Review

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Hepatic endothelial dysfunction and abnormal angiogenesis: New targets in the treatment of portal hypertension

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Portal hypertension is the main cause of complications in patients with chronic liver disease. Over the past 25 years, progress in the understanding of the pathophysiology of portal hypertension was followed by the introduction of an effective pharmacological therapy, consisting mainly of treatments aimed at correcting the increased splanchnic blood flow. It is only recently that this paradigm has been changed. Progress in our knowledge of the mechanisms of increased resistance to portal blood flow, of the formation of portal-systemic collaterals, and of mechanisms other than vasodilatation maintaining the increased splanchnic blood flow have opened entirely new perspectives for developing more effective treatment strategies. This is the aim of the current review, which focuses on: (a) the modulation of hepatic vascular resistance by correcting the increased hepatic vascular tone due to hepatic endothelial dysfunction, and (b) correcting the abnormal angiogenesis associated with portal hypertension, which contributes to liver inflammation and fibrogenesis, to the hyperkinetic splanchnic circulation, and to the formation of portal-systemic collaterals and varices.

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 need for 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 of 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 [1,2]. 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 [3]. Some of these agents (NSBB, terlipressin) act by decreasing the cardiac index and causing splanchnic arterial vasoconstriction [2], while the effects of somatostatin are more linked to suppressing glucagon secretion [4] and facilitating adrenergic vasoconstricition [5].

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Modulation of intra-hepatic endothelial dysfunction

Increased intra-hepatic resistance to portal blood flow is the primary factor leading to portal hypertension in cirrhosis. Much of the increased intra-hepatic 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 intra-hepatic resistance may represent 30–40% of the total increased intra-hepatic vascular resistance in cirrhosis. Contractile elements influencing the hepatic vascular bed can be located at sinusoidal as well as extrasinusoidal levels and include vascular smooth muscle cells of the intra-hepatic vasculature (i.e. small portal venules in portal areas) [7], activated hepatic stellate cells (HSCs) (pericyte cells located in the perisinusoidal space of Disse with extensions that wrap around the sinusoids and reduce its caliber after contraction) [8,9], and hepatic myofibroblasts

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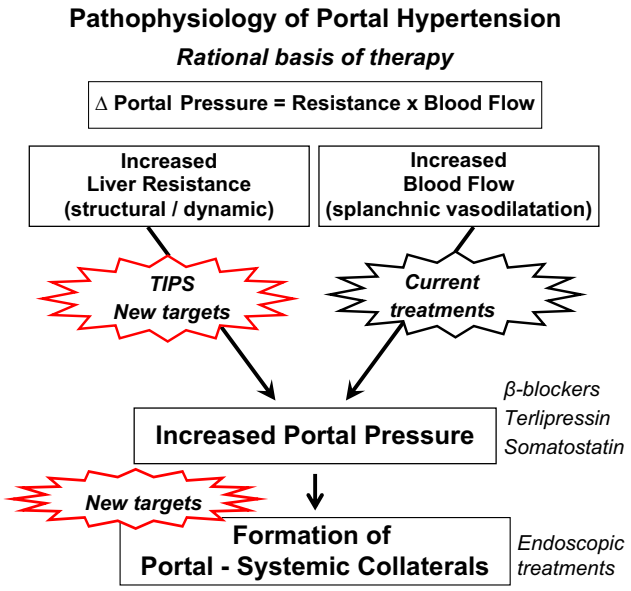

Fig. 1. Rationale basis for the treatment of portal hypertension. While current treatments are based on the use of drugs that decrease blood flow by attenuating the splanchnic vasodilatation (*old paradigm*), new treatments aim at correcting the increased hepatic vascular resistance and the formation of portal-systemic collaterals (*new paradigm*).

that may compress the regenerating nodules or venous shunts within the fibrous septa. It is now clear that vasoactive mediators, either vasoconstrictors or vasodilators, may modulate intrahepatic vascular resistance either in health or during liver disease, whatever the location where they act.

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" [10,11]. The hepatic vascular bed of cirrhotic livers also exhibits endothelial dysfunction [12]. Indeed, contrary to what happens in normal livers, the cirrhotic liver cannot accommodate the increased portal blood flow caused by the post-prandial hyperemia, which determines an abrupt post-prandial increase in portal pressure [13]. 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) [12,14]. 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 intra-hepatic resistance of the cirrhotic liver. Such imbalance between endogenous vasoconstrictor and vasodilator factors observed in the cirrhotic liver is quite similar to that found in the systemic circulation in patients with arterial hypertension. This is why the extensive advances in cardiovascular research have contributed enormously to an improved knowledge of the pathophysiology of portal hypertension. The main factors involved are described below.

