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REVIEW

Physiopathology of splanchnic vasodilation in portal hypertension

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Abstract

In liver cirrhosis, the circulatory hemodynamic alterations of portal hypertension significantly contribute to many of the clinical manifestations of the disease. In the physiopathology of this vascular alteration, mesenteric splanchnic vasodilation plays an essential role by initiating the hemodynamic process. Numerous studies performed in cirrhotic patients and animal models have shown that this splanchnic vasodilation is the result of an important increase in local and systemic vasodilators and the presence of a splanchnic vascular hyporesponsiveness to vasoconstrictors. Among the molecules and factors known to be potentially involved in this arterial vasodilation, nitric oxide seems to have a crucial role in the physiopathology of this vascular alteration. However, none of the wide variety of mediators can be described as solely responsible, since this phenomenon is multifactorial in origin. Moreover, angiogenesis and vascular remodeling processes also seem to play a role. Finally, the sympathetic nervous system is thought to be involved in the pathogenesis of the hyperdynamic circulation associated with portal hypertension, although the nature and extent of its role is not completely understood. In this review, we discuss the different mechanisms known to contribute to this complex phenomenon.

Key words: Liver cirrhosis; Portal hypertension; Splanchnic vasodilation; Hyperdynamic circulation; Sympathetic nervous system

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INTRODUCTION

Portal hypertension is defined as a pathological increase in portal vein pressure and it is diagnosed when the hepatic venous pressure gradient (HVPG) is above the normal range (1-5 mm Hg). HVPG is assessed by a hepatic hemodynamic study through a suprahepatic vein catheterization and estimates the difference of pressure between the portal vein and the inferior cava vein. Liver cirrhosis is the most frequent cause of portal hypertension in western countries. When HVPG increases to 10 mm Hg or more, portal hypertension of cirrhosis results in severe complications including ascites, hepatorenal syndrome, hepatic encephalopathy and haemorrhage from esophageal varices[1,2]. Two main factors contribute

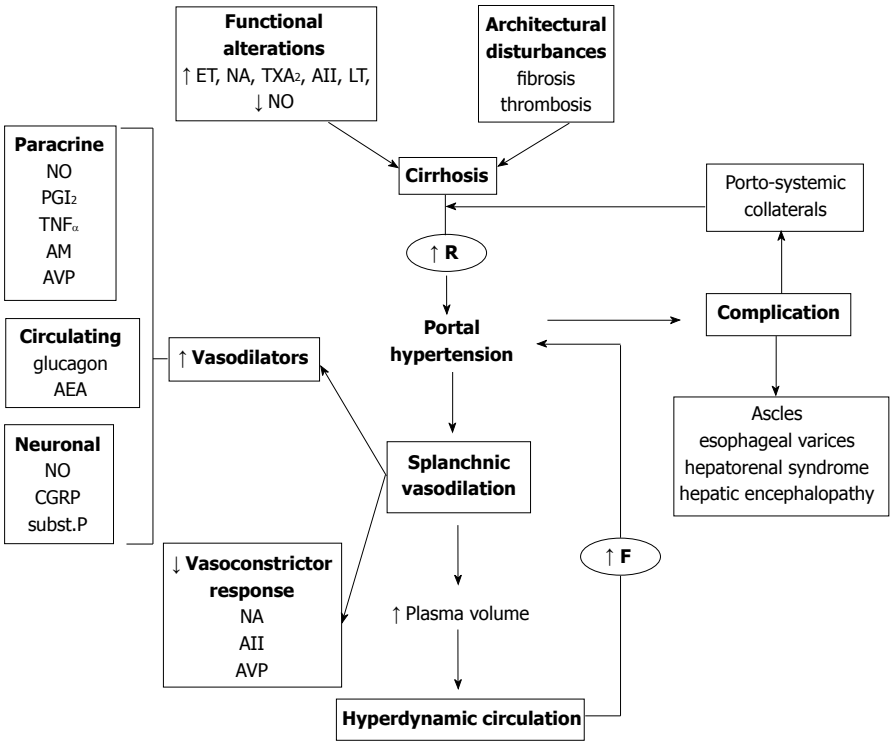

Figure 1 Physiopathology of portal hypertension. In cirrhosis, the initiating factor leading to portal hypertension is an increase in intrahepatic vascular resistance (R), whereas the increase in portal blood flow (F) is a secondary phenomenon that maintains and worsens the increased portal pressure, giving rise to the hyperdynamic circulation syndrome. The different factors implicated in the distinct mechanisms of portal hypertension are shown. AⅡ: angiotensin Ⅱ; AEA: anandamide; AM: adrenomedullin; CGRP: calcitonine gene related peptide; CO: carbon monoxide; ET: endothelin; H_2S: hydrogen sulfide; LT: leukotrienes; NE: norepinephrine; NO: nitric oxide; PGI_2: prostacyclin; SP: substance P; TXA_2: thromboxane A_2.

to establish and maintain portal hypertension: the vascular resistance due to difficult outflow of portal blood to the hepatic veins and the increased splanchnic blood flow (hyperdynamic syndrome). Portal hypertension is also associated with the formation of porto-systemic venous collaterals in an attempt to decompress the portal venous system[3,4]. However, this collateral circulation leads to the generation of varices which contribute to the morbidity and mortality of the disease.

