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Molecular abnormalities in portal hypertension: diagnostic, prognostic and therapeutic implications

Juan G. Abraldes*, Juan Carlos García-Pagán and Jaime Bosch

Hepatic Hemodynamic Laboratory, Liver Unit, Hospital Clínic-IDIBAPS and Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd), Hospital Clínic, Barcelona, Spain

Introduction

Portal hypertension is a very frequent and dreadful complication of chronic liver disease. Its consequences, mainly bleeding from gastro-esophageal varices and portal hypertensive gastropathy, ascites spontaneous bacterial peritonitis, hepatorenal syndrome, hepatopulmonary syndrome, portopulmonary hypertension, hyperkinetic syndrome and hepatic encephalopathy, carry a poor prognosis and represent the first cause of death and liver transplantation in patients with cirrhosis[1].

Over the past 25 years, progress in the understanding of the pathophysiology of portal hypertension was followed by the introduction of effective pharmacological therapy, consisting mainly on the continued oral administration of non-selective beta-blockers (propranolol, nadolol) for the prevention of first or recurrent variceal bleeding, and on the short-term intravenous infusion of terlipressin, somatostatin or somatostatin analogs for acute variceal bleeding[1]. These treatments were aimed at correcting the increased splanchnic blood flow that was shown at this moment to contribute to maintain and aggravate portal hypertension[2]. It is only recently that this paradigm has been changed. Progress in our knowledge of the mechanisms of increased resistance to portal blood flow have opened new perspectives for developing more effective treatment strategies. This is the focus of the current review, which addresses the new advances in the modulation of hepatic vascular resistance, emphasizing those strategies that have been (or are being) tested in humans.

*Corresponding author.
E-mail: jgon@clinic.ub.es (J.G. Abraldes).

Increased hepatic resistance in cirrhosis

Increased intrahepatic resistance to portal blood flow is the primary factor leading to portal hypertension in cirrhosis. Much of the increased intrahepatic resistance is the mechanical consequence of the architectural disturbances caused by the cirrhotic process. However, in recent years it has become clear that on top of these alterations there is an active contraction of several elements in the liver that further contribute to increase resistance. It has been claimed that this dynamic and reversible component of intrahepatic resistance may represent 30 to 40% of the total increased intrahepatic vascular resistance in cirrhosis. There are not convincing studies that had quantified the magnitude of this functional component in patients with cirrhosis, and it is not well known whether its importance changes along the natural history of cirrhosis. This finding set the rationale for the treatment of portal hypertension with vasodilators.

There is not a full agreement on the contractile structures that account for the increased vascular tone within the liver. What is clear is that several vasoconstrictors and vasodilators modify hepatic resistance. These substances can be of hepatic origin and act in a paracrine fashion (nitric oxide (NO), prostacyclin, hydrogen sulfide (H_2S), carbon monoxide (CO), endothelin, locally produced angiotensin II, thromboxane, leucotriens), can arrive to the liver from the systemic circulation (circulating angiotensin II, vasopressin or norepinephrine) or can be of neural origin (norepinephrine). It is not well known which of these systems is more relevant but is clear that in cirrhosis there is an imbalance between vasoconstrictive and vasodilating forces, characterized by an abundance of vasoconstrictors and a deficient production and deficient response to vasodilators. These abnormalities

are amplified by the fact that, as compared to the normal liver, the hepatic vascular bed of the cirrhotic liver exhibits an increased response to vasoconstrictors, and a deficient response to vasodilators. In addition, most vasoconstrictors have profibrogenic actions, while vasodilators have antifibrogenic properties, so the effects of this imbalance go beyond the increase in intrahepatic vascular tone.

