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Review

Arachidonic acid metabolites and endothelial dysfunction of portal hypertension

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ABSTRACT

Increased resistance to portal flow and increased portal inflow due to mesenteric vasodilatation represent the main factors causing portal hypertension in cirrhosis. Endothelial cell dysfunction, defined as an imbalance between the synthesis, release, and effect of endothelial mediators of vascular tone, inflammation, thrombosis, and angiogenesis, plays a major role in the increase of resistance in portal circulation, in the decrease in the mesenteric one, in the development of collateral circulation. Reduced response to vasodilators in liver sinusoids and increased response in the mesenteric arterioles, and, viceversa, increased response to vasoconstrictors in the portal-sinusoidal circulation and decreased response in the mesenteric arterioles are also relevant to the pathophysiology of portal hypertension. Arachidonic acid (AA) metabolites through the three pathways, cyclooxygenase (COX), lipoxygenase, and cytochrome P450 monooxygenase and epoxygenase, are involved in endothelial dysfunction of portal hypertension. Increased thromboxane-A2 production by liver sinusoidal endothelial cells (LSECs) via increased COX-1 activity/expression, increased leukotriens, increased epoxyeicosatrienoic acids (EETs) (dilators of the peripheral arterial circulation, but vasoconstrictors of the portal-sinusoidal circulation), represent a major component in the increased portal resistance, in the decreased portal response to vasodilators and in the hyper-response to vasoconstrictors. Increased prostacyclin (PGI2) via COX-1 and COX-2 overexpression, and increased EETs/heme-oxygenase-1/K channels/gap junctions (endothelial derived hyperpolarizing factor system) play a major role in mesenteric vasodilatation, hyporeactivity to vasoconstrictors, and hyper-response to vasodilators. EETs, mediators of liver regeneration after hepatectomy and of angiogenesis, may play a role in the development of regenerative nodules and collateral circulation, through stimulation of vascular endothelial growth factor (VEGF) inside the liver and in the portal circulation. Pharmacological manipulation of AA metabolites may be beneficial for cirrhotic portal hypertension.

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1. Introduction

Portal hypertension, a common complication of chronic liver diseases, is defined as an increase in portal pressure above normal values of 10 mmHg. It is the result of an increase in portal vascular resistance and an elevated portal inflow (Fig. 1), and it causes the development of esophageal varices, ascites and hepatorenal syndrome, the hepatopulmonary syndrome, porto-pulmonary hypertension, encephalopathy [1–5]. Hypotension, low systemic vascular resistance, and reduced sensitivity to vasoconstrictors are common features of the hyperdynamic syndrome in portal hypertension, and are pathogenetic factors [3].

Endothelial cell (EC) signaling plays a pivotal role in the control of flow and pressure in the splanchnic circulation, and, on the other hand, the altered hemodynamic profile in portal hypertension, influences EC signaling, structure, and function in cirrhotics [6–9]. Endothelial-derived dilating factors, nitric oxide (NO), carbon monoxide (CO), prostacyclin (PGI2), and endothelial-derived hyperpolarizing factors (EDHF) are released from the arterial endothelium in response to both humoral and mechanical stimuli and can profoundly affect the function of the underlying vascular smooth muscle. Endothelial-derived contracting factors such as endothelin (ET), angiotensin II, thromboxaneA2 (TxA2), leukotrienes (LTs), are powerful vasoconstrictors released from the endothelium that are hormonally and mechanically induced, and can also affect vascular smooth muscle cells tone [8,9].

2. Endothelial function and dysfunction

Since the discovery in 1980 that acetylcholine (ACh) requires the presence of ECs to elicit vasodilation [10], the importance of the EC layer for vascular homeostasis has been increasingly appreciated. Dysfunction of the endothelium has been implicated in the pathophysiology of different forms of cardiovascular disease, including hypertension, coronary artery disease, chronic heart failure, peripheral artery disease, diabetes, and chronic renal failure. ECs sense mechanical stimuli, such as pressure and shear stress, and hormonal stimuli, such as vasoactive substances, releasing agents that regulate vasomotor function, trigger inflammatory processes, and affect hemostasis. The endothelium also contributes to mitogenesis, angiogenesis, vascular permeability, and fluid balance.

Endothelial dysfunction was initially identified as impaired vasodilation to specific stimuli such as ACh or bradykinin. A broader understanding of the term would include vasodilation, vasoconstriction, a proinflammatory and prothrombic state.

