, location, location. *Anna Rev Physiol* 2002; 64: 749-774 [PMID: 11826287 DOI: 10.1146/annurev.physiol.64.081501.155952]

Chen PF, 1713-1715 [PMID: 17127362 DOI: 10.2741/2127]

Chen ZP, Mitchell KI, Michell BJ, Stapleton D, Rodriguez-Crespo I, Witters LA, Power DA, Ortiz de Montellano PR, Kemp BE. AMP-activated protein kinase phosphorylation of endothelial NO synthase. *FEBS Lett* 1999; 443: 285-289 [PMID: 10025949 DOI: 10.1016/S0014-5793(98)01705-0]

Fleming I, Fisslthaler B, Dimmeler S, Kemp BE, Busse R. Phosphorylation of eNOS (Ser495) regulates Ca2+/calmodulin-dependent endothelial nitric oxide synthase activity. *Circ Res* 2001; 88: E68-E75 [PMID: 11397791 DOI: 10.1161/01.hrr.101.092677]

Michell BJ, Harris MB, Chen ZP, Ju H, Venema VJ, Blackstone MA, Huang W, Venema RC, Kemp BE. Identification of regulatory sites of phosphorylation of the bovine endothelial nitric-oxide synthase at serine 617 and serine 635. *J Biol Chem* 2002; 277: 4234-4235 [PMID: 12171920 DOI: 10.1074/jbc.M205144200]

Bates TE, Loesch A, Burnstock G, Clark JB. Mitochondrial nitric oxide synthase: a ubiquitous regulator of oxidative phosphorylation. *Biochem Biophys Res Comm* 1996; 218: 1713-1715 [DOI: 10.1016/j.wjg.2015.01.837]
48 Gupta TK, Toruner M, Chung MK, Groszmann RJ. Endothelial dysfunction and decreased production of nitric oxide in the intraportal microcirculation of cirrhotic rats. Hepatology 1998; 28: 926-931 [PMID: 9755227 DOI: 10.1002/hep.5082800405]

49 Petermann H, Vogl S, Schulze E, Dargel R. Chronic liver injury alters basal and stimulated nitric oxide production and 3H-thymidine incorporation in cultured sinusoidal endothelial cells from rats. J Hepatol 1999; 31: 284-292 [PMID: 10453942 DOI: 10.1016/S0168-8278(99)80226-8]

50 Shah V, Toruner M, Haddad F, Cadelina G, Papapetroupooulos A, Choo K, Sessa WC, Groszmann RJ. Impaired endothelial nitric oxide synthease activity associated with enhanced caveolin binding in experimental cirrhosis in the rat. Gastroenterology 1999; 117: 1222-1228 [PMID: 10535886 DOI: 10.1016/S0016-5085(99)70408-7]

51 Liu S, Premont RT, Kontos CD, Zhu S, Rockey DC. A crucial role for GRK2 in regulation of endothelial cell nitric oxide synthesis function in portal hypertension. Nat Med 2005; 11: 952-958 [PMID: 16142423 DOI: 10.1038/rmm1289]

52 Mohammad M, Thabut D, Cazals-Hatem D, Galbois A, Rudler M, Bonnefont-Rousselot D, Moreau R, Lecureur B, Zhou Q, Schmidt M, Jakobs KH, Sauerbruch T, Hufnagel M, Newby DE. Inducible nitric oxide synthase activity contributes to the regulation of peripheral vascular tone in patients with cirrhosis and ascites. Gut 2006; 55: 542-546 [PMID: 16290335 DOI: 10.1136/gut.2005.076562]

53 Hennenberg M, Biecker E, Trebicka J, Jochem K, Zhou Q, Schmidt M, Jakobs KH, Sauerbruch T, Hufnagel M, Newby DE. RhoA/Rho-kinase signaling contributes to vascular hypococontractility and vasodilatation in cirrhotic rats. Gastroenterology 2006; 130: 838-854 [PMID: 16535023 DOI: 10.1053/j.gastro.2005.11.029]