Endothelial dysfunction in the cirrhotic liver

In normal conditions, the endothelium is able to generate vasodilatory factors in response to increases in blood volume, blood pressure or vasoconstrictor agents, in an attempt to prevent or attenuate the concomitant increase in intravascular pressure. In several pathological conditions there is an impairment in this endothelium-dependent vasodilatation, a condition that has been named as "endothelial dysfunction"[3,4]. The hepatic vascular bed of cirrhotic livers also exhibits endothelial dysfunction[5]. Indeed, contrary to what happens in normal livers, the cirrhotic liver can not accommodate the increased portal blood flow caused by the postprandial hyperemia, which determines an abrupt postprandial increase in portal pressure[6]. In addition, in experimental models of cirrhosis, endothelial dysfunction has been further characterized by showing that the cirrhotic liver exhibits an impaired response to the endothelium-dependent vasodilator acetylcholine (ACh)[5,7]. In patients with cirrhosis it has been shown that plasma surrogate markers of endothelial dysfunction, such as Von Willebrand factor (vWF), are increased in patients with cirrosis, correlate with liver dysfunction and the degree of portal hypertension, and independently predict the clinical outcome[8]. This suggests that endothelial dysfunction has pathogenic relevance beyond increasing hepatic resistance and portal pressure.

Endothelial dysfunction in cirrhosis has been attributed to reduce nitric oxide (NO) bioavailability and to increased vasoconstrictor COX-1 derived prostanoids and it is also thought to be implicated in the pathogenesis of the dynamic component of the increased intrahepatic resistance of the cirrhotic liver. The main factors involved are described below.

Cyclooxigense derived prostanoids

Cyclooxygenase (COX) is the key enzyme in the biosynthetic pathway leading to prostaglandins (PGs) and thromboxane (TX) from arachidonic acid (AA)[9]. COX-1 is constitutively expressed but it can also be stimulated by different factors[10,11]. COX-2 is the inducible isoform of COX that is usually expressed after stimulation with proinflammatory agents[12]. However, it has also been shown to be constitutively expressed in some tissues, including the liver[7,13] and the mesenteric vascular bed[14].

Several evidences support the involvement of COX-1 derived prostanoids promoting the increase in resistance to portal blood flow of cirrhotic livers. Indeed, the hyperresponse of the hepatic vasculature of cirrhotic livers to the vasoconstrictor alfa1-agonist methoxamine is associated with an overproduction of thromboxane A2 (TXA2) by COX-1 and it is completely corrected by pre-treating the livers with non-selective COX blockers, COX-1 selective blockers or TXA2 receptor antagonists[13]. Similarly, it has been demonstrated that the endothelial dysfunction of cirrhotic livers, is also associated with an increased production of TXA2 and completely prevented by selective COX-1 blockers and TXA2 antagonists. These results suggest that an increased production of a COX-1 derived vasoconstrictor prostanoid, probably TXA2, is at least in part, responsible for the presence of endothelial dysfunction[7].

Sinusoidal endothelial cells are the major contributor to the increased production of vasoconstrictor prostanoids (TXA2, and probably also prostaglandin H2)[15]. Moreover, the increased phospholipase A2 activity observed in cirrhotic rat livers, by increasing AA bioavailability, is an additional mechanism contributing to the increased generation of vasoconstrictor prostanoids[15] On the other hand, it has been suggested that Kupffer cells activation is also involved in the increased portal pressure of fibrotic livers via TXA2 generation[16]. All these findings suggest that in cirrhotic livers there is an over-activation of the COX-1 pathway with an increased production of their vasoconstrictor-derived compounds, and that correcting these abnormalities will have beneficial effects in the hepatic circulation.

Reduced nitric oxide bioavailability within the cirrhotic liver

Nitric oxide (NO) is the natural ligand for soluble guanylate cyclase and is responsible for an increase in cyclic guanosine monophosphate (cGMP), the final agent responsible for the relaxation of the vascular wall through the extrusion of cytosolic Ca2+. Endothelial NO synthase (eNOS) is responsible for most of the vascular NO produced in a reaction where L-arginine is oxidized to L-citrulline and NO[17]. In cirrhotic liver, there is a reduced NO bioavailability that plays a major role increasing intrahepatic vascular resistance and thereby worsening portal hypertension. Decreased NO production occurs despite a normal expression of eNOS mRNA and normal levels of eNOS protein[5,18] and has been attributed, at least in part, to reduced eNOS activity caused by several posttranslational alterations in the regulation of the enzyme such as increased caveolin expression, or a defect of the essential cofactor of eNOS, tetrahydrobiopterin (BH4), decreased eNOS phosphorylation, and increased levels of asymmetric dimethylarginine among others[2]. According with these pathophysiological abnormalities several efforts to improve NO bioavailability within the liver have been attempted.