Endothelial dysfunction in liver sinusoidal endothelial cells (LSECs), and possibly in portal venules, decreases the production of vasodilators, and favours vasoconstriction. EC dysfunction in the splanchnic and systemic arterial circulation overproduces vasodilator molecules, leading to arterial vasodilatation. In addition, portal hypertension leads to the formation of portosystemic collateral vessels.

This review will examine the contributory role of AA metabolites in endothelial dysfunction of portal hypertension, considering the hepatic venous dysfunction and splanchnic arterial dysfunction separately.

3. Porto-sinusoidal circulation

There are several potential morphological sites for regulating blood flow through the sinusoids: the various segments of the afferent portal venules and hepatic arterioles, the sinusoids themselves, the central and hepatic venules. All these vessels contain several types of contractile cells and respond to pharmacologic agents [11]. The principal site of regulation of blood flow through the sinusoids is thought to reside in the sinusoid itself, where the major blood pressure drop occurs in the liver. The sinusoidal lining cells are responsive to a variety of pharmacologic substances. By contracting (or swelling), they may selectively reduce the patency of the sinusoid lumen and act like sphincters. The participation of perisinusoidal, stellate cells (far-storing, Ito cells) in regulating sinusoidal diameter has also been reported. ET-1 has been shown to cause contraction of isolated stellate cells in culture and to narrow the lumens of sinusoids in isolated perfused livers, although the principal site of vasoconstriction elicited by ET-1 was shown to be the preterminal portal venule [11].

Hepatic sinusoidal endothelium is discontinuous and possesses large (100–200 nm) membrane-bound, nondiaphragmed round cytoplasmic holes or fenestrae (occupying 6–8% of the endothelial surface) [12]. The fenestrae are arranged in sieve plates, which are approximately 0.1 μm in diameter and comprise 20–50 aggregated pores. Sinusoidal ECs also display gaps and lack an organized basement membrane. The sinusoidal endothelium functions as a selective sieve allowing for passage of small particles (up to medium-sized chylomicrons) from blood to hepatocytes via the space of Disse, and also contributes to vasomotor tone [13]. Cirrhosis is characterized by phenotype changes of LSEC, with capillarization, i.e. loss of fenestrae and abnormal deposition of a basement membrane matrix on the abluminal face, and endotheliopathy. Further, LSEC dysfunction results in increased inflammation due to impaired immune tolerance and defenestration [14–16]. One of the first demonstrations that vasoconstriction in the cirrhotic hepatic portal circulation contributes to portal hypertension, was given by Bhathal and Grossman in 1985 [17]. They showed that the isolated perfused cirrhotic liver has a mean resistance which is approximately 110% higher than the normal liver. The vascular tone of the normal liver is minimal as assessed by the response to a variety of vasodilator agents, including sodium nitroprusside, magnesium sulphate, papaverine hydrochloride, and cytochalasin B. In contrast, these agents reduce the perfusion resistance of the cirrhotic livers by approximately 15%. Prostaglandin E1 produces a lesser fall in resistance which nevertheless is greater in cirrhotic livers than controls. Gupta et al. [18] showed that after preconstricting the intrahepatic microcirculation with methoxamine, vasorelaxation to cumulative doses of receptor-mediated endothelial agonist, ACh, and to receptor-independent endothelial agonist, calcium ionophore A23187, is significantly less in cirrhotic livers as compared with normal livers. The impaired vasorelaxation is a result of a decrease in both NO-mediated and non-NO-mediated components of vasorelaxation. In human cirrhotic portal hypertension, NO donors are effective in reducing hepatic resistance to portal flow [19].

Furthermore, also vasoconstriction to endothelin-1 (ET-1) [20] methoxamine [21], leukotriene-D4 [22] is increased in the cirrhotic portal hepatic circulation.

EC dysfunction seen in the portal intrahepatic/sinusoidal microcirculation contributes to the increased intrahepatic vascular resistance, and, thus, to portal hypertension. LSECs have far-reaching effects regulating liver functions, including blood clearance, vascular tone, immunity, hepatocyte growth [23] and angiogenesis/sinusoidal remodeling [24]. Thus, LSEC dysfunction results in a pathology contributing to impaired vasomotor control (primarily vasoconstrictive), inflammation, fibrosis, liver regeneration [23] and pathological angiogenic/sinusoidal remodeling [24]. These factors facilitate the development of cirrhosis and portal hypertension [13].