COX derived prostanoids

Cyclooxygenase (COX) is the key enzyme in the biosynthetic pathway leading to the production of prostaglandins (PGs) and thromboxane (TX) from arachidonic acid (AA) [15]. COX-1 is constitutively expressed but it can also be stimulated by different factors [16,17]. COX-2 is the inducible isoform of COX that is usually expressed after stimulation with proinflammatory agents [18]. However, it has also been shown to be constitutively expressed in some tissues, including the liver [14] [19] and the mesenteric vascular bed [20].

Much evidence supports the involvement of COX-1 derived prostanoids promoting the increase in resistance to portal blood flow of cirrhotic livers. Indeed, the hyper-response 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 pretreating the livers with non-selective COX blockers, COX-1 selective blockers, or TXA2 receptor antagonists [19]. 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 [14].

Sinusoidal endothelial cells are the major contributor to the increased production of vasoconstrictors prostanoids (TXA2, and probably also prostaglandin H2) [21]. 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 [21]. On the other hand, it has been suggested that Kupffer cell activation is also involved in the increased portal pressure of fibrotic livers via TXA2 generation [22]. 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 on hepatic circulation.

Reduced nitric oxide (NO) bioavailability within the cirrhotic liver

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 Ca^{2+}. 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 [23]. In the cirrhotic liver, there is a reduced NO bioavailability that plays a major role in increasing intra-hepatic vascular resistance and thereby worsening portal hypertension. Decreased NO production occurs despite a normal expression of eNOS mRNA and normal levels of eNOS protein [12,24], 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 [25,26] (Table 1). In accordance with these pathophysiological abnormalities several efforts

Table 1. Mechanism for decreased hepatic NO synthesis in cirrhosis.

Decrease eNOS expression	⟶	Not proven
Decrease eNOS activity	⟶	Well demonstrated
↓		
Intra-hepatic endothelial dysfunction		**Counteracted by**
Increased caveolin expression (cholestasis)		
Decreased Akt-p dependent eNOS phosphorylation		Statins
Decreased production of BH4		Supplements
Scavenging of NO by O^- (reduced SOD activity)		Antioxidant/SOD
Increased levels of asymmetric-DMA		

to improve NO bioavailability within the liver have been attempted.

The 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 [15]. NCX-1000, a NO-releasing derivative of ursodeoxycolic acid (UDCA), was designed to selectively target intra-hepatic circulation by delivering NO only to the intra-hepatic site, with the aim to effectively reduce the baseline hepatic venous pressure gradient (HVPG) and to counteract the post-prandial increase in portal pressure, without adverse effects on systemic and splanchnic circulation. Results from experimental models were promising [27]. 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 [28]. Thus, this agent failed to prove efficacious for the treatment of portal hypertension. This was mainly due to poor bioavailability, and to the lack of a specific intra-hepatic vasodilatory effect [28]. The search for an effective way of specifically supplementing NO to liver circulation, thus reducing portal pressure without affecting systemic arterial pressure, is still warranted.

Other strategies to correct the intra-hepatic NO deficiency have been based on either overexpressing NOS by transfecting the liver with adenovirus encoding eNOS [29,30], nNOS [31], or constitutively active Akt [32], by enhancing eNOS activity by simvastatin (a HMG-CoA reductase inhibitor) [33,34], by decreasing NO scavenging by means of antioxidants [35,36], by administering the eNOS cofactor tetrahydrobiopterin [37] or scavenging NO by reactive oxygen species (ROS) due to oxidative stress [38] (Table 1). The possible role of statins, tetrahydrobiopterin (BH4), and oxidative stress are the strategies more extensively studied.

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 intra-hepatic vascular resistance, while maintaining or enhancing hepatic blood flow [39]. 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 [29,31,32,39,40].