PHYSIOPATHOLOGY OF PORTAL HYPERTENSION

Applying the Ohm's law in the portal venous system: $\Delta P = Q \times R$, then the portal pressure gradient (ΔP), is the result of the product of the blood flow within the entire portal venous system (Q) and the vascular resistance of the same vascular system (R), including the hepatic vascular bed and the porto-systemic collaterals. Thus, portal hypertension is caused by an increase in blood flow, an increase in resistance or a combination of both. The initial mechanism that leads to portal hypertension in liver cirrhosis is an increase in hepatic resistance, mainly as a result of a mechanical occlusion. In later stages, an increase in splanchnic blood flow leads to the hyperdynamic circulation state, which in turn contributes to the maintenance and aggravation of many of the complications of cirrhosis and portal hypertension (Figure 1)[5].

INCREASED VASCULAR RESISTANCE TO PORTAL BLOOD FLOW

The vascular resistance to portal blood flow is dependent on two factors: the intrahepatic resistance and the resistance generated by the collateral circulation. The increased intrahepatic vasculature resistance (IHVR) to portal blood flow is the main and primary factor of portal hypertension secondary to liver cirrhosis (Figure 1).

Intrahepatic resistance

Classically, structural distortion of the intrahepatic vasculature, as a consequence of fibrosis, scaring and vascular thrombosis, has been considered the only cause of the increased IHVR. The cellular mechanisms involved in fibrosis formation and cirrhosis are well known. In response to hepatocellular injury, hepatic stellate cells are activated and their phenotype changes from a quiescent one to a myofibroblast-like cell. As a result of hepatic stellate cell activation, collagenization (capillarization) of the Space of Disse occurs, and the injured liver becomes cirrhotic[6,7]. The pioneering work by Bathal and Groszmann[8], based on a perfused rat liver model, demonstrated that in addition to the structural changes, a dynamic component, represented by contractile elements of the hepatic vascular bed, might contribute to the increased intrahepatic vascular tone. It has been suggested that this modifiable component represents 40% of the total IHVR[9]. In cirrhosis, an increased production of vasoconstrictors and a deficient release of vasodilators, in combination to an exaggerate response to vasoconstrictors and an impaired vasodilatory response of the hepatic vascular bed, are the mechanisms responsible for the increased dynamic component of IHVR[10].

Among all overexpressed vasoconstrictors[11-14], endothelin (ET) seems to play a particularly important role in the enhanced vascular tone in liver cirrhosis. Patients with liver cirrhosis present elevated ET-1 and ET-3 plas-

ma concentrations[15]. Moreover, not only hepatic ET-1 levels, but also ET receptor density are increased in the cirrhotic rat liver[16]. The ET_A receptor, found on vascular smooth muscle cells, causes vasoconstriction, whereas the ET_B receptor subtype located on endothelial cells induces vasorelaxation by stimulating endothelial nitric oxide synthase (eNOS)[17]. Several studies have been focused on ET blockade therapies. However, in contrast to what might be expected, ET_B receptor stimulation by ET_B agonist administration, resulted in an increased portal pressure in cirrhotic rats[18]. The effect of ET_A antagonists in reducing portal pressure of cirrhotic rats remains controversial[18,19]. In addition to endothelin, other contributing factors to the increased IHVR are the products of S-lipoxygenase (cysteinyl-leukotriene) and the cyclooxygenase pathways (thromboxane A2), angiotensin Ⅱ and the sympathetic system[11,12,14].

In addition to the exaggerated production of vasoconstrictors, the intrahepatic production of vasodilators, mainly nitric oxide (NO), remains insufficient in the cirrhotic liver[20,21]. NO is a potent vasodilator produced from L-arginine by different NOS. Although in the liver both eNOS and inducible NOS (iNOS) isoforms can be active, the insufficient hepatic NO production observed in cirrhosis has been attributed to the endothelial isoform[22,23]. Because mRNA and protein levels of eNOS are found in equal amounts in cirrhotic and normal livers, this NO-deficient production has been attributed to a post-translational dysfunction in eNOS activity[20,24,25]. On one hand, an increased expression of caveolin (an eNOS inhibitory protein)[24], and on the other hand, a decrease in eNOS phosphorilation due to abnormal Akt (protein kinase B) signalling[26], are the mechanisms that might explain the reduced eNOS activity in liver cirrhosis. In addition to a decreased NOS activity, an increased NO degradation has also been suggested to be responsible for the diminished NO bioavailability. Since superoxide (O_2^-) is able to react with NO to generate peroxinitrite ($ONOO^-$), NO bioavailability can be substantially reduced if the O_2^- levels are increased as a consequence of a decrease in superoxide dismutase activity. Indeed, a portal injection of adenovirus containing superoxide dismutase encoding gene reduces portal pressure by increasing NO bioavailability in cirrhotic rats[27].