54 Rockey D. The cellular pathogenesis of portal hypertension: stellate cell contractility, endothelin, and nitric oxide. Hepatology 1997; 25: 2-5 [PMID: 8985256 DOI: 10.1053/hep.200502010]

55 Anegawa G, Watanaka H, Yoshida D, Konishi K, Yamaguchi S, Kinjo N, Taketomi A, Hashizume M, Shimokawa H, Maehara Y. Defective endothelial nitric oxide synthase signaling is mediated by rho-kinase activation in rats with secondary biliary cirrhosis. Hepatology 2008; 47: 966-977 [PMID: 18160763 DOI: 10.1002/hep.22089]

56 Fernández-Varo G, Melgar-Lemeses P, Casals G, Pauta M, Arroyo V, Morales-Ruíz M, Ros J, Jiménez W. Inactivation of extrahepatic vascular Akt improves systemic hemodynamics and sodium excretion in cirrhotic rats. J Hepatol 2010; 53: 1041-1048 [PMID: 20800923 DOI: 10.1016/j.jhep.2010.05.031]

57 Fukumura D, Gohongi T, Kadambi A, Iizumi Y, Ang J, Yun CO, Buerk DG, Huang PL, Jain RK. Predominant role of endothelial nitric oxide synthase in vascular endothelial growth factor-induced angiogenesis and vascular permeability. Proc Natl Acad Sci USA 2001; 98: 2604-2609 [PMID: 11226286 DOI: 10.1073/pnas.041359198]

58 Abraides JG, Ivaki Y, Loureiro-Silva M, Haq O, Sessa WC, Groszmann RJ. Mild increases in portal pressure upregulate vascular endothelial growth factor and endothelial nitric oxide synthase in the intestinal microcirculatory bed, leading to a hyperdynamic state. Am J Physiol Gastrointest Liver Physiol 2006; 290: G980-G987 [PMID: 16603731 DOI: 10.1152/ajpgi.00336.2005]

59 Tazi KA, Barrière E, Moreau R, Sellerij P, Sagnières D, Moreau P, Lebrec D. Role of shear stress in aortic eNOS up-regulation in rats with biliary cirrhosis. Gastroenterology 2002; 122: 1869-1877 [PMID: 12055594 DOI: 10.1010/gast.2002.33586]

60 Kajita M, Murata T, Horiguchi K, Iizuka M, Hori M, Ozaki H. iNOS expression in vascular resident macrophages contributes to circulatory dysfunction of splanchnic vascular smooth muscle contractions in portal hypertensive rats. Am J Physiol Heart Circ Physiol 2011; 300: H1021-H1031 [PMID: 21193589 DOI: 10.1152/ajpheart.00563.2009]

61 Moreau R, Barrière E, Tazi KA, Lardeux B, Dargère D, Urbanowicz W, Poirel O, Chauvelot-Moachon L, Guimont MC, Berneau D, Lebrec D. Terlipressin inhibits in vivo aortic iNOS expression induced by lipopolysaccharide in rats with biliary cirrhosis. Hepatology 2002; 36: 1070-1078 [PMID: 12395316 DOI: 10.1053/jhep.2002.36501]

62 Malysh E, Tazi KA, Moreau R, Lebrec D. Discrepancy effects of inducible nitric oxide synthase modulation on systemic and splanchic endothelial nitric oxide synthase activity and expression in cirrhotic rats. J Gastroenterol Hepatol 2007; 22: 2195-2201 [PMID: 18031380 DOI: 10.1111/j.1440-1746.2006.06068.x]

63 Huang HC, Wang SS, Chang CC, Lee FY, Lin HC, Hou MC, by vitamin E in cirrhotic rats. Liver Int 2012; 32: 48-57 [PMID: 22089317 DOI: 10.1111/j.1447-8231.2011.02651.x]
Teng TH, Chen YC, Lee SD. Evolution of portal-systemic collateral vasospasm response in endotoxemic portal hypertensive rats. Shock 2009; 32: 503-508 [PMID: 19295490 DOI: 10.1097/SHK.0b013e3181a6f68]