A first attempt was the exogenous *administration of NO donors*. In that regard, the administration of nitrates such as isosorbide-5-mononitrate has been shown to decrease portal pressure. The major concern with the use of these drugs in patients with advanced cirrhosis is that, by reducing arterial blood pressure, they may promote the activation of endogenous vasoactive systems that finally may lead to water and sodium retention. NCX-1000, a NO-releasing derivative of ursodeoxycolic acid (UDCA), was designed to

target selectively the intrahepatic circulation by delivering NO only at intrahepatic site, with the aim to effectively reduce baseline hepatic venous pressure gradient (HVPG) and to counteract the postprandial increase in portal pressure, without adverse effects on the systemic and splanchnic circulation. Results from experimental models were promising[19]. However, in a recent study in cirrhotic patients with portal hypertension, NCX-1000 treatment did not reduce baseline HVPG while it decreased hepatic blood flow and systolic blood pressure. In addition, NCX-1000 did not modify the post-prandial increase in HVPG[20]. Thus, this agent failed to prove efficacious for the treatment of portal hypertension. This was mainly due to a poor bioavailability, and to the lack of a specific intrahepatic vasodilatory effect[20]. The search for an effective way of specifically supplementing NO to the liver circulation, thus reducing portal pressure without affecting systemic arterial pressure, is still warranted.

Other strategies to correct the intrahepatic NO deficiency have been based on either overexpressing NOS by transfecting the liver with adenovirus encoding eNOS[21,22], nNOS[23], or constitutively active Akt[24], by enhancing eNOS activity by simvastatin (a HMG-CoA reductase inhibitor)[25,26], by decreasing NO scavenging by means of antioxidants[27,28], by administering the eNOS cofactor tetrahydrobiopterin[29] or scavenging NO by reactive oxygen species (ROS) due to oxidative stress[30]. The possible role of statins, tetrahydrobiopterin (BH4) and oxidative stress are the strategies more extensively studied.

New strategies to decrease hepatic resistance

The role of statins in the treatment of portal hypertension

The ideal drug for portal hypertension was recently pictured as one that should reduce portal pressure by decreasing intrahepatic vascular resistance, while maintaining or enhancing hepatic blood flow[31]. Other desirable actions would be an antifibrotic effect and a capacity to improve liver function. A drug that would be able to increase nitric oxide bioavailability in the liver would fulfil many of these requirements.

HMG-CoA reductase inhibitors, commonly called statins, are widely used lipid lowering drugs that have additional beneficial effects over the peripheral vasculature by enhancing NO production in endothelial cells[32]. This occurs by enhancing both the expression of the endothelial nitric oxide synthase (eNOS) and its activity at the posttranslational level, by acting on multiple mechanisms modulating eNOS activity. This led us to hypothesize that statins could be useful to enhance NO production at the liver circulation[26] and, thus, they could have potential for the treatment of portal hypertension. Indeed, consistent experimental[25,33] and human studies[26] suggested that statins were able to decrease intra-hepatic vascular resistance and improve flow-mediated vasodilation of liver vasculature in the cirrhotic liver by selectively enhancing endothelial NO production at the liver, without further enhancing arterial vasodilation. In addition, statins have been shown to inhibit hepatic RhoA/Rho kinase signalling, which would decrease hepatic stellate cells (HSC) contraction by a NO independent (and, thus, endothelium independent) mechanism[33]. Altogether, this indicates that these drugs could behave as true liver-selective vasodilators[25,26,33]. Subsequently, a multicenter double-blind randomized controlled trial, including 59 cirrhotic patients with severe portal hypertension, demonstrated that one-month simvastatin administration significantly decreases HVPG without inducing arterial hypotension[34]. This occurred without modifications on liver blood flow, suggesting that simvastatin did reduce hepatic vascular resistance. The magnitude of the HVPG reduction caused by simvastatin was moderate (-8%), but was present regardless of whether patients were on treatment with non-selective beta-adrenergic blockers. Moreover, the effect of simvastatin was slightly greater in those taking beta-blockers (-11%), suggesting that both drugs, which act by different mechanisms of action, have additive effects reducing portal pressure. This might be related to the fact that simvastatin, by increasing NO availability at the sinusoidal circulation, counteracts the effects of beta-blockers increasing liver resistance due to unopposed alpha-adrenergic driven vasoconstriction. Another positive effect of simvastatin was a marked improvement in hepatic clearance of indocyanine green, which suggests that the reduction in hepatic vascular tone caused by simvastatin improves the effective liver perfusion of the hepatocytes, with an ensuing beneficial effect on liver function[34]. It is important to note that this effect, of potential clinical relevance, is neither observed with beta-blockers, alone nor associated with organic nitrates, nor with any other drug used to treat portal hypertension. Adverse events were not different between placebo and simvastatin, a finding that is in keeping with the increasing number of studies reporting the safety of statins in patients with chronic liver diseases, but the long-term safety of statins in cirrhosis needs to be specifically assessed.