Oxidative stress is a main cause of EC dysfunction. Patients with cirrhosis have elevated oxidative stress [25] and administration of the antioxidant vitamin C markedly attenuates postprandial increases in portal pressure, suggesting that increased oxidative stress in cirrhotic patients contributes to portal hypertension. In

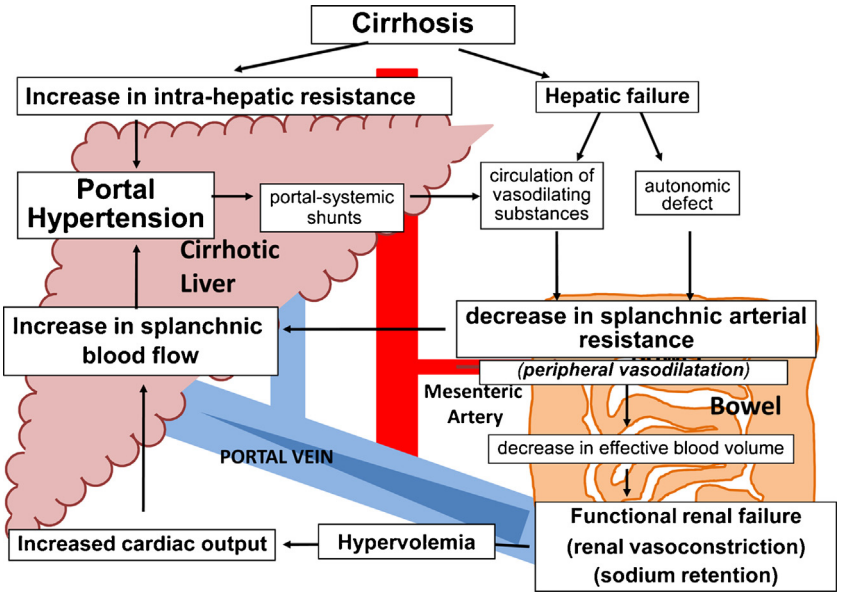

Fig. 1. Pathophysiology of portal hypertension of cirrhosis.

the LSECs of cirrhotic livers, there is increased production of the superoxide radical (O_2^-). In cirrhotic rats, the superoxide dismutase mimetic, tempol, causes a reduction in portal resistance [26]. Xanthine oxidase and cyclooxygenase (COX), but not NADPH oxide, have been shown to be the primary cause of these elevated O_2^- levels.

Sinusoidal distortion resulting from fibrosis/cirrhosis alters the flow pattern in the intrahepatic microcirculation. LSECs sense these hemodynamic changes and influence cellular functions by changing their gene expression. LSECs play an important role in the regulation of hepatic vascular tone by releasing vasoactive molecules [27,28]. As a consequence of LSEC dysfunction, they decrease the production/bioavailability of vasodilator molecules and increase vasoconstrictor molecules, thereby increasing intrahepatic resistance.

NO and CO are important vasodilators regulating vascular tone in the intrahepatic microcirculation. In cirrhotic livers, NO and CO levels decrease and contribute to the increased intrahepatic vascular resistance [13].

Metabolites of AA, through the different pathways, are produced and/or metabolized by ECs, and influence endothelial function. They have been extensively studied in relation portal endothelial dysfunction [1513] (Fig. 2).

3.1. Cyclooxygenase-dependent AA metabolites

They include compounds with vasodilating properties, like PGI2 and with vasoconstricting action, like PGH2 and TxA2.

First of all, concerning COX itself, two isoforms are encoded by two separate genes. One is a constitutive enzyme, COX-1, which is expressed in many tissues, including ECs. In contrast, COX-2 is not constitutively expressed, but can be induced rapidly and transiently in a variety of cells, including vascular ECs, by physical stimuli and a number of pro-inflammatory agents. In cirrhotic livers there is overexpression of COX-2, but not of COX1 [29]. However, COX-1 is overexpressed in SEC as it is present in >50% of cirrhotic SEC compared to 20% in normal livers, also with increased intensity [29]. Furthermore, a downregulation of COX-1 expression is present in hepatic stellate cells (HSC) of cirrhotic livers. Mohammed et al. [30] have partially different results, showing that COX-2 is absent from normal liver but is highly expressed in cirrhosis, mainly in the inflammatory, sinusoidal, vascular endothelial, and biliary epithelial cells, and that low amounts of COX-1 are expressed in both normal and cirrhotic livers, exclusively in sinusoidal and vascular endothelial cells, with no differences seen between normal and cirrhotic livers.