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 [41–46]. 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 [43,47,48] (Fig. 2). This led us to hypothesize that statins could be useful for enhancing NO production in the liver circulation [34] and, thus, they could have potential for the treatment of portal hypertension. Indeed, consistent experimental [33,49] and human studies [34] 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 in 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 cell (HSC) contraction by a NO independent (and, thus, endothelium independent) mechanism [49]. Altogether, this indicates that these drugs could behave as true liver-selective vasodilators [33,34,49]. 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 [50]. 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 (Fig. 3). This might be related to the fact that simvastatin, by increasing NO availability in the sinusoidal circulation, counteracts the effects of beta-blockers increasing liver resistance due to unopposed alpha-adrenergic driven vasoconstriction [51]. 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 perfusion of the hepatocytes, with an ensuing beneficial effect on liver function [50]. It is important to note that this effect, of potential clinical relevance, is observed neither 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 [52–60], 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 reduction in portal pressure. A recent study showed that the continuous administration of atorvastatin prevented liver inflammation and hepatic stellate cell activation induced by Angiotensin-II infusion [61].

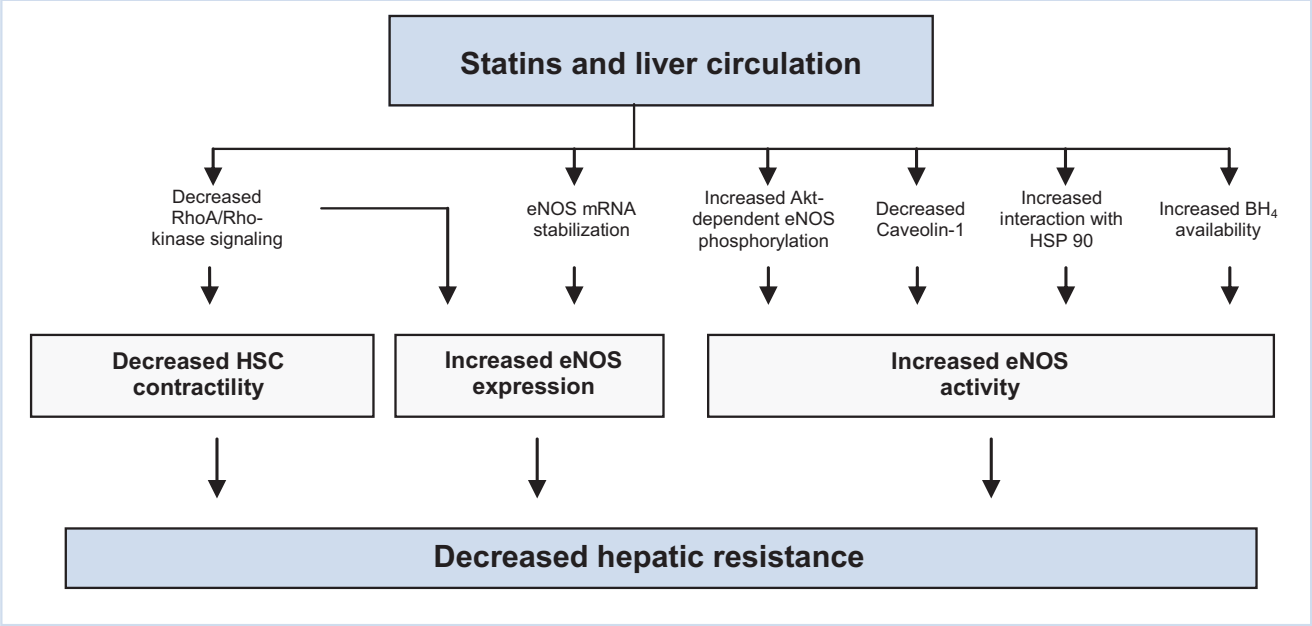

Fig. 2. Mechanisms mediating the decrease in hepatic resistance by statins. Statins increase eNOS expression and activity. The most immediate effect of statins on endothelial NO production is an increase in eNOS phosphorylation at Ser 1177/1179, with subsequent increased activity [43]. This is mediated by the activation of the phosphatidyl inositol 3-kinase (PI3K)/Akt pathway [43] that leads to an increase in Akt phosphorylation at Ser473 with ensuing eNOS phosphorylation [47]. Statins reduce the expression of the eNOS inhibitory protein caveolin-1 [86] and increase the interaction of eNOS with its stimulatory protein Hsp90 [86]. These effects occur slower than the PI3K/Akt pathway activation. Statins also increase the expression of GTP cyclohydrolase I (GTPCH) [87], the rate-limiting enzyme for *de novo* synthesis of tetrahydrobiopterin (BH_4), a cofactor that increases eNOS activity by preventing eNOS uncoupling and, thus, superoxide generation. Statins also upregulate eNOS expression by increasing eNOS mRNA stability [48]. Lastly, statins inhibit hepatic RhoA/Rho kinase signalling, which increases eNOS expression and decreases hepatic stellate cell (HSC) contractility [49].