Additional data suggest that statins might have other beneficial effects on cirrhosis, beyond the observed portal pressure reduction. Recent studies showed that the continuous administration of atorvastatin prevented the liver inflammation and hepatic stellate cells activation induced by Angiotensin-II infusion[35], and the development of fibrosis in a model of biliary cirrhosis[36]. Further, a recent large randomized controlled trial showed that rosuvastatin significantly decreased the occurrence of venous thromboembolism[37], which could be relevant in patients with cirrhosis, who are at increased risk of portal vein thrombosis[38,39]. Preliminary data from our lab in preclinical murine models suggest that statins might prevent the liver microvascular dysfunction induced by endotoxemia, and in that way protect from liver dysfunction in sepsis[40]. Whether this might translate in clinical benefits, preventing the development of acute-on-chronic liver failure associated with sepsis, deserves further evaluation.

The next step to take is to evaluate the potential of statins in patients with cirrhosis in randomized controlled trials with clinical end-points. The first scenario in which statins have clear potential is as an adjunct to beta-blockers and banding in the secondary prophylaxis of variceal bleeding. In a recent trial bleeding-free survival in patients

treated with the combination of drugs+endoscopic band ligation was 53% at two years, which is clearly unsatisfactory. This might be improved by adding a drug that enhances the portal pressure-lowering effect of beta-blockers and that have the potential to improve liver function, and this drug could be a statin. An ongoing RCT (NCT01095185) is currently evaluating this hypothesis.

Another potential scenario is the prophylaxis of the development of varices or clinical decompensation, since no drug has proved effective so far in these situations. However, due to the small risk of variceal formation (around 6% per year) or of clinical decompensation, to prove a benefit of statins in this setting would require a large trial with a very long follow-up, or the use of surrogate end-points, such as HVPG response. Along these lines, another ongoing trial (NCT01282398) is testing whether, in patients with compensated cirrhosis and mild portal hypertension (HVPG between 6 and 10 mmHg), the treatment of portal hypertension with simvastatin may prevent progression of portal hypertension and prevent the development of clinically significant portal hypertension (defined by a HVPG ≥ 10 mmHg).

Tetrahydrobiopterin supplementation

Tetrahydrobiopterin (BH4) is an essential cofactor for the adequate generation of NO by NOS enzymes[41,42]. If adequate quantities of BH4 are not present, a situation known as NOS uncoupling takes place and the production of NO is decreased[43]. Studies from our lab have shown that in cirrhotic livers there is a deficiency of BH4, secondary to a reduction in the expression and activity of Guanosine-5'-triphosphate cyclohydrolase I (GTPCHI), the limiting enzyme in BH4 synthesis, which is associated with decreased NOS activity and NO availability[29,44]. In cirrhotic rats, administration of BH4 during 3 days increased liver NOS activity and cGMP levels and significantly reduced portal pressure. Amelioration of portal hypertension was associated with a normalization of arterial pressure. These data support the concept that tetrahydrobiopterin supplementation may represent a new and effective therapeutic strategy for portal hypertension[29,44]. This is currently being evaluated in a randomized pilot study, in which patients with cirrhosis receive sapropterin (an oral analogue of tetrahydrobiopterin) or placebo for two weeks and portal pressure and other hemodynamic parameters are evaluated before and after the administration of the drug (NCT01456286).