Collateral circulation

The increase in portal pressure leads to the appearance of direct connections between the portal blood vessels and the general circulation. This attempt to decompress the portal venous system leads to severe complications, such as hepatic encephalopathy and the formation of esophageal varices. Taking into account that porto-systemic shunting diverts a large quantity of portal blood flow away from the liver, the vascular resistance of these vessels might contribute importantly to increasing vascular resistance of the portal venous system. Porto-systemic collaterals formation, which involves both neovascularisation and opening existing vessels[28,29], has been suggested to be angiogenic-dependent. Angiogenesis is mediated mainly by the vascular endothelial growth factor (VEGF). Fernandez *et al* demonstrated that anti-VEGF receptor-2 monoclonal antibody prevented porto-systemic collateral vessel formation in portal hypertensive mice[30,31]. Furthermore, since NAD(P)H is required for VEGF-induced angiogenesis, NAD(P)H oxidase blockade significantly reduced porto-systemic collateral formation[32]. The same authors have demonstrated that portal hypertensive rats treated with signalling inhibitors of VEGF and platelet derived growth factor (PDGF) significantly reduce their porto-systemic collaterization[33]. Also, the use, in experimental rat models of portal hypertension, of Sorafenib, a potent inhibitor of proangiogenic VEGF receptor-2 and PDGF receptor-β, induced an important decrease in splanchnic neovascularisation and in the extent of porto-systemic collaterals, along with a marked attenuation of hyperdynamic splanchnic and systemic circulations[34].

INCREASED SPLANCHNIC BLOOD FLOW

The splanchnic circulation is the main vascular bed responsible for the reduction in vascular resistance in the portal hypertensive state. An increase in splanchnic blood flow in portal hypertension is the result of a marked vasodilation of arterioles in splanchnic organs, which drain blood into the portal venous system[35]. The increase in blood flow in splanchnic organs and the subsequent increase in portal venous inflow, together with an increased resistance to portal inflow, maintains and aggravates the portal hypertensive syndrome[9]. An increased production or activation of vasodilatory mediators and systems, and a decreased vascular reactivity to vasoconstrictors (Figure 1), are probably responsible for this splanchnic hyperaemia (vasodilation). In addition, increased angiogenesis probably collaborates in increasing the splanchnic blood inflow[30,31].

Hyperdynamic circulation

The hyperdynamic circulatory state of portal hypertension is characterized by splanchnic and peripheral vasodilation, increased plasma volume and increased cardiac output[5]. The hyperdynamic splanchnic circulation is mediated in part by arterial vasodilation, but this vasodilation alone is not sufficient to cause the circulation to become hyperdynamic. It is the combination of arterial vasodilation and blood volume expansion that produces optimal conditions for maintaining the hyperdynamic circulatory state in portal hypertension[35,36] (Figure 1). The arterial vasodilation in the peripheral and splanchnic circulation leads to a decrease in central blood volume. This relative arterial hypovolemia leads to the stimulation of cardiopulmonary volume receptors and arterial baroreceptors, activating the sympathetic nervous system, the renin-angiotensin-aldostern system and arginin-vasopresin (antidiuretic hormone). Mediators from these

systems result in sodium and water retention by the kidneys, and consequently, plasma volume expansion. Sodium retention is due to increased tubular reabsorption of sodium, mediated by receptors for aldosterone, angiotensin and alpha-adrenergic stimuli. The decrease in water excretion is due to increased secretion of antidiuretic hormone[37].

The harmful effects of hyperdynamic circulation are not restricted to the hepatosplanchnic circulation. The hyperdynamic circulation also affects the cardiac (increase cardiac output), the pulmonary (hepatopulmonary syndrome) and the cerebral circulation (acute hepatic coma)[38,39]. Other organs such as the kidney and the brain (chronic encephalopathy) appear to be indirectly affected by the vasodilation in the other circulatory beds[5].

Animal models

The development of experimental models to study the hemodynamic alterations of portal hypertension has been of critical importance for the understanding of this syndrome. The pioneering work of Chojkier and Groszmann in establishing the partial portal-vein ligated (PVL) model has been a basic element in understanding portal hypertension pathophysiology[3,40]. In this model, the portal vein is isolated and a stenosis is created by a single ligature around a 20-gauge blunt-tipped needle lying along the portal vein. Subsequent removal of the needle yields a calibrate stenosis of the portal vein.

The PVL model reproduces all systemic and hemodynamic abnormalities detected in portal hypertension and the circulatory hyperdynamic state: portal pressure and portal flow increase, appearance of porto-systemic shunts, splanchnic vasodilation with splanchnic arteriolar resistance reduction and splanchnic flow increase, systemic vasodilation with arterial hypotension, total peripheral resistance reduction and cardiac output increase[40]. This model is extraordinarily homogenous, reproducible and has highly predictable chronobiology that permits the elucidation of the sequence of events involved in the generation of the hyperdynamic syndrome[41,42]. Porto-systemic shunting is detectable at two days after PVL surgery and the percentage of portal blood inflow diverted to collaterals approaches 100% after 1 wk[42]. Circulation becomes hyperdynamic 4-5 d after PVL, and 1 wk after portal vein ligation, rats present the complete range of portal hypertensive alterations with hyperdynamic circulatory syndrome and porto-systemic shunting formation.

Although the PVL model is easy to use and reproducible, the experimental rat models of cirrhosis generated by different mechanisms (basically by carbon tetrachloride administration and bile duct ligation) are probably more similar to human cirrhosis, since in addition to displaying all the hemodynamic alterations of portal hypertension, they present the metabolic, infectious and other complications of advanced liver disease[36,43]. Results obtained in PVL rats are usually tested in these models of cirrhosis.