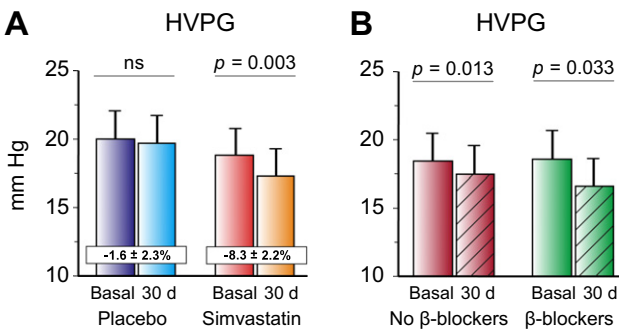

Fig. 3. Double-blind randomized controlled trial of simvastatin vs. placebo for portal hypertension in patients with cirrhosis. (A) HVPG decreased significantly in patients receiving simvastatin, but not in those receiving placebo. (B) In the group of patients treated with simvastatin, the decrease in portal pressure was observed both in patients under no treatment and in patients under continuous propranolol administration, suggesting an additive effect with non-selective beta-blockers. (*modified from Abraldes* et al., *Gastroenterology 2009*).

Furthermore, a recent large randomized controlled trial showed that rosuvastatin significantly decreased the occurrence of venous thromboembolism [62], which could be relevant in patients with cirrhosis, who are at increased risk of portal vein thrombosis [63,64].

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 secondary prophylaxis of variceal bleeding. In a recent trial, bleeding-free survival in patients treated with a 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 has the potential to improve liver function – this drug could be a statin. 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 the benefit of statins in this setting would require a large trial with a very long follow-up, which is unlikely to be done in the absence of commercial interests or strong support from public agencies.

Tetrahydrobiopterin supplementation

Tetrahydrobiopterin (Fig. 4)(BH4) is an essential cofactor for the adequate generation of NO by NOS enzymes [14–16]. If adequate quantities of BH4 are not present, a situation known as NOS uncoupling takes place and the production of NO is decreased. Studies from our lab [13] 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 (GTP-CHI), the limiting enzyme in BH4 synthesis, which is associated with decreased NOS activity and NO availability. In cirrhotic rats, administration of BH4 for three days increased liver NOS activity and cGMP levels and significantly reduced portal pressure. Amelioration of portal hypertension was associated with a normaliza-

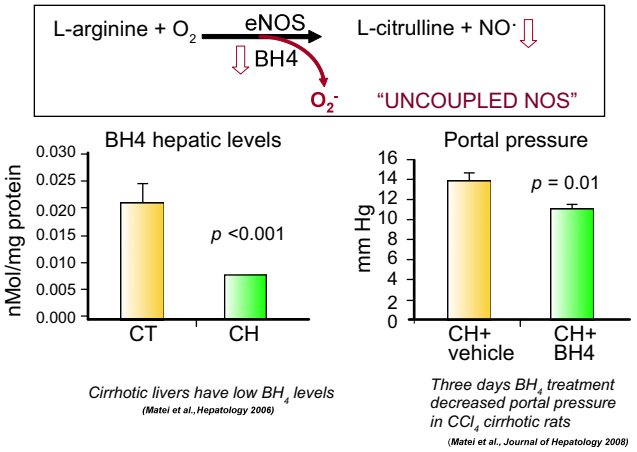

Fig. 4. Tetrahydropiopterin (BH4) is an essential cofactor for eNOS. Decreased BH4 levels in the cirrhotic liver determine eNOS uncoupling, which results in decreased NO synthesis and increased release of superoxide radicals, which in turn can react with NO, further decreasing the bioavailability of NO. Supplementation with exogenous BH4 decreases portal pressure in cirrhotic rats. (*data from Matei* et al. *Hepatology 2006, and Matei* et al., *Journal of Hepatology 2008*).

tion of arterial pressure. These data support the concept that tetrahydrobiopterin supplementation may represent a new and effective therapeutic strategy for portal hypertension. [37,65].