It has been shown that the hyperesponse to the α1-adrenergic vasoconstrictor, methoxamine, in cirrhotic livers, is associated with an increased production of TXA2 [31], a vasoconstrictor prostanoid implicated in the pathophysiology of endothelial dysfunction in other vascular beds [32–34]. Blocking TXA2 (by a prostaglandin H2/TXA2 receptor blocker, SQ-29548) or inhibiting COX-1 activity (by a COX-1 inhibitor, SC-560), but not the increased COX2, corrects the hyperesponse to methoxamine of cirrhotic livers [35,36]. Thus, despite abundant hepatic COX-2 expression, the increased response to methoxamine of cirrhotic livers is dependent of COX-1.

AA produces a dose-dependent increase in portal pressure, associated with increased TXA2 release. These responses are significantly greater in cirrhotic livers. COX-1 inhibition and PGH2/TXA2 receptor blockade, but not TXA2 synthase inhibition, markedly attenuate portal pressure response to AA of cirrhotic livers. Incubation of SEC from cirrhotic livers with AA promotes an increase in TxB2, which is not observed with cells from normal rats. Furthermore, production of PGI2 is not different. Additionally, cirrhotic livers exhibit significantly increased phospholipase-A2 (cPLA2) activity, although cPLA2 expression is similar. Thus, COX-1 dependent metabolites acting on PGH2/TxA2 receptor mediate in part the increased vasoconstriction to AA [37].

Graupera et al. have shown that in cirrhotic livers there is a peculiar response to ACh: there is hyporeactivity to low concentrations, and a paradoxical vasoconstriction to higher concentrations [35]. The response in cirrhotic livers is associated with a high release of TxB2 and is improved, or even normalized, by the nonselective COX inhibitor, indomethacin, by the selective COX-1 inhibitor SC-560 and by TxA2 blockade, while COX2 inhibition does not correct endothelial dysfunction. Thus, it appears that LSEC dysfunction is related to an increased TxA2 production via COX1 activation.

As far as the mechanism of action of COX1 dependent metabolites is concerned, Rosado et al. [38] have shown that in control and cirrhotic LSEC, COX activation with AA reduces NO bioavailability and increases O_2^- levels. These effects are abolished by pre-treating LSEC with the non-selective COX inhibitor, indomethacin. In control, but not in cirrhotic LSEC, scavenging of O_2^- by superoxide dismutase (SOD) incubation partially restores the decrease in NO

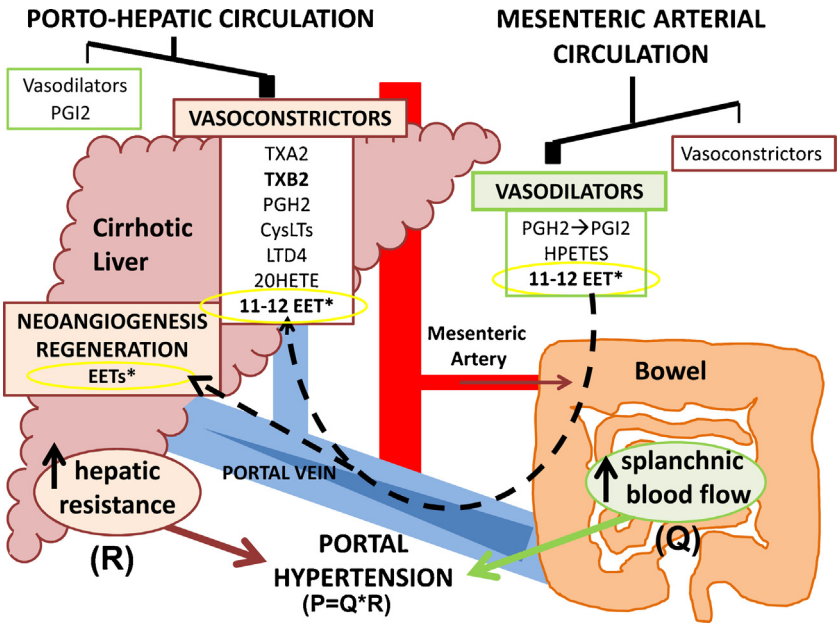

Fig. 2. Differential effects of arachidonic acid (AA) metabolites via the cyclooxygenase (TxA2, PGH2, PGI2), lipoxygenase (LTD4, HPETES; CysLTs), and cytochrome P450 (EETs, 20-HETE) pathways on porto-sinusoidal and mesenteric arterial circulations. The different metabolites participate in increasing portal pressure (PP) by increasing hepatic resistance to portal flow (R) and increasing mesenteric arterial blood flow (Q).