Antioxidant therapy

In several vascular disorders it has been demonstrated than an increase in the reactive oxygen species (ROS) superoxide (O_2^-), by rapidly reacting with NO[45], promotes a marked reduction in NO bioavailability followed by an increase in vascular tone[46-49]. Our group has recently demonstrated that this also happens in the cirrhotic liver. This further demonstrates that in chronic liver disease, reduced intrahepatic NO bioavailability is not only the consequence of a reduction in its production by eNOS synthase but also to an increase scavenging by increased superoxide.

We have recently shown that increased O_2^- levels in the cirrhotic liver is associated with reduced superoxide dismuatse (SOD) activity, the enzyme dismutating O_2^- to H_2O_2, suggesting that this maybe its mechanism. Furthermore, this study clarified that reduced SOD activity is due to decreased protein expression of the cytoplasmic and mitochondrial SOD, but not of the extracellular SOD isoform[30]. Interestingly, the study demonstrated that cyclooxygenase (COX) or xanthine oxidase (XO) inhibition markedly reduced intrahepatic O_2^- levels, which points out that these enzymatic systems are potential sources of O_2^- in cirrhosis[30]. Thus an increased production of superoxide and a diminished degradation are the causes of the increased superoxide levels observed in the cirrhotic livers. Increased O_2^- in cirrhotic livers was associated with a significant increase in nitrotirosinated proteins, a well recognized marker of the reaction of O_2^- with NO[30]. The relationship between NO bioavailability and O_2^- content in the liver is further supported by our experiments in sinusoidal endothelial cells (SEC) demonstrating that NO bioavailability is modulated by O_2^-. Indeed, increasing O_2^- content in SEC by incubating with a SOD inhibitor was associated with a marked fall in NO bioavailability[30]. Further, abolition of the increase in O_2^- using SOD supplementation was followed by a partial restoration in NO bioavailability.

All these data strongly suggests that oxidative stress may contribute to reduced NO bioavailability in cirrhotic livers, and emphasize that antioxidant therapy, by removing O_2^- from the cirrhotic livers, could be a new therapeutic strategy to improve intrahepatic NO bioavailability and to ameliorate hepatic vascular tone in cirrhosis. In that regard, transfection of cirrhotic rats with portal hypertension with adenovirus codifying extracelular SOD (EcSOD) resulted in a marked reduction in O_2^-, in enhanced NO bioavailability (as estimated by the hepatic levels of cGMP), and decreased liver nitrotyrosinated proteins[27]. These molecular effects were associated with a significant improvement in the endothelium-dependent vasodilatation to acetylcholine and, more importantly, promoted a significant reduction of portal pressure in vivo without significant changes in MAP[27]. Reduction in portal pressure averaged 13.3%. This is similar to that observed in other studies aimed at reducing portal pressure in cirrhotic rats through other strategies, such as the administration of non-selective beta-blockers[50].

This study provided evidence, for the first time in vivo, that decreasing hepatic O_2^- levels by increasing SOD activity (i.e. an antioxidant treatment) may represent an effective strategy to improve NO bioavailability within the liver. Further evidence supporting this concept comes from studies supplementing ascorbic acid in patients with cirrhosis[28]. Ascorbic acid (vitamin C), is a potent antioxidant that has consistently been shown to improve NO-dependent vasodilatation in vascular beds of patients with conditions characterized by marked endothelial dysfunction, such as hypertension, diabetes, hypercholesterolemia and coronary heart disease. In these conditions, the beneficial effect of acute ascorbic acid administration has been attributed to its capacity for neutralizing ROS, mainly superoxide (O). Of note, a study from our group has shown that cirrhotic patients had significantly lower ascorbic acid levels and higher malondialdehyde levels (MDA: a serum marker of

oxidative stress) than healthy controls. Ascorbic acid significantly reduced MDA levels and effectively improved intrahepatic endothelial dysfunction, blunting the postprandial increase in portal pressure[28].