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 [66], promotes a marked reduction in NO bioavailability followed by an increase in vascular tone [67–70]. Our group has recently demonstrated that this also happens in the cirrhotic liver. This further demonstrates that in chronic liver disease, reduced intra-hepatic NO bioavailability is due not only to the consequence of a reduction in its production by eNOS synthase, but also to an increase in scavenging by increased levels of 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 [38]. Interestingly, the study demonstrated that cyclooxygenase (COX) or xanthine oxidase (XO) inhibition markedly reduced intra-hepatic O_2^- levels, which points out that these enzymatic systems are potential sources of O_2^- in cirrhosis [38]. 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 [38]. 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 [38]. Further, abolition of the increase in O_2^- using SOD supplementation was followed by a partial restoration in NO bioavailability.

All these data strongly suggest 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 intra-hepatic NO bioavailability and to ameliorate hepatic vascular tone in cirrhosis. In that regard, transfection of cirrhotic rats with portal hypertension with adenovirus encoding extracellular 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 [35]. 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 [35]. 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 [71] (Fig. 5).

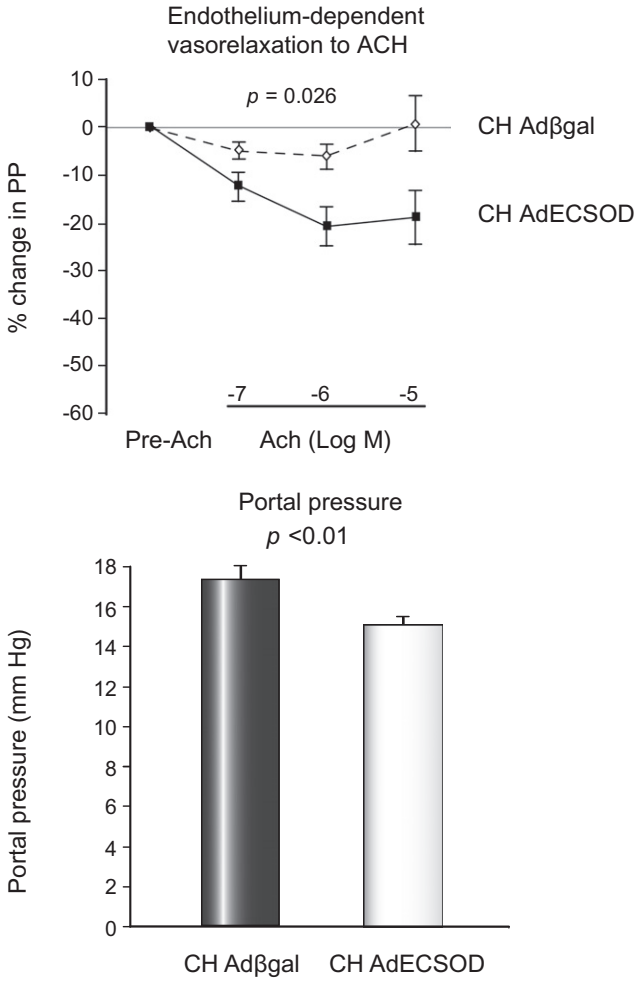

Fig. 5. Transfection with adenovirus encoding EC-SOD improves the endothelial dependent vasorelaxation in perfused cirrhotic livers (*upper panel*) and decreases portal pressure *in vivo* in cirrhotic rats (*lower panel*). (*Modified from Laviña* et al. *Gut 2009*).

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 support for this concept comes from studies supplementing ascorbic acid in patients with cirrhosis [36]. 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 of neutralizing ROS, mainly superoxide (O_2^-). 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 intra-hepatic endothelial dysfunction, blunting the post-prandial increase in portal pressure [36], which encourages performing further studies testing antioxidants as adjunctive therapy in the treatment of portal hypertension.

Role of angiogenesis in portal hypertension

As already mentioned, the two main hemodynamic factors leading to the portal hypertensive syndrome are the increased resistance to portal blood flow through the cirrhotic liver, and the development of a hyperdynamic splanchnic circulatory state. In this state, an increase in blood flow in splanchnic organs drains into the portal vein and a subsequently increases portal venous inflow which represents a significant factor maintaining and worsening portal hypertension [1,2].