Wiest R, Cadelina G, Milstien S, McCuskey RS, Garcia-Tsao G, Groszmann RJ. Bacterial translocation up-regulates GTP-cyclohydrolase I in mesenteric vasculature of cirrhotic rats. Hepatology 2003; 38: 1508-1515 [PMID: 14647062]

Wiest R, Das S, Cadelina G, Garcia-Tsao G, Milstien S, Groszmann RJ. Bacterial translocation in cirrhotic rats stimulates eNOS-derived NO production and impairs mesenteric vascular contractility. J Clin Invest 1999; 104: 1223-1233 [PMID: 1054521 DOI: 10.1172/JCI9783]

Sastre E, Balfagón G, Revuelta-López E, Aller MA, Nava MP, Arias J, Blanco-Rivero J. Effect of short- and long-term portal hypertension on adrenergic, nitricergic and sensory functioning in rat mesenteric artery. Clin Sci (Lond) 2012; 122: 337-348 [PMID: 21999248 DOI: 10.1042/CS20110030]

Juzrik L, Froh M, Straub RH, Schölerich J, Wiest R. Up-regulation of nNOS and associated increase in nitricergic vasoconstriction in superior mesenteric arteries in pre-portal hepatic hypertension. J Hepatol 2005; 43: 258-265 [PMID: 15963596 DOI: 10.1016/j.jhep.2005.02.036]

Kaneda K, Ekatasink W, Sogawa M, Matsumura A, Cho A, Kawada N. Endothelin-1-induced vasocostriction causes a significant increase in portal pressure of rat liver: localized constrictive effect on the distal segment of preterminal portal venules as revealed by light and electron microscopy and serial reconstruction. Hepatology 1998; 27: 735-747 [PMID: 9500702 DOI: 10.1002/hep.10270315]

Zhang JX, Pegoli W, Clemens MG. Endothelin-1 induces direct constriction of hepatic sinusoids. Am J Physiol 1994; 266: G646-G632 [PMID: 8179001]

Kawada N, Tran-Thi TA, Klein H, Decker K. The contrac
tional vascular escape from norepinephrine-induced constric
tion in cirrhosis. J Clin Invest 1999; 104: 1223-1233 [PMID: 1054521 DOI: 10.1172/JCI9783]

Mittal MK, Gupta TK, Lee FY, Sieber CC, Groszmann RJ. Nitric oxide modulates hepatic vascular tone in normal rat liver. Am J Physiol 1994; 266: G416-G422 [PMID: 7943239]

Zhang B, Borderie D, Sogni P, Soubraune O, Houssin D, Calmus Y. NO-mediated vasodilation in the rat liver. Role of hepatocytes and liver endothelial cells. J Hepatol 1997; 26: 1348-1355 [PMID: 920623 DOI: 10.1016/S0168-8278(97)80471-0]

Ming Z, Han C, Lautt WW. Nitric oxide mediates hepatic arterial vascular escape from norepinephrine-induced constric
tion. Am J Physiol 1999; 277: G1200-G1206 [PMID: 10608017]

Loureiro-Silva MR, Cadelina G, Groszmann RJ. Nitric oxide modulates both pre-sinusoidal and sinusoidal responses to phenylephrine in normal rat liver. Gastroenterology 2001; 120: 1354-A9 [DOI: 10.1053/j.gastro.2000.08043-1]

Zhang ZQ, Qiu JF, Luo M, Sun YW, Zhao G, Chen W, Liu H, Wu YZ. Liposome-mediated gene transfer of endothelial nitric oxide synthase to cirrhotic rat liver decreases intrahepatic vascular resistance. J Gastroenterol Hepatol 2008; 23: e487-e493 [PMID: 18070013 DOI: 10.1111/j.1440-1746.2007.05244.x]

Yu Q, Shao R, Qian HS, George SE, Rockey DC. Gene transfer of the neuronal NO synthase isofrom to cirrhotic rat liver ameliorates portal hypertension. J Clin Invest 2000; 105: 741-748 [PMID: 10722442 DOI: 10.1172/JCI7997]