A recent study provided further evidence on the potential of antioxidants for the treatment of portal hypertension. This was a phase II, double-blind, randomized, controlled study that evaluated the effects of supplementing a meal with dark chocolate, rich in flavonoids (potent antioxidants), on meal stimulated hepatic hemodynamics in cirrhosis. This study showed that dark chocolate supplementation improved hepatic vasorelaxation and blunted the post-prandial increase in HVPG[1].

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References

1. Bosch J, Berzigotti A, García-Pagan JC, Abraldes JG. The management of portal hypertension: rational basis, available treatments and future options. J Hepatol. 2008;48 Suppl 1:S68-92.
2. Groszmann RJ, Abraldes JG. Portal hypertension: from bedside to bench. J Clin Gastroenterol. 2005;39:S215.
3. Harrison DG. Cellular and molecular mechanisms of endothelial cell dysfunction. J Clin Invest. 1997;100:2153-7.
4. Harrison DG. Endothelial function and oxidant stress. Clin Cardiol. 1997;20:II-7.
5. Gupta TK, Toruner M, Chung MK, Groszmann RJ. Endothelial dysfunction and decreased production of nitric oxide in the intrahepatic microcirculation of cirrhotic rats. Hepatology. 1998;28:926-31.
6. Bellis L, Berzigotti A, Abraldes JG, Moitinho E, García-Pagan JC, Bosch J, et al. Low doses of isosorbide mononitrate attenuate the postprandial increase in portal pressure in patients with cirrhosis. Hepatology. 2003;37:378-84.
7. Graupera M, García-Pagan JC, Parés M, Abraldes JG, Roselló J, Bosch J, et al. Cyclooxygenase-1 inhibition corrects endothelial dysfunction in cirrhotic rat livers. J Hepatol. 2003;39:515-21.
8. La Mura V, Reverter JC, Flores-Arroyo A, Raffa S, Reverter E, Seijo S, et al. Von Willebrand factor levels predict clinical outcome in patients with cirrhosis and portal hypertension. Gut. 2011;60:1133-8.
9. Miyamoto T, Ogino N, Yamamoto S, Hayaishi O. Purification of prostaglandin endoperoxide synthetase from bovine vesicular gland microsomes. J Biol Chem. 1976;251:2629-36.
10. Smith WL. Prostanoid biosynthesis and mechanisms of action. Am J Physiol. 1992;263:F181-91.
11. Smith WL, Garavito RM, DeWitt DL. Prostaglandin endoperoxide H synthases (cyclooxygenases)-1 and -2. J Biol Chem. 1996;271:33157-60.
12. Smith WL, Langenbach R. Why there are two cyclooxygenase isozymes. J Clin Invest. 2001;107:1491-5.
13. Graupera M, García-Pagan JC, Abraldes JG, Peralta C, Bragulat M, Corominola H, et al. Cyclooxygenase-derived products modulate the increased intrahepatic resistance of cirrhotic rat livers. Hepatology. 2003;37:172-81.
14. Potenza MA, Botrugno OA, De Salvia MA, Lerro G, Nacci C, Marasciulo FL, et al. **Endothelial COX-1 and -2 differentially affect reactivity of MVB in portal hypertensive rats. Am J Physiol Gastrointest Liver Physiol. 2002;283:G587-94.**
15. Gracía-Sancho J, Lavina B, Rodríguez-Vilarrupla A, García-Caldero H, Bosch J, García-Pagan JC. Enhanced vasoconstrictor prostanoid production by sinusoidal endothelial cells increases portal perfusion pressure in cirrhotic rat livers. J Hepatol. 2007;47:220-7.