Another characteristic disturbance of portal hypertension is the formation of an extensive network of portosystemic collateral vessels, which include the esophageal and gastric varices [1,2]. In addition to varices and bleeding, collateral vessels result in shunting of portal blood into the systemic circulation, causing high systemic concentrations of several substances normally metabolized by the liver, such as drugs, toxins, hormones, and bacteria, which contribute to major complications of chronic liver disease, including portosystemic encephalopathy and sepsis [1,2]. Traditionally, the increased splanchnic blood flow has been attributed to an overproduction of endogenous vasodilators and a decreased vascular reactivity to vasoconstrictors [1,2], while the formation of collaterals has been considered a mechanical consequence of the increased portal pressure that will result in the opening and dilatation of pre-existing vascular channels at sites of anatomical communications between the portal and systemic circulations [1,2].

However, recent studies from our laboratory challenge these concepts by demonstrating that angiogenesis, the formation of new blood vessels [72–75], plays a pivotal role in the development and maintenance of splanchnic hyperemia and portosystemic collateralization. Importantly, growing evidence suggests that pathological angiogenesis is also involved in the establishment of the abnormal angioarchitecture distinctive of the cirrhotic liver, and is also intimately related to fibrogenesis [76,77]. Therefore, angiogenesis could also contribute to an increased intra-hepatic resistance in portal hypertension and liver cirrhosis.

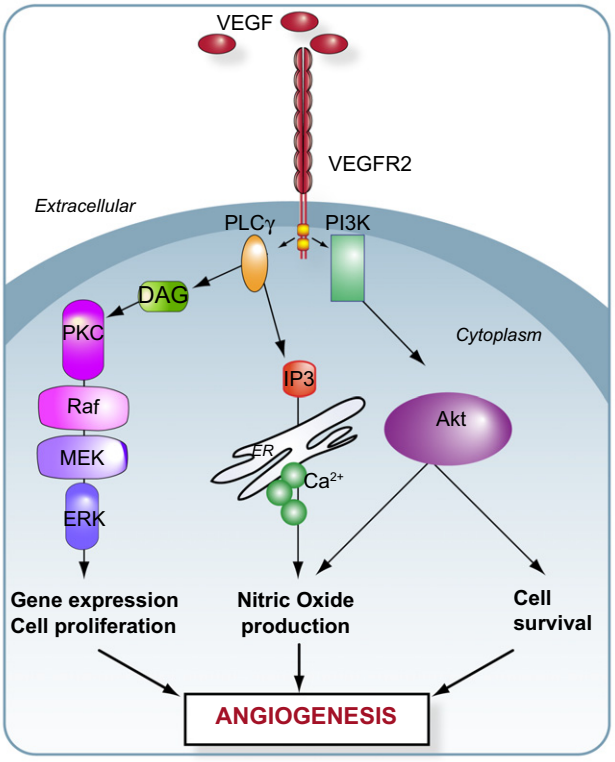

Fig. 6. Schematic illustration of the vascular endothelial growth factor (VEGF) signalling pathway. There is now much evidence that VEGF receptor-2 (VEGFR-2) is the major mediator of VEGF-driven responses in endothelial cells and it is considered to be a crucial signal transducer in both physiologic and pathologic angiogenesis. The binding of VEGF to VEGFR-2, a protein tyrosine kinase that is directly activated by ligand-receptor interaction, allows the receptor to activate a range of signal transduction molecules, such as phospholipase C gamma (PLCγ) and phosphatidylinositol 3-kinase (PI3K). Activation of PLCγ cleaves phosphatidylinositol 4,5-bisphosphate (PIP2) to form diacylglycerol (DAG) and D-myo-inositol-1,4,5-trisphosphate (IP3). DAG binds to and activates protein kinase C (PKC), which plays a crucial role in VEGF mitogenic signalling via the Raf-MEK-ERK (mitogen activated protein kinase kinase/extracellular signal-regulated kinase) pathway. IP3 regulates intracellular Ca^{2+} by binding to the IP3 receptor on the endoplasmic reticulum and stimulating Ca^{2+} release from the endoplasmic reticulum. Free intracellular Ca^{2+} can bind to calmodulin, and this Ca^{2+}-calmodulin complex activates endothelial nitric oxide synthase (eNOS), which in turn increases nitric oxide (NO) synthesis. Upon ligand binding to VEGFR-2, PI3K is also activated leading to activation of the protein kinase Akt, which in turn phosphorylates eNOS and increases NO production. Akt activation can also promote endothelial cell survival.