MECHANISMS OF SPLANCHIC VASODILATION

The arterial vascular tone is determined by the balance between the effects of vasoactive molecules acting on the vascular smooth muscle. As mentioned, an increased concentration of circulatory vasodilators and an enhanced endothelial production of local vasodilators, as well as a decreased vascular responsiveness to endogenous vasoconstrictors have been observed in splanchnic vessels in portal hypertension[36] (Figure 1). Among the molecules and factors known to be potentially involved in this arterial vasodilation, none of them can be described as solely responsible, since this phenomenon is multifactorial in origin[44].

Nitric oxide

NO, an endothelial-derived relaxing factor, has been recognized as the most important vasodilator molecule that mediates the excessive arterial vasodilation observed in portal hypertension[45]. Its involvement, initially suggested by Vallance and Moncada[46], has been confirmed by a number of studies. In cirrhotic patients, increased levels of nitrates and nitrites, degradation products of NO oxidation[47], have been observed. In the splanchnic vascular bed of rats with portal hypertension an overproduction of NO responsible for vasopressor hyposensitivity has been clearly demonstrated[48]. Furthermore, inhibition of NO production reduces porto-systemic shunting and largely prevents the development of the hyperdynamic circulation[49]. NO is produced from L-arginine by the family of NOS enzymes, forming the free radical NO and citrulline as byproducts[50]. NO has a short life and is rapidly oxidized to the stable, inactive end-products, nitrite and nitrate[51]. The mechanism by which NO causes vasodilation is through the stimulation of soluble guanylyl cyclase (sGC) to generate cyclic guanosine monophosphate (cGMP) in vascular smooth muscle[52] (Figure 2). Three isoforms are known to produce NO: constitutively expressed isoforms, eNOS[53] and neuronal NOS (nNOS)[54], and iNOS[55] which, surprisingly, does not appear to be involved in the increased NO production in cirrhosis[56]. The major enzymatic source of the vascular NO overproduction has been shown to be eNOS[57]. In animal models (PVL rats) at least, it has been observed that eNOS upregulation precedes the hyperdynamic circulatory changes[45]. More recent evidence suggests that nNOS is also upregulated in aorta[58] and mesenteric arteries[59], playing a role in the development/maintenance of the hyperdynamic splanchnic circulation in experimental cirrhosis.

In endothelial cells, eNOS is activated by calcium/calmodulin (Ca^{2+}/CaM) in response to an elevation of cytosolic Ca^{2+} and by phosphorilation of eNOS at several sites[60,61]. Initial up-regulation of eNOS starts at the post-translational level by Akt-mediated eNOS phosphorilation[62], which increases its activity at any Ca^{2+} concentration[63]. During early cirrhosis, this pathway is

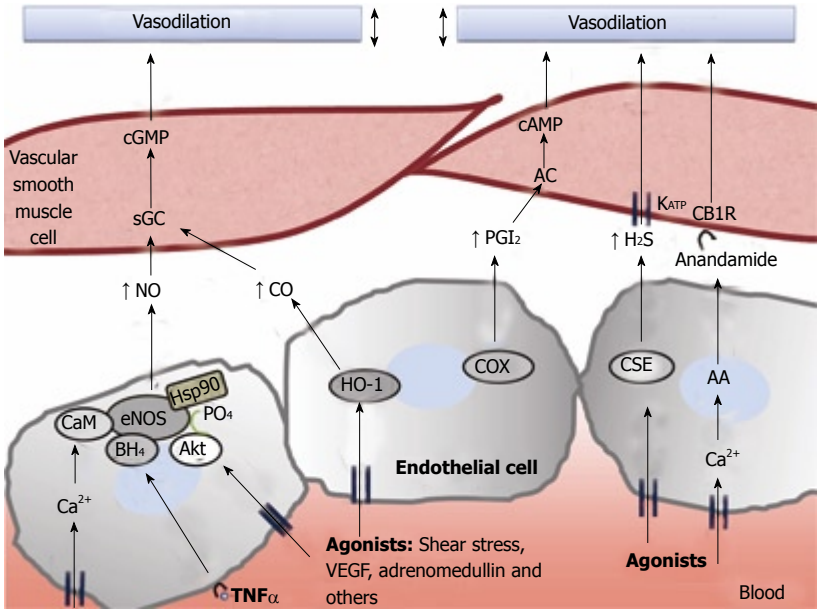

Figure 2 Molecular pathways associated to splanchnic vasodilation. Vasoactive molecules involved in the regulation of vascular tone in the arteries of the splanchnic circulation. Nitric oxide (NO), carbon monoxide (CO), prostacyclin (PGI_2) or hydrogen sulfide (H_2S), generated through different pathways in endothelial cells, cause vasodilation in vascular smooth muscle cells by either activating soluble guanylate cyclase (sGC) to generate cyclic guanosine monophosphate (cGMP), by stimulating adenylate cyclase (AC) and generation of cyclic adenosine monophosphate (cAMP) or through the opening of K_{ATP} channels. Also, anandamide activates endothelial cannabinoid 1 receptors (CB1R) provoking vasodilation. AA: arachidonic acid; AC: adenylyl cyclase; Akt: protein kinase B; BH4: tetrahydrobiopterin; CaM: calmodulin; CSE: cystathionine-γ-lyase; COX: cyclooxygenase; eNOS: endothelial nitric oxide synthase; HSP90: heat shock protein 90; IP3: inositol triphosphate; TNFα: tumor necrosis factor α; VEGF: vascular endothelial growth factor.

stimulated by different forms of stimuli such as vascular endothelial growth factor (VEGF), inflammatory cytokines, and mechanical forces by shear stress[63-65]. This latter mechanism involves an increased interaction of eNOS with the positive regulator molecular chaperone heat shock protein 90 (Hsp90)[66]. Later, in advanced stages of portal hypertension, bacterial translocation activates eNOS through a tumor necrosis factor-α dependent increase in tetrahydrobiopterine, an essential cofactor of eNOS[67,68] (Figure 2). It is worth remarking that, according to several studies, other mechanisms such as changes in subcellular localization of eNOS[69], S-nitrosilation[70,71] or asymmetric dimethylarginine degrading enzyme might be involved in the regulation of eNOS activity[72].