Van de Casteele M, Omasta A, Janssens S, Roskams T, Desmet V, Nevens F, Fevery J. In vivo gene transfer of en
dotheilial nitric oxide synthase decreases portal pressure in anaesthetised carbon tetrachloride cirrhotic rats. Gut 2002; 51: 440-445 [PMID: 1217197] DOI: 10.1136/gut.31.3.440]

Rockey DC, Chung J. Inducible nitric oxide synthase in rat hepatic lipocytes and the effect of nitric oxide on lipocyte contractility. J Clin Invest 1995; 95: 1199-1206 [PMID: 7533786 DOI: 10.1172/JCI17769]

Rockey DC, Houssen CN, Friedman SL. Activation-depen
dent contractility of rat hepatic lipocytes in culture and in vivo. J Clin Invest 1993; 92: 1795-1804 [PMID: 8408632 DOI: 10.1172/JCI116769]

Perri RE, Langer DA, Chatterjee S, Gibbons SJ, Gadgil J, Cao S, Farrugia G, Shah VH. Defects in cGMP-PKG pathway contribute to impaired NO-dependent responses in hepatic stellate cells upon activation. Am J Physiogastrointest Liver Physiol 2006; 290: G535-G542 [PMID: 16269521 DOI: 10.1152/ajpgi.00297.2005]

Failli P, DeFRANCO RM, Caligiuri A, Gentilini A, Romanelli RG, Marra F, Batignani G, Guerra CT, Laffi G, Gentilini P, Pinzani M. Nitrovasodilators inhibit platelet-derived growth factor-induced proliferation and migration of activated hu
mn hepatic stellate cells. Gastroenterology 2000; 119: 479-492 [PMID: 10903883]

Vorobioff J, Bredfeldt JE, Groszmann RJ. Increased blood flow through the portal system in cirrhotic rats. Gastroente
orology 1984; 87: 1129-1126 [PMID: 6479534]

Groszmann RJ. Hyperdynamic circulation of liver disease 40 years later: pathophysiology and clinical consequences. Hepatology 1994; 20: 1359-1363 [PMID: 7927273 DOI: 10.1002/hep.1840200538]

Yang YY, Huang YT, Lee KC, Lee FY, Lee TY, Hou MC, Lin HC, Lee SD. Chronic administration of ursodeoxycholic acid decreases portal pressure in rats with biliary cirrhosis. Clin Sci (Lond) 2009; 116: 71-79 [PMID: 18479249 DOI: 10.1042/CS20080075]

Leifeld F, Fiebelbach M, Dumoulin FL, Speidel N, Sauerbruch T, Spengler U. Inducible nitric oxide synthase (iNOS) and endothelial nitric oxide synthase (eNOS) expression in fulminant hepatic failure. J Hepatol 2002; 37: 613-619 [PMID: 12599227 DOI: 10.1016/S0168-8278(02)00271-4]

Rodriguez-Vilarrupla A, Fernandez M, Bosch J, Garcia-Pagán JC. Current concepts on the pathophysiology of portal hypertension. An\n\n10.1172/JCI116769

Wiest R, Shah V, Sessa WC, Groszmann RJ. NO overproduction by eNOS precedes hyperdynamic splanchic circulation in portal hypertensive rats. Am J Physiol 1999; 276: G1043-G1051 [PMID: 10198549]

Theodorakis NG, Wang YN, Wu JM, Maluccio MA, Sitzmann J, Skill NJ. Role of endothelial nitric oxide synthase in the development of portal hypertension in the carbon tetrachloride-induced liver fibrosis model. Am J Physiogastrointest Liver Physiol 2009; 297: G792-G799 [PMID: 19628654 DOI: 10.1152/ajpgi.00229.2009]

Theodorakis NG, Wang YN, Skill NJ, Metz MA, Cahill PA, Redmond EM, Sitzmann J. The role of nitric oxide synthase isoforms in extrahepatic portal hypertension: studies in gene-knockout mice. Gastroenterology 2003; 124: 1500-1508 [PMID: 12738088 DOI: 10.1016/S0016-5085(03)00820-4]