Evidence supporting a role for angiogenesis in the pathogenesis of portal hypertension includes recent investigations demonstrating that VEGF, a potent angiogenic factor [36], is overexpressed in splanchnic organs from portal hypertensive animals [37,66]. The VEGF signalling cascade is schematically shown in Fig. 6.

The expression of VEGF receptor-2 (VEGFR-2) and the endothelial cell marker CD31 [67] is also increased in the splanchnic territory in experimental models of portal hypertension [37,66]. These and other studies provided evidence of increased VEGF-driven splanchnic angiogenesis in portal hypertensive animals and in cirrhotic patients [68–71].

The precise mechanism triggering VEGF-dependent angiogenesis in portal hypertension remains speculative, but several factors known to occur in portal hypertension, such as tissue

hypoxia, cytokines, and mechanical stress have been shown to promote VEGF expression in various tissues and cell types [32,33].

Recent studies have determined the effects of several angiogenesis inhibitors, with different modes of action, in experimental models of portal hypertension [74,78]. These studies demonstrated that treatment with VEGFR-2 blockers from the induction of portal hypertension markedly decreased the formation of portosystemic collateral vessels (by 50–65%) and reduced splanchnic vascularization and portal venous inflow in rodent models of portal hypertension [74,78]. These findings indicate that angiogenesis is involved in the development of the portosystemic collateral and in the maintenance of an increased portal venous inflow, and strongly support that agents interfering with the VEGF/VEGFR-2 signalling pathway may potentially be used to prevent these complications of portal hypertension.

Furthermore, recent studies have shown that therapeutic strategies directed at inhibiting angiogenesis may have clinical importance in the treatment of established portal hypertension in chronic liver disease [75,79] and angiogenesis in liver diseases [80]. In this regard, it should be noted that in the process of neovascularization, VEGF plays a predominant role in the initial stages of formation of new blood vessels, activating the proliferation of endothelial cells and formation of endothelial tubules, while maturation of the newly formed vessels is mainly modulated by the proangiogenic growth factor platelet-derived growth factor (PDGF). To revert established new blood vessels, multi-target agents or a combination of agents blocking both VEGF and PDGF is required. For example, the administration of a low dose of the multi-targeted receptor tyrosine kinase inhibitor sorafenib has produced multi-fold beneficial effects in experimental models of portal hypertension and cirrhosis, with a 25% reduction in portal pressure, attenuation of hyperdynamic splanchnic and systemic circulations, a significant decrease in the extent of portosystemic collaterals, and a remarkable improvement in liver damage and intra-hepatic fibrosis, inflammation, and angiogenesis [75] (Fig. 7). Of note, sorafenib has been approved in several countries worldwide for treatment of renal [81] and hepatocellular carcinoma [82]. The latter is a common complication of advanced cirrhosis, which means that sorafenib is currently being used in patients with portal hypertension.

The potential efficacy of anti-angiogenic drugs in portal hypertensive and cirrhotic patients will need, however, a very careful evaluation since the side effects of these drugs can be serious [83,84]. For instance, it will be necessary to define a safe and optimal dose for portal hypertensive patients, which likely will be much lower than that required to treat patients with cancer [82]. Of note, in our experimental studies we have used doses of anti-angiogenic agents over one order of magnitude lower than those used clinically for cancer, which had no noticeable adverse effects in mice or rats. Another issue of concern is that the formation of new blood vessels is not only required for growth and development, but it is also an important natural process in the adult body used for healing and reproduction [83]. Treatments that block angiogenesis could therefore potentially interfere with these physiological processes [83]. These may be partly overcome by