In summary, different mechanisms such as complex protein-protein interactions and posttranslational modifications have been reported to up-regulate eNOS in portal hypertension[73].

Other paracrine vasodilators

In addition to NO, other local paracrine/autocrine vasodilators have been described as possibly being involved in the pathogenesis of the hyperdynamic circulation associated with portal hypertension (Figures 1 and 2).

Carbon monoxide: Carbon monoxide (CO) is a gaseous molecule produced by heme oxygenase (HO) during heme metabolism to biliberdin Ⅸ[74]. CO, in a similar manner to NO in cirrhosis, is believed to relax smooth muscle cells through the activation of NO-dependent sGC, resulting in an increased production of cGMP. Although CO is a far less potent mediator than NO[75], a role in vasodilation of portal hypertension has been suggested[76]. CO-induced vasodilation can also occur *via* Ca^{2+}-activated potassium channels[77]. In portal hypertension, an inducible isoform of HO, HO-1, has been shown to be up-regulated in systemic and splanchnic arterial circulation[78], although the mechanisms of activation remain to be fully understood.

Prostacyclin (PGI_2): Prostacyclin is synthesized by cyclooxygenase and released from the endothelium to promote smooth muscle relaxation by activating adenylyl cyclase and augmenting the intracellular level of cyclic adenosine monophosphate[79]. Increased levels of circulating PGI_2 have been observed in patients with cirrhosis[80] and in portal hypertensive rabbits[81], supporting a role for prostaglandins in the pathogenesis of the hyperdynamic circulatory syndrome.

Hydrogen sulfide (H_2S): Recent evidence has suggested a role for H_2S, a potent vasodilator, in the development of hyperdynamic circulation in cirrhosis[82]. This is based on the observation that in cirrhosis, endotoxaemia leads to upregulation of the enzyme cystathionine-γ-lyase, responsible for H_2S production, which causes vasodilation through the opening of K_{ATP} channels[83].

Circulating vasodilators

Early studies in the physiopathology of portal hypertension focused on the role of circulating vasodilator substances of splanchnic origin accumulated as a consequence of reduced hepatic metabolism and/or increased porto-systemic shunting. The strongest evidence is for glucagon, whereas other substances described here have not been extensively investigated[84,85].

Glucagon: Numerous studies have demonstrated elevated plasma glucagon levels in patients with cirrhosis and in portal hypertensive rat models. Glucagon seems to promote vasodilation by relaxing the vascular smooth muscle and decreasing its sensitivity to endogenous vasoconstrictors, although the exact mechanism remains to be elucidated[86].

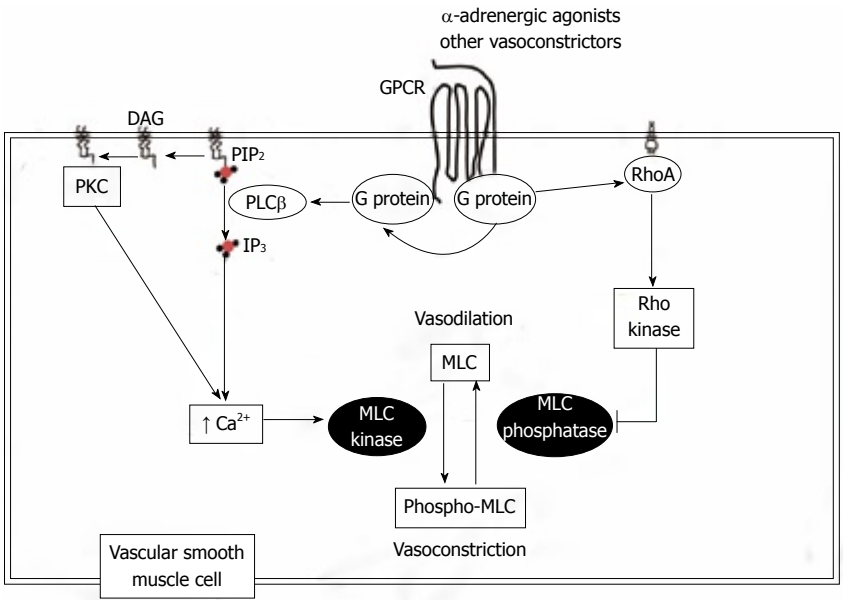

Figure 3 Vasodilation/contractile signaling in vascular smooth muscle cells. The contractile state of vascular smooth muscle depends on the phosphorilation state of myosin light chains (MLCs). Under normal conditions, contractile agonists activate G protein couple receptors (GPCR). These receptors subsequently activate downstream effectors such as phospholipase C (PLC) and GTPase RhoA, leading to the increase of MLC phosphorilation *via* the activation of MLC kinase or the inhibition of MLC phosphatase. DAG: diacylglycerol; IP3: inositol triphosphate; PIP_2: Phosphatidylinositol 4,5-bisphosphate; PKC: protein kinase C.