bioavailability promoted by COX activation. NO supplementation produces a significant and parallel reduction in PGI2 and TXA2 production in control LSEC, whereas it only reduces TXA2 production in cirrhotic SEC. By contrast, in control and cirrhotic LSEC, NO inhibition does not modify COX expression or activity. In cirrhotic LSEC, COX inhibition increases NO bioavailability and NO supplementation induces a reduction in TXA2. Cirrhotic LSEC exhibits a significantly higher TxA2 synthase expression compared with control LSEC, whereas PGI2 synthase expression is unchanged. Rodriguez et al. [39] have shown that CCl4 cirrhotic rats treated with the peroxisome proliferator-activated receptor α (PPARα) activator, fenofibrate, have a significantly lower portal pressure (−29%) and higher arterial pressure than those treated with vehicle. These effects are associated with an improved vasodilatory response to ACh, mediated by a reduction in COX-1 expression and TXB2 production and a significant increase in NO bioavailability in LSEC.

Also the positive effect on endothelial dysfunction of the antioxidant, resveratrol [40] has been shown to be associated with decreased TxA2 production and increased endothelial NO.

Concerning the receptors associated with the negative effects of COX-1 metabolites, Rosado et al. [41] have shown that the TxA2/prostaglandin-endoperoxide (TP) receptor antagonist, terutroban, decreases hepatic vascular tone and portal pressure in rats with cirrhosis due to carbon tetrachloride (CCl4) or bile duct ligation (BDL). Thus, in cirrhotic livers endothelial dysfunction is caused, at least in part, by an increased PGH2/TxA2 synthesis associated with increased AA release and mediated by COX-1 increased activity in LSECs, with no apparent alteration in PGI2 synthesis.

3.2. Lipoxygenases-dependent AA metabolites

Lipoxygenases (LOs) are a family of non-heme containing enzymes that dioxygenate poliunsatured fatty acids to hydroperoxyl metabolites. Three major LO isoforms include 5-LO, 12-LO and 15-LO, which correspond to the carbon position of AA. Different LOs produce various hydroperoxyeicosatetraenoic acids (HPETEs) and hydroxyeicosatetraenoic acids (HETEs) and further products such LTs and lipoxins (LX). 5-LO is mainly involved in LTs production and 12/15 LOs are mainly involved in 12HETE and LX production [42,43]. LOs and LX have structural similarities and appear to have some complementary biological activities [44]. While LTs are involved in pro-inflammatory pathways activation and in smooth muscle contraction, LX have been implicated in the generation of anti-inflammatory lipid mediators and in vasodilatation. 12- and 15-LO have been described in ECs of many vascular beds [11], while the presence of 5-LO in ECs is controversial; however ECs can metabolize and participate to the production of LTs.

LTs are potent mediators of numerous biological events, including vascular permeability and smooth muscle contraction [45,46]. LTs are metabolized by the liver [47] and secreted into bile by an adenosine triphosphate-dependent organic anion transporter in the canalicular membrane [48]. In addition the liver itself produces LTs.

Titos E et al. [49] demonstrated that hepatocytes from rats with carbon tetrachloride–induced cirrhosis show an enhanced generation of cysteinyl-LTs as compared to normal rats, but their ability to synthesize cysteinyl-LTs from exogenous LTA4 was found to be similar. They also observed that LTD4 administration significantly increased portal pressure in anesthetized rats. These observations led to the idea that the “limiting step” for hepatic production of cys-LTs is the production of LTA4 by activated KCs. In the same year Graupera et al. [22] showed that CCl4-induced cirrhotic livers have markedly increased expression of 5-LO–positive cells. At this regard Steib et al. [50] showed that the activation of isolated KCs with zymosan or LPS produced Cys-LTs and that the activation of KCs in isolated perfused cirrhotic livers causes an increase in portal pressure. He also observed that the infusion of Cys-LTs increases portal pressure and, vice versa, treatment with the Cys-LT1 receptor inhibitor montelukast reduces portal pressure in two different rat-models of liver cirrhosis obtained by bile duct ligation and thioacetamide application. Different results were obtained by Graupera et al. [22] who found a hyper-response to LTD4 administration in CCl4-induced livers while 5 LO inhibition by AA-861 administration produced a marked and significantly greater reduction in portal pressure as compared to controls. No effect on portal