Wei CL, Hon WM, Lee KH, Kho HE. Chronic administra
tion of aminoguanidine reduces vascular nitric oxide production and attenuates liver damage in bile duct-ligated rats. Liver Int 2005; 25: 647-656 [PMID: 15910502 DOI: 10.1111/j.1478-3231.2005.01063.x]

Bauer TM, Schwacha H, Steinbrückner B, Brinkmann FE, Ditzen AK, Aponte JJ, Pelz K, Berger D, Kist M, Blum HE. Small intestinal bacterial overgrowth in human cirrhosis
Hu LS et al. Nitric oxide and portal hypertension is associated with systemic endotoxemia. Am J Gastroenterol 2002; 97: 2364-2370 [PMID: 12358257 DOI: 10.1111/j.1520-2649.2002.05791.x]

Guainer C, Soriano G, Tomas A, Bulbena O, Novella MT, Balanzó J, Vilallonga F, Mourelle M, Moncada S. Increased serum nitrite and nitrate levels in patients with cirrhosis: relationship to endotoxemia. Hepatology 1993; 18: 1139-1143 [PMID: 8225520 DOI: 10.1002/hep.1840180520]

Albillos A, de la Hera A, González M, Moya JL, Calleja JL, Monserrat J, Ruiz-del-Arbo L, Alvarez-Mon M. Increased lipopolysaccharide binding protein in cirrhotic patients with marked immune and hemodynamic derangement. Hepatology 2003; 37: 208-217 [PMID: 12500206 DOI: 10.1053/jhep.2003.50038]

Morales-Ruiz J, Jiménez W, Pérez-Sala D, Ros J, Leivas A, Lamas S, Rivera F, Arroyo V. Increased nitric oxide synthase expression in arterial vessels of cirrhotic rats with ascites. Hepatology 1996; 24: 1481-1486 [PMID: 8938184 DOI: 10.1002/hep.1840240719]

Bhimani EK, Sumanovski LT, Arroyo AG, Sánchez-Madrid F, Moreno-Otero R, Sistierra E, Garcia-Pras E, Tiani C, Miquel R, Bosch J, Fernández-Madrid A. Increased NO synthase activity in prehepatic portal hypertensive rats. J Hepatol 2005; 43: 47-54 [PMID: 16005121 DOI: 10.1016/j.jhep.2004.05.006]

Fernández-Madrid A, Semela D, Bruix J, Colle I, Pinzani M, Bosch J. Angiogenesis in liver disease. J Hepatol 2009; 50: 604-620 [PMID: 19157625 DOI: 10.1016/j.jhep.2008.12.011]

Bosch J, Abraldes JG, Fernández-Madrid A, García-Pagán JC. Hepatic endothelial dysfunction and abnormal angiogenesis: new targets in the treatment of portal hypertension. J Hepatol 2010; 53: 558-567 [PMID: 20561700 DOI: 10.1016/j.jhep.2010.03.021]

Fernández-Madrid A, Vizzutti F, García-Pagán JC, Rodes J, Bosch J. Anti-VEGF receptor-2 monoclonal antibody prevents portal-systemic collateral vessel formation in porto-hypertensive mice. Gastroenterology 2004; 126: 886-894 [PMID: 14988842 DOI: 10.1053/j.gastro.2003.12.012]

Mejías M, García-Pras E, Tiani C, Miquel R, Bosch J, Fernández-Madrid A. Beneficial effects of sorafenib on splanchnic, intrahepatic, and portocollateral circulations in portal hypertensive and cirrhotic rats. J Hepatol 2009; 49: 1245-1256 [PMID: 19137887 DOI: 10.1016/j.jhep.2010.03.021]

Zhao Y, Wang Y, Wang Q, Liu Z, Liu Q, Deng X. Hepatic stellate cells produce vascular endothelial growth factor via phospho-p44/42 mitogen-activated protein kinase/cyclooxygenase-2 pathway. Mol Cell Biochem 2012; 359: 217-223 [PMID: 21863308 DOI: 10.1007/s10140-011-0116-x]

Medina J, Arroyo AG, Sánchez-Madrid F, Moreno-Otero R. Angiogenesis in chronic inflammatory liver disease. Hepatology 2004; 39: 1185-1195 [PMID: 15122744 DOI: 10.1002/hep.20215]