Endocannabinoid: The contribution of the endocannabinoid system in the development of splanchnic vasodilation has been described in several studies proposing various mechanisms. The main endocannabinoid mediator is anandamide, a product of arachinoid acid metabolism. Endocannabinoids activate endothelial cannabinoid 1 receptors and vanilloid receptor 1 causing pronounced vasodilation in BDL rats[87]. Anandamide levels are increased in monocytes in cirrhosis and over-activation of cannabinoid 1 receptors induce mesenteric NO production by eNOS in mesenteric vessels from portal hypertensive rats[87,88].

Adrenomedullin: In a similar way to endocannabinoids, increased peptide adrenomedullin levels have been found in plasma of cirrhotic rats[89] and patients[90]. Adrenomedullin is a vasoactive peptide known to contribute to enhancement of eNOS activity causing vasodilation. This peptide phosphorylates and activates Akt and increases cGMP production in rat aorta, probably promoting vasorelaxation through production of NO[91].

Endothelium-derived hyperpolarizing factor: Endothelium-derived hyperpolarizing factor (EDHF) has been shown to be an important endothelium-dependent vasodilator in resistance vessels of eNOS knockout mice[92]. Its role becomes more significant when the production of NO is inhibited, because NO seems to inhibit the release of EDHF[93].

Other endogenous humoral vasodilators including atrial-natriuretic peptide, whose levels tend to increase in advanced stages of liver cirrhosis with ascites[94], adenosine, histamine, bile salts, calcitinin gene related protein (CGRP) and substance P, have been proposed to play a role in the arterial vasodilation in portal hypertension[95] (Figure 1).

Contracting signalling alterations

In cirrhosis and portal hypertension, the majority of the vessels are dilated despite systemic activation of vasoconstrictors[96-98]. This splanchnic resistance to vasoconstrictor agents can be attributed to vascular hyporesponsiveness[99,100], explaining why the hyperdynamic circulation increases with progression of the disease despite the stimulation of renin-angiotensin, sympathetic nervous system and vasopressin release. Impaired responsiveness to vasoconstrictors is involved both in the increased vasodilation of splanchnic territories and in vasoconstriction of essential end-organs, triggering the severe complications of cirrhosis.

The contractile state of vascular smooth muscle depends essentially on myosin light chain (MLC) phosphorylation and is regulated via activation of MLC kinase or inhibition of MLC phosphatase[101,102] (Figure 3). In contrast, pathways leading to vasorelaxation decrease MLC phosphorylation *via* deactivation of MLC kinase or activation of MLC phosphatases[103-106]. All vasoconstrictor receptors belong to the superfamily of guanine nucleotide-binding protein (G-protein)-coupled receptors (GPCR). Stimulation of GPCR on the vascular smooth muscle cell activates G proteins and consequently their down stream effectors, phospholipase C β (PLCβ) and the small GTPase, RhoA. PLCβ hydrolyzes phosphatidylinositol 4,5-biphosphate into inositol triphosphate (IP3) and diacylglycerol (DAG). IP3 diffuses in the cytosol and DAG remains in the plasma membrane activating protein kinase C. Both products cause an increase in intracellular calcium in vascular smooth muscle cells. The released calcium initiates a cascade of intracellular events, causing MLC phosphorilation and resulting in cross-bridging of actin and myosin, leading to contraction[101,102]. In addition, the parallel cascade of G-protein-induced RhoA activation subsequently activates Rho kinase causing inhibition of MLC phosphatase, enhanced MLC phosphorilation and eventually vascular contraction.

In several studies both in animal models and human tissues the diminished contractile response to α_1-ad-

renergic agonists or other vasoconstrictors persisted after removal of the endothelium or pharmacological inhibition of endogenous NO production. It is also known that vascular hyporeactivity is not caused by a down regulation of receptors to most relevant endogenous vasoconstrictors or by a decrease in their affinity. These vasoconstrictor receptors are actually increased in the hepatic artery. Therefore, the defective contractile signalling should be at the subreceptor level[107,108]. Recent evidence suggests that during portal hypertension these contracting signalling pathways are altered early after receptor stimulation, most probably at the level of Gα effectors. In rats with secondary biliary cirrhosis induced by bile duct ligation, it has been observed that impaired response to α-adrenoreceptor stimulation involves a reduced activation of PLCβ and consequently, a diminished formation of inositol phosphates[109], as well as reduced activation of RhoA with subsequently defective Rho kinase activation[110]. Moreover, this impairment in PLCβ and RhoA activation is resistant to endothelium denudation or pharmacological NOS inhibition[109,110], supporting the existence of defects in receptor-mediated activation of contraction.

The impaired response to contractile agonists occurring in portal hypertension has been also explained by desensitization of GPCRs by receptor-desensitising proteins, namely G-protein-coupled receptor kinase 2 (GRK-2) and β-arrestin 2. These receptor-desensitising proteins have been found to be up-regulated in aortas from BDL rats as well as in hepatic arteries from patients with cirrhosis, inducing desensitisation of angiotensin II receptor[111]. Moreover, it is known that the GRK-2/β-arrestin 2 system also induces desensitization of a variety of different receptors and that GRK-2/β-arrestin 2 mediated receptor desensitisation is initiated in response to exaggerated receptor stimulation[112,113]. It seems possible that elevated plasma levels of angiotensin Ⅱ and catecholamines, which are well established in cirrhosis, are responsible for the onset of these processes in hypocontractile vessels[114,115].