16. Steib CJ, Gerbes AL, Bystron M, Op den Winkel M, Hartl J, Roggel F, et al. Kupffer cell activation in normal and fibrotic livers increases portal pressure via thromboxane A(2). J Hepatol. 2007;47:228-38.
17. Moncada S, Higgs A. The L-arginine-nitric oxide pathway. N Engl J Med. 1993;329:2002-12.
18. Shah V, Toruner M, Haddad F, Cadelina G, Papapetropoulos A, Choo K, et al. Impaired endothelial nitric oxide synthase activity associated with enhanced caveolin binding in experimental cirrhosis in the Rat. Gastroenterology. 1999;117:1222-8.
19. Fiorucci S, Antonelli E, Brancaleone V, Sanpaolo L, Orlandi S, Distrutti E, et al. NCX-1000, a nitric oxide-releasing derivative of ursodeoxycholic acid, ameliorates portal hypertension and lowers norepinephrine-induced intrahepatic resistance in the isolated and perfused rat liver. J Hepatol. 2003;39:932-9.
20. Berzigotti A, Bellot P, De Gottardi A, García-Pagan JC, Gagnon C, Spenard J, et al. **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;105:1094:101.**
21. Van de Casteele M, Omasta A, Janssens S, Roskams T, Desmet V, Nevens F, et al. In vivo gene transfer of endothelial nitric oxide synthase decreases portal pressure in anaesthetised carbon tetrachloride cirrhotic rats. Gut. 2002;51:440-5.
22. Shah V, Chen AF, Cao S, Hendrickson H, Weiler D, Smith L, et al. Gene transfer of recombinant endothelial nitric oxide synthase to liver in vivo and in vitro. Am J Physiol Gastrointest Liver Physiol. 2000;279:G1023-30.
23. Yu Q, Shao R, Qian HS, George SE, Rockey DC. Gene transfer of the neuronal NO synthase isoform to cirrhotic rat liver ameliorates portal hypertension. J Clin Invest. 2000;105:741-8.
24. Morales-Ruiz M, Cejudo-Martín P, Fernández-Varo G, Tugues S, Ros J, Angeli P, et al. Transduction of the liver with activated Akt normalizes portal pressure in cirrhotic rats. Gastroenterology. 2003;125:522-31.
25. Abraldes JG, Rodríguez-Vilarrupla A, Graupera M, Zafra C, García-Caldero H, García-Pagan JC, et al. Simvastatin treatment improves liver sinusoidal endothelial dysfunction in CCl4 cirrhotic rats. J Hepatol. 2007;46:1040-6.
26. Zafra C, Abraldes JG, Turnes J, Berzigotti A, Fernández M, García-Pagan JC, et al. Simvastatin enhances hepatic nitric oxide production and decreases the hepatic vascular tone in patients with cirrhosis. Gastroenterology. 2004;126:749-55.
27. Lavina B, Gracía-Sancho J, Rodríguez-Vilarrupla A, Chu Y, Heistad DD, Bosch J, et al. Superoxide dismutase gene transfer reduces portal pressure in CCl4 cirrhotic rats with portal hypertension. Gut. 2009;58:118-25.
28. Hernández-Guerra M, García-Pagan JC, Turnes J, Bellot P, Deulofeu R, Abraldes JG, et al. Ascorbic acid improves the intrahepatic endothelial dysfunction of patients with cirrhosis and portal hypertension. Hepatology. 2006;43:485-91.
29. Matei V, Rodríguez-Vilarrupla A, Deulofeu R, Colomer D, Fernández M, Bosch J, et al. The eNOS cofactor tetrahydrobiopterin improves endothelial dysfunction in livers of rats with CCl4 cirrhosis. Hepatology. 2006;44:44-52.
30. Gracía-Sancho J, Lavina B, Rodríguez-Vilarrupla A, García-Caldero H, Fernández M, Bosch J, et al. Increased oxidative stress in cirrhotic rat livers: A potential mechanism contributing to reduced nitric oxide bioavailability. Hepatology. 2008;47:1248-56.