pressure was observed after the administration of Cys LTs receptor 1 selective antagonist MK-571. These discordances on LTs effect on portal pressure could be related to the effect of LTs on different LTs receptors. Two receptors have been identified (CysLT1 and CysLT2). Both are G-protein-coupled receptors and are responsible of intracellular calcium flux regulation [51]. Only CysLT2 is expressed in ECs and regulates microvascular permeability. CysLT produced by hepatocytes and KCs are reported to be responsible of the increase of intrahepatic vascular resistance in normal and expecially in cirrhotic livers where their production is increased [52]. In cirrhotic rats hepatocyte-derived cysteinyl-LTs act in a paracrine fashion on nearby nonparenchymal liver cells by increasing Ca^{2+} concentration and inducing contraction of hepatic stellate cells (HSC) [22]. Administration of LT D4 results in marked HSC contraction coupled with increased $[Ca^{2+}]_i$. HSC are located in the perisinusoidal space of Disse beneath the endothelial barrier. They have long cytoplasmic processes which run parallel to the sinusoidal endothelial wall. Thus, HSC contraction may play a role in blood flow regulation in the normal and pathological liver [53].

As concerns the role of endothelial cells in LTs metabolism in portal hypertension, it is known that endothelial cells do not contain 5-LO and, thus, are unable to generate LTA4 from arachidonate. However, ECs play an important role in LT synthesis because of their ability to metabolize LTA4, derived from activated polymorphonuclear leukocytes (PMN-L), to LTC4 through LTA4 hydrolase [54,55]. Under physiologic conditions, ECs are in constant contact with circulating PMN-L, which are known to generate substantial amounts of LTA4. PMN-L adhesion to TNF alpha-stimulated ECs enhances LTB4 production by PMN-L [56]. The regulation of LTs production from PMN-L by ECs involves other molecular pathways involving AA metabolism, in particular the endothelial production of PGI2 acts as a feedback regulator of PMN-L LT synthesis as reported by Feinmark et al. [57]. It is also known that a variety of endogenous products of arachidonate metabolism including 5-lipoxygenase-derived LTC4 and LTD4 can lead to the generation and release of PGI2 by human ECs [58,59].

As already described, ECs have receptor for LTs (CysLT2R). CysLTs induce EC contraction and endothelial barrier disruption [60]. Thus they increase intrahepatic vascular permeability that prompts hepatic edema, that favours cholestasis and reduced CysLT elimination [61], and increased traslocation of bacterial products that, as already described [22], causes a further increase in Cys LT production through the activation of KCs. Moreover cys-LTs induce inflammatory signals through CysLT2R. CysLT2R activation potentiates TNFa-induced attachment of leukocytes to endothelial monolayer via up-regulation of VCAM-1 [60] and, thus, a further increase in LT production by PMN-L [56].

In summary, increased CysLTs production in portal hypertension seems to be related to hepatic cellular damage and to sinusoidal endothelial dysfunction with an indirect effect on vascular tone. These data are very relevant to the recent hypothesis of a role for superimposed inflammation and bacterial translocation in the pathogenesis and severity of portal hypertension [62]. Furthermore, other two LTB selective receptors have been identified [62,63] and, although little is known about LTB receptors in the human normal and diseased vascular walls, they may be responsible of a direct vascular effect also in splanchnic vascular bed.

3.3. Cytochrome P450-dependent AA metabolites

Besides COX and LOX, there is another prominent enzymatic pathway for which AA is the substrate: the cytochrome P450 (CYP) system [64]. This eicosanoid pathway consists of two main branches: ω-hydroxylases, which convert arachidonic acid into 19- and 20-hydroxyeicosatetraenoic acids (19- and 20-HETE), and epoxygenases, which convert it to epoxyeicosatrienoic acids (EETs) [64,65]. EETs are metabolized by soluble epoxide hydrolase (sEH) to the corresponding dihydroxyeicosatrienoic acids [64].