Another observation contributing to the understanding of the dysregulation of contractile signalling in portal hypertension has come from the recent studies on increased release and enhanced effect of neuropeptide Y (NPY) on adrenergic mesenteric contraction in PVL rats[116]. By itself, NPY mediates no direct vasoconstriction, but potentiates NE-evoked vasoconstriction in the mesenteric vasculature through the Y1 specific receptor. Enhanced release of NPY may represent a compensatory mechanism to counterbalance arterial vasodilation by restoring the efficacy of endogenous catecholamines, especially in states of high alpha1-adrenergic activity.

Nervous system and portal hypertension

Histological studies have revealed that vascular smooth muscle is innervated by neurons containing NOS immunoreactivity[54], as well as by those containing tyrosine hydroxylase and choline acetyltransferase[117]. These efferent post-ganglionic neurons, identified as nitrergic, noradrenergic and cholinergic, control vasoconstriction of vascular smooth muscle cells from blood vessels. Functionally, nitrergic nerves are more important in vascular tone control than cholinergic nerves, which only play a role in modulating adrenergic and nitrergic nerve functions[118].

In the mesenteric circulation, both in humans and rodents, vasoconstriction induced by the sympathetic nervous system (SNS) is mainly mediated by post-synaptic α_1-adrenoreceptors[119]. Indeed, α_1-adrenoreceptor stimulation is the major mechanism through which the SNS regulates vascular tone. It has been shown that stimulation of perivascular nerves in blood vessels evokes vasoconstriction. This vasoconstriction is blocked by tetrodotoxin (neurotoxin), prazosin (α_1-adrenoceptor antagonist), guanethidine (adrenergic neuron blocker) or 6-hydroxydopamine (neurotoxin that destroys adrenergic neurons)[120,121]. Thus, the vascular tone of peripheral blood vessels might be controlled mainly by sympathetic adrenergic nerves through the release of the neurotransmitter norepinephrine (NE). Moreover, different investigations have also shown that other agents like NPY and adenosine triphosphate are also released in the SNS, acting as co-transmitters of NE and potentiating its action[122].

There are a large number of publications evaluating the role of SNS in human cirrhosis. Increased systemic levels of catecholamines have been found in many studies, tending to increase when liver disease worsens[114,115]. These elevated levels are a result of an increased production of NE (increased plasma levels of NE, spillover of NE from the neuroeffector junctions and muscle sympathetic nervous activity), rather than a decreased clearance[123,124]. However, the origin of this SNS-hyperactivity is not homogeneous, since there are organs or tissues in which increased NE production has not been found. One of the main sites of NE overproduction is the kidney[124,125]. Another important site of NE production is muscle, with many studies showing increased muscle sympathetic nerve traffic[126,127]. There are also regional differences, the upper limb seems to release increased amounts of NE, but the lower limb does not[125,128]. Also, in contrast to the increased sympathetic nerve traffic found in muscles, the skin seems to present a normal level of sympathetic activity[127].

It seems quite clear that the adrenergic system plays a role in the cardiovascular, homeostatic and metabolic dysfunction present in advanced liver disease and that in cirrhosis and portal hypertension there is a global overactivity of this system. What is more questionable is whether this SNS-hyperactivity takes place everywhere and especially in mesenteric vessels. In this regard, our group has recently demonstrated an important down-regulation, both at the transcriptional and translational level, of many proteins implicated in adrenergic neurotransmission in the superior mesenteric artery from PVL and cirrhotic rats[129]. This adrenergic inhibition is accompanied by a remarkable regression/atrophy of the

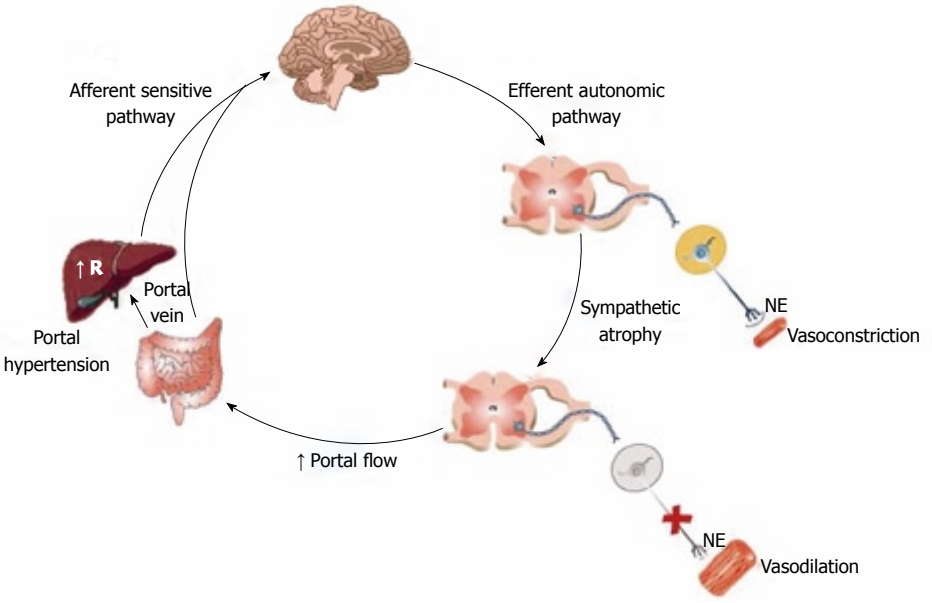

Figure 4 Hypothesis regarding the mechanisms and effects of the sympathetic post-ganglionic atrophy in splanchnic vasodilation. The afferent stimulus of portal hypertension, originating from pressure increases in portal or mesenteric vessels or microvasculature, reaches the brain stem cardiovascular nuclei through the afferent nerves. From there, post-ganglionic sympathetic nerve regression are mediated by efferent sympathetic nerves, leading to neurotransmission inhibition and vasoconstriction impairment mediated by norepinephrine (NE).