31. Bosch J, Abraldes JG, Groszmann RJ. Current management of portal hypertension. J Hepatol. 2003;38:S54-68.
32. Kureishi Y, Luo Z, Shiojima I, Bialik A, Fulton D, Lefer DJ, et al. The HMG-CoA reductase inhibitor simvastatin activates the protein kinase Akt and promotes angiogenesis in normocholesterolemic animals. Nat Med. 2000;6:1004-10.
33. Trebicka J, Hennenberg M, Laleman W, Shelest N, Biecker E, Schepke M, et al. **Atorvastatin lowers portal pressure in cir**rhotic rats by inhibition of RhoA/Rho-kinase and activation of endothelial nitric oxide synthase. Hepatology. 2007;46:242-53.
34. Abraldes JG, Albillos A, Banares R, Turnes J, González R, García-Pagan JC, et al. Simvastatin lowers portal pressure in patients with cirrhosis and portal hypertension: a randomized controlled trial. Gastroenterology. 2009;136:1651-8.
35. Moreno M, Ramalho LN, Sancho-Bru P, Ruiz-Ortega M, Ramalho F, Abraldes JG, et al. Atorvastatin attenuates angiotensin II-induced inflammatory actions in the liver. Am J Physiol Gastrointest Liver Physiol. 2009;296:G147-56.
36. Trebicka J, Hennenberg M, Odenthal M, Shir K, Klein S, Granzow M, et al. Atorvastatin attenuates hepatic fibrosis in rats after bile duct ligation via decreased turnover of hepatic stellate cells. J Hepatol. 2010;53:702-12.
37. Glynn RJ, Danielson E, Fonseca FA, Genest J, Gotto AM Jr, Kastelein JJ, et al. A randomized trial of rosuvastatin in the prevention of venous thromboembolism. N Engl J Med. 2009;360:1851-61.
38. Francoz C, Belghiti J, Vilgrain V, Sommacale D, Paradis V, Condat B, et al. Splanchnic vein thrombosis in candidates for liver transplantation: usefulness of screening and anticoagulation. Gut. 2005;54:691-7.
39. Zocco MA, Di Stasio E, De Cristofaro R, Novi M, Ainora ME, Ponziani F, et al. Thrombotic risk factors in patients with liver cirrhosis: correlation with MELD scoring system and portal vein thrombosis development. J Hepatol. 2009;51:682-9.
40. La Mura V, Pasarin M, García-Pagan JC, Abraldes JG, Bosch J. Effects of simvastatin administration on liver microvascular dysfunction induced by LPS. J Hepatol. 2011;54 Suppl 1:S48.
41. Alp NJ, Channon KM. Regulation of endothelial nitric oxide synthase by tetrahydrobiopterin in vascular disease. Arterioscler Thromb Vasc Biol. 2004;24:413-20.
42. Katusic ZS, D'Uscio LV. Tetrahydrobiopterin: mediator of endothelial protection. Arterioscler Thromb Vasc Biol. 2004;24:397-8.
43. Wever RM, Van Dam T, Van Rijn HJ, De Groot F, Rabelink TJ. Tetrahydrobiopterin regulates superoxide and nitric oxide generation by recombinant endothelial nitric oxide synthase. Biochem Biophys Res Commun. 1997;237:340-4.
44. Matei V, Rodríguez-Vilarrupla A, Deulofeu R, García-Caldero H, Fernández M, Bosch J, et al. Three-day tetrahydrobiopterin therapy increases in vivo hepatic NOS activity and reduces portal pressure in CCl4 cirrhotic rats. J Hepatol. 2008;49:192-7.
45. Huie RE, Padmaja S. The reaction of no with superoxide. Free Radic Res Commun. 1993;18:195-9.
46. Cai H, Harrison DG. Endothelial dysfunction in cardiovascular diseases: the role of oxidant stress. Circ Res. 2000;87:840-4.
47. Lum H, Roebuck KA. Oxidant stress and endothelial cell dysfunction. Am J Physiol Cell Physiol. 2001;280:C719-41.
48. Jay D, Hitomi H, Griendling KK. Oxidative stress and diabetic cardiovascular complications. Free Radic Biol Med. 2006;40:183-92.
49. Heistad DD. Oxidative stress and vascular disease: 2005 Duff lecture. Arterioscler Thromb Vasc Biol. 2006;26:689-95.
50. Pizcueta MP, De Lacy AM, Kravetz D, Bosch J, Rodés J. Propranolol decreases portal pressure without changing portocollateral resistance in cirrhotic rats. Hepatology. 1989;10:953-7.
51. De Gottardi A, Berzigotti A, Seijo-Rios S, D'Amico M, Abraldes JG, García-Pagán JC, et al. Dark chocolate attenuates the post-prandial increase in HVPG in patients with cirrhosis and portal hypertension. J Hepatol. 2010;52:S9.