20-HETE is a potent vasoconstrictor that contributes to the development hypertension [66], cerebral vasospasm and ischemia reperfusion injury [64]. It has also been reported to stimulate the migration and proliferation of vascular smooth muscle cell induced by angiotensin II, vascular endothelial growth factor (VEGF), and platelet-derived growth factor (PDGF) in vitro [67]. 20-HETE biosynthesis is primarily localized to the smooth muscle cells [68]. Frisbee et al. [69] first suggested that 20-HETE plays a role in endothelial function through interaction with NO. In their study, they showed that 20-HETE attenuates the effect of ACh-induced relaxation in cremasteric arterioles. Further analysis revealed that 20-HETE interferes with the NO-dependent component of ACh-induced relaxation without affecting the NO-independent component of the relaxing response to ACh. This suggests that 20-HETE interferes with NO synthesis and/or bioavailability. On the other hand, recent studies [68] have shown that 20-HETE activates the renin-angiotensin system via induction of vascular angiotensin converting enzyme (ACE). In ECs, 20-HETE is a potent inducer of ACE expression. ACE induction by 20-HETE brings about increases in Ang II levels and its actions through the AT1R in vascular smooth muscle and ECs. Such interactions may constitute, at least in part, the mechanism by which 20-HETE causes hypertension [68].

The only information on the role of 20HETE in portal hypertension comes from our study that shows that in the hepatic portal circulation, 20HETE is a weak vasoconstrictor, and its effect is mediated by COX activation [65]. Furthermore, inhibition of 20HETE does not influence the portal resistance in cirrhotic livers.

EETs are potent endothelium-derived vasodilators, in particular in the coronaries [70]. Interestingly, in the portal circulation, similarly to pulmonary arteries, they show the opposite effect, i.e. they cause vasoconstriction [65], which is not dependent metabolism by COX. In the normal liver, 11,12EET participates in the vasoconstricting effect of ET1, as EET inhibition causes a decrease in its effect. Also the vasoconstricting effect of AA on portal circulation is mediated in part by EETs, as it is decreased by treatment with miconazole [71]. It is not known whether EETs cause portal-sinusoidal vasoconstriction directly or indirectly through an interaction with ET-1, and this could be one of the mechanisms involved in the portal vasoconstricting effect. Otherwise, it is possible that in the portal-sinusoidal circulation there is an anomalous response to EETs, similarly to what happens in the lung. It has been demonstrated that, in pulmonary vascular bed, EETs induce vasoconstriction instead of vasodilatation. As described by Loot AE et al. [72] in pulmonary smooth muscle cells EETs enhance Ca^{2+} influx by stimulating the translocation of specific transient receptor potential channels (TRPC6) to caveolin-rich areas in the plasma membrane, potentiating the contractile response. Moreover EETs amplify the activation of a mithocondrial subpopulation of Ca^{2+} sensitive potassium channels (BK) by stimulating the association of the α and β1 subunits [73]. The EET-induced activation of this population of BK channels results in the loss of mitochondrial membrane potential and depolarization of the pulmonary artery smooth muscle cells. A similar mechanism could be hypothesized in portal smooth muscle cells and/or stellate cells (Fig. 3).

Plasma levels of EETs are increased in cirrhotic patients, as shown by Sacerdoti et al. [74], but the source of EETs has not been identified. They may come from the mesenteric arterial endothelial cells and, thus, getting directly into the portal circulation, they cause vasoconstriction. Inhibition of EETs with miconazole causes a decrease in portal resistance in cirrhotic livers [71], and this may open a new possibility in the treatment of cirrhotic patients.