sympathetic innervation in the whole mesenteric vascular bed. However, this nervous atrophy is not present in other vascular territories such as the renal arteries[130]. The down-regulation of the mesenteric adrenergic system has been interpreted as a local consequence of portal hypertension that might contribute to aggravating splanchnic vasodilation, which is responsible for a generalized sympathetic overactivity, especially in muscles and kidneys. The observation that alpha-adrenergic agonists, such as norepinephrine and midodrine, are effective in the treatment of hepatorenal syndrome[131,132], the ultimate consequence of arterial vasodilation in cirrhosis, suggests that, at least in some areas, the adrenergic activity rather than overactivated, might be suppressed. The recent observation that NPY restores adrenergic superior mesenteric artery hyporeactivity in PVL rats[116], would also point to a deficient local adrenergic tone in portal hypertension. Also, Joh and co-workers[133] have demonstrated that using antagonists to α-adrenergic receptors, the response to vasoconstrictor blockade in portal hypertensive animals differed drastically from normal rats. Unlike the response of normal rats, α-adrenergic blockade produced essentially no change in intestinal microvascular dimensions, while vasopressin or angiotensin II blockade was associated with arteriolar dilation. These data suggest that loss of adrenergic vascular tone could be a very important functional vasoconstrictor defect in portal hypertension.

The neural pathway controlling the cardiovascular system includes the primary afferent innervation (sensory neurons), the brain stem medullary cardiovascular nuclei, and the effector arm composed of sympathetic and parasympathetic efferent nerves[134,135]. Considering this system, the signal responsible for the post-ganglionic sympathetic nerve regression suggested by our studies probably originates in preganglionic neurons or other neurons with a synaptic connection to post-ganglionic neurons. The afferent stimulus originating from pressure increases in portal or mesenteric vessels or microvasculature would reach the central nuclei through the afferent nerves and from there to the sympathetic ganglia[136,137] (Figure 4). In this context, it is important to mention that several studies suggest that by pharmacologically eliminating the primary afferent nerves by capsaicin administration, the development of hemodynamic alterations is prevented as well as ascites formation, in PVL and cirrhotic rats[138-141]. In addition, these afferent sensory nerves once activated by peripheral stimuli can also release the transmitter content (the vasodilator peptides substance P and CGRP) from their peripheral terminals in innervated tissue to elicit functional responses[142]. It has been described that periarterial nerve stimulation in rat mesenteric resistance arteries produces neurogenic vasodilation mediated by CGRP[143], and that CGRP release suppresses sympathetic nerve mediated vasoconstriction[143]. Finally, different studies showing high levels of substance P and CGRP in patients with cirrhosis and liver failure have suggested that these neuronally generated vasodilators could play a role in splanchnic vasodilation of portal hypertension[144,145].

Perivascular presence of nNOS–containing nerves, so called nitrergic nerves, has been demonstrated in numerous vascular beds and multiple species. These nNOS immunoreactive fibers play an important role in regulating vascular tone, mediating neurogenic vasodilation by releasing NO. Up-regulation of n-NOS has been recently demonstrated in mesenteric arteries of PVL rats[58,146]. This nNOS activation seems to mediate an increased neural NO-mediated vasodilatation and might be an additional pathway for mesenteric smooth muscle relaxation in portal hypertension. Moreover, the non-selective NOS inhibition by L-NAME (N-(G)-nitro-L-arginine methyl ester) and the selective inhibition of nNOS by L-VNIO (vinyl-L-N-5-(1-imino-3-butenyl)-L-ornithine), increase the induced adrenergic vasoconstriction in rat mesenteric arteries in response to periarterial

nerve stimulation[147]. These findings strongly suggest that endogenous NO also modulates the neurogenic release of NE from adrenergic nerve terminals.

CONCLONSION

The increase in splanchnic flow that contributes to portal hypertention results from persistent mesenteric vasodilation together with angiogenesis. Studies in animal models and in patients have shown that splanchnic arterial vasodilation is a multifactorial phenomenon. In addition to overproduction of vasodilators (especially nitric oxide), defects in the contractile signalling pathways in smooth muscle cells in response to vasoconstrictor stimulation contribute to vascular hyporesponsiveness to endogenous vasoconstrictors. In addition, sympathetic atrophy seems to participate in the late stages of portal hypertension. It is reasonable to suggest that mesenteric sympathetic atrophy decreases the vascular tone of the mesenteric tree, allowing an increased activity of vasodilatory mediators (humoral and nervous). However, little is known about possible interactions between participating pathways and mechanisms, and further efforts are needed to clarify this essential component of portal hypertension.

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