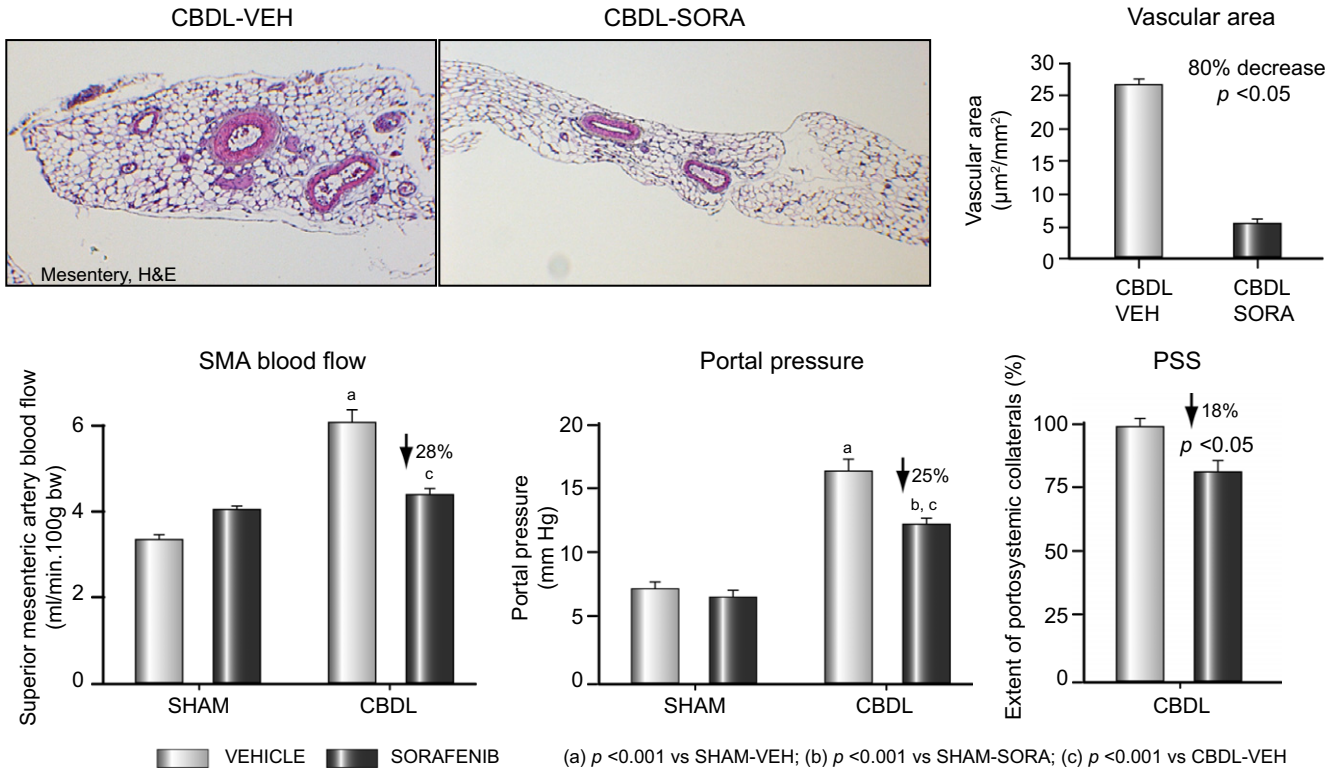

Fig. 7. Effects of continued treatment with sorafenib (SORA) or vehicle (VEH) on splanchnic neovascularization (*top panel*), and on superior mesenteric artery (SMA) blood flow, portal pressure, and portosystemic collateralization (PSS) in common bile duct ligated (CBDL) cirrhotic rats (*bottom panel*). This treatment also resulted in decreased liver fibrosis as a consequence of reduced activation of hepatic stellate cells and diminished inflammation (data not shown). (*reproduced from Mejias* et al., *Hepatology 2009*).

using anti-angiogenic agents that do not interfere with physiological angiogenesis since they act predominantly on pathological angiogenesis. It has been suggested that drugs blocking placental growth factor (PLGF) driven angiogenesis may accomplish this goal in portal hypertensive models [61,84,85].

Despite these drawbacks, however, angiogenesis remains a very tempting and innovative target in portal hypertension therapy, and researchers are exploring new agents and approaches to maximize the beneficial effects of anti-angiogenic therapies in cirrhosis.

Key points

- Increased hepatic resistance is the primary cause of portal hypertension in chronic liver diseases
- Increased hepatic resistance has two components, the first is structural while the second is functional
- The molecular pathways of both components are being delineated
- Both are (almost) totally reversible in experimental models, which opens new perspectives for therapeutic applications
- Angiogenesis plays an important role in the pathogenesis of portal hypertension, modulating hepatic stellate cells activation and fibrogenesis, splanchnic vasodilation and formation of portal-systemic collaterals
- Because of the multiple factors involved, a rational therapy should target several pathophysiological mechanisms (*multitarget therapy*)
- Translation of these advances into clinical practice is soon anticipated

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Conflict of interests

The Authors who have taken part in this study do not have a relationship with the manufacturers of the drugs involved either in the past or present and did not receive funding from the manufacturers to carry out their research.

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