Sieber CC, Sumanovski LT, Stumm M, van der Kooij M, Battegay E. In vivo angiogenesis in normal and portal hypertensive rats: role of basic fibroblast growth factor and nitric oxide. J Hepatol 2001; 34: 644-650 [PMID: 11434609 DOI: 10.1016/S0168-8278(00)00647-7]

Sumanovski LT, Battegay E, Stumm M, van der Kooij M, Sieber CC. Increased angiogenesis in portal hypertensive rats: role of nitric oxide. Hepatology 1999; 29: 1044-1049 [PMID: 10094944 DOI: 10.1002/hep.10190436]

Ahmad S, Hewett PW, Wang P, Al-Ani B, Cudmore M, Fusiwas T, Haigh J, le Noble F, Wang L, Mukhopadhyay D, Ahmed A. Direct evidence for endothelial vascular endothelial growth factor receptor-1 function in nitric oxide-mediated angiogenesis. Circ Res 2006; 99: 715-722 [PMID: 16946136 DOI: 10.1161/01.RES.0000243899.40606.b9]

Huang HC, Wang SS, Hsin IF, Chang CC, Lee FY, Lin HC, Chung CL, Lee YJ, Hsieh HG, Lee SD. Cannabinoid receptor 2 agonist ameliorates mesenteric angiogenesis and portosystemic collaterals in cirrhotic rats. Hepatology 2012; 56: 248-258 [PMID: 22290687 DOI: 10.1002/hep.25564]

Huang HC, Chu CJ, Lee FY, Chang FY, Wang SS, Lin HC, Hou MC, Chan CC, Wu SL, Chen CT, Lee SD. Chronic inhibition of nitric oxide ameliorates splanchnic hyposensitivity to giprectin in a hemorrhage-transfused rat model of portal hypertension. Scand J Gastroenterol 2000; 35: 1308-1313 [PMID: 11199372 DOI: 10.1080/0036552004536674]

Pizzieta P, Pique JM, Fernández M, Bosch J, Rodés J, Whittle BJ, Moncada S. Modulation of the hyperdynamic circulation of cirrhotic rats by nitric oxide inhibition. Gastroenterology 1992; 103: 1909-1915 [PMID: 1451984]

Forrest EH, Jones AL, Dillon JF, Walker J, Hayes PC. The effect of nitric oxide synthase inhibition on portal pressure and azysso blood flow in patients with cirrhosis. J Hepatol 1995; 23: 254-258 [PMID: 8550988 DOI: 10.1016/0168-8278(95)80464-8]

González-Abraldes JG, García-Pagán JC, Bosch J. Nitric oxide and portal hypertension. Metab Brain Dis 2002; 17: 311-324 [PMID: 12625080]

Groszmann RJ. Beta-adrenergic blockers and nitrovasodilators for the treatment of portal hypertension: the good, the bad, the ugly. Gastroenterology 1997; 113: 1794-1797 [PMID: 9352888 DOI: 10.1053/gast.1997.v113.as13.97131794]

Dudenhoeffer AA, Loureiro-Silva MR, Cadelina GW, Gupta T, Groszmann RJ. Bioactivation of nitroglycerin and vaso-motor response to nitric oxide are impaired in cirrhotic rat livers. Hepatology 2002; 36: 388-395 [PMID: 12415406 DOI: 10.1053/jhep.2002.342739]

Bosch J, Abraldes JG, Groszmann R. Current management of portal hypertension. J Hepatol 2003; 38 Suppl 1: S54-S68 [PMID: 12599186 DOI: 10.1016/S0168-8278(02)00430-0]

Fiorucci S, Antonelli E, Morelli O, Mencarelli A, Casini A, Mello T, Palazzetti B, Tallet D, del Soldato P, Morelli A. NCX-1000, a nitric oxide-releasing derivative of glibenclamide, lowers norepinephrine-induced intrahepatic resistance in cirrhotic rats. J Hepatol 2003; 39: 939-959 [PMID: 14642608 DOI: 10.1016/S0168-8278(03)00393-3]

Loureiro-Silva MR, Cadelina GW, Iwakiri Y, Groszmann RJ. A liver-specific nitric oxide donor improves the hepatic vascular response to both portal blood flow increase and methoxamine in cirrhotic rats. J Hepatol 2003; 39: 940-946 [PMID: 14642609 DOI: 10.1053/jhep.2003.09.018]

Benzingi A, Bellot P, De Gottiardi A, García-Pagán JC, Gagnon C, Spénard J, Bosch J, NCX-1000, a nitric oxide-releasing derivative of UDCA, does not decrease portal pressure in patients with cirrhosis: results of a randomized, double-blind, dose-escalating study. Am J Gastroenterol 2010; 105: 1094-1101 [PMID: 19920806 DOI: 10.1038/ajg.2009.661]

Saavedra JE, Billar TR, Williams DL, Kim YM, Watkins SC, Keever LK. Targeting nitric oxide (NO) delivery in vivo. Design of a liver-selective NO donor prodrug that blocks tumor...
necrosis factor-alpha-induced apoptosis and toxicity in the liver. *J Med Chem* 1997; 40: 1947-1954 [PMID: 9207935 DOI: 10.1021/jm9701031]

134 Qu W, Liu J, Fuquay R, Saavedra JE, Keefer LK, Waalkes MP. The nitric oxide prodrug, V-PYRRO/NO, mitigates arsenic-induced liver cell toxicity and apoptosis. *Cancer Lett* 2007; 256: 238-245 [PMID: 17658681 DOI: 10.1016/j.canlet.2007.06.009]

135 González R, Cruz A, Ferrín G, López-Cillero P, Fernández-Rodríguez R, Briceño J, Gómez MA, Rután S, Mata Mde L, Martínez-Ruiz A, Marin J, Muntané J. Nitric oxide mimics transcriptional and post-translational regulation during α-tocopherol cytoprotection against glycochenodeoxycholate-induced cell death in hepatocytes. *J Hepatol* 2011; 55: 133-144 [PMID: 21145864 DOI: 10.1016/j.jhep.2010.10.022]

136 DeLeve LD, Wang X, Kanel GC, Ito Y, Bethea NW, McCuskey MK, Tokes ZA, Tsai J, McCuskey RS. Decreased hepatic nitric oxide production contributes to the development of rat sinusoidal obstruction syndrome. *Hepatology* 2003; 38: 900-908 [PMID: 14512877]

137 Moal F, Veal N, Vuillemin E, Barrière E, Wang J, Fizanne L, Oberti F, Douay O, Gallois Y, Bonnefont-Rousselot D, Rousselet MC, Cales P. Hemodynamic and antifibrotic effects of a selective liver nitric oxide donor V-PYRRO/NO in bile duct ligated rats. *World J Gastroenterol* 2006; 12: 6639-6645 [PMID: 17079777]

138 Edwards C, Feng HQ, Reynolds C, Mao L, Rockey DC. Effect of the nitric oxide donor V-PYRRO/NO on portal pressure and sinusoidal dynamics in normal and cirrhotic mice. *Am J Physiol Gastrointest Liver Physiol* 2008; 294: G1311-G1317 [PMID: 18356534 DOI: 10.1152/ajpgi.00368.2007]

139 Biecker E, Trebicka J, Kang A, Hennenberg M, Sauerbruch T, Heller J. Treatment of bile duct-ligated rats with the nitric oxide synthase transcription enhancer AVE 9488 ameliorates portal hypertension. *Liver Int* 2008; 28: 331-338 [PMID: 18290775 DOI: 10.1111/j.1478-3231.2008.01664.x]

140 Das A, Mukherjee P, Singla SK, Guturu P, Frost MC, Mukhopadhyay D, Shah VH, Patra CR. Fabrication and characterization of an inorganic gold and silica nanoparticle mediated drug delivery system for nitric oxide. *Nanotechnology* 2010; 21: 305102 [PMID: 20610873 DOI: 10.1088/0957-4484/21/30/305102]