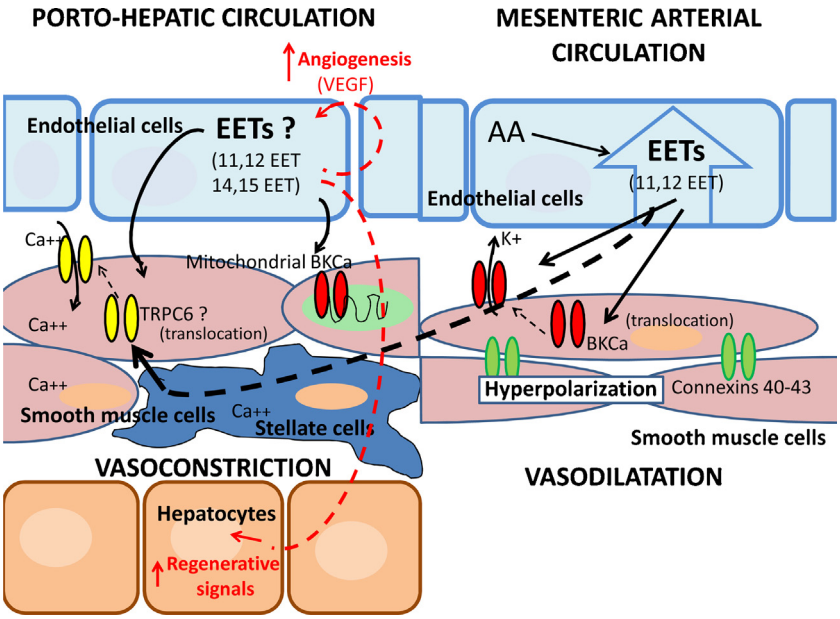

Fig. 3. Simplified mechanisms of opposite effects of epoxyeicosatrienoic acids (EETs) in mesenteric arteries (vasodilatation) and porto-sinusoidal circulation (vasoconstriction). In mesenteric arteries, EETs, produced by ECs, cause relaxation, acting through voltage gated K-channel (BKCa) and gap junctions, in particular connexins 40–43, in smooth muscle cells. In portal venules/sinusoids, EETs, produced by ECs and/or coming from the mesenteric circulation, cause an increase in resistance. It is hypothesized that the mechanism of vasoconstriction is similar to that shown in the lung, through the translocation of specific transient receptor potential channels (TRPC6) to caveolin-rich areas in the plasma membrane of smooth muscle cells and/or stellate cells, and/or the amplification of the activation of a mithocondrial subpopulation of Ca^{2+} sensitive potassium channels (BK).

In the pathophysiology of cirrhosis and portal hypertension, liver regeneration plays a fundamental role, causing alterations in the vascular architecture. Histological analyses of cirrhotic livers indicate an increased number of vessels in the fibrotic septa and surrounding regenerative nodules [75]. This observation has led to the hypothesis that activated HSCs and/or other myofibroblasts promote angiogenesis in liver cirrhosis activating LSECs by releasing angiogenic factors, such as angiopoietins [24,76] and VEGF [77]. ECs play a critical role in the development of a vascular network, tissue growth, and regenerative processes, including liver regeneration. Normal tissue and organ regeneration require an active microvascular endothelium that provides trophic support [78,79]. For instance, the liver requires angiocrine signals from the sinusoidal endothelium [23]. It has been recently shown that EETs contribute to liver regeneration after partial hepatectomy, by interacting with VEGF [80]. After 2/3 hepatectomy plasma 14,15-, 11,12-, 8,9-, and 5,6-EET are elevated by 300%, 305%, 225%, and 222%, respectively. Similarly, human donors undergoing partial liver resections for living-related liver transplantation have a 1.5- to 3-fold increase in 14,15-EET levels 1–7 d after liver hepatectomy. Mice with endothelial expression of either human epoxygenases CYP2C8 or CYP2J2 and mice with global disruption of the gene that encodes soluble epoxide hydrolase (sEH-null) [80], have significantly increased endothelial EETs. Hepatectomy performed in transgenic mice has about 30% increase in liver/body weight compared to wt controls. Also, 11,12-EET and 14,15-EET stimulate liver EC proliferation from 45% to 81%, respectively. The hypothesis of a role of EETs in liver regeneration is confirmed by the reduced regeneration of the liver after EETs inhibition. Thus, liver regeneration appears to be regulated via an EET-dependent mechanism which acts through VEGF signaling. It is possible that increased EETs play a role also in the development of regenerative nodules in cirrhosis. These data allow to hypothesize that EETs may participate in the development of portal hypertension also through the development of regenerative nodules which cause the architectural derangement of the liver that is the main cause of increased resistance to portal flow. Irregular flow patterns generated as a result of intussusceptive angiogenesis might contribute to an increase in resistance within the intrahepatic circulation, leading to portal hypertension.

4. Mesenteric/peripheral arterial circulation

An increase in portal pressure triggers mechanical signals that induce diverse vascular changes in the splanchnic and systemic circulation: arterial vasodilatation, and formation of porto-systemic collateral vessels. These vascular changes increase blood flow to the portal vein, thereby exacerbating portal hypertension [27,28,81,82]. Alterations in EC function are central to the vascular changes seen in the splanchnic and systemic circulation during portal hypertension.

There is agreement on arterial vasodilatation in the splanchnic circulation and increased production of vasodilator molecules in ECs, while there is disagreement on vasodilatation in the systemic circulation [83], as splanchnic arterial vasodilatation is a prerequisite for the development of the hyperdynamic circulatory syndrome observed in patients with cirrhosis and portal hypertension. This syndrome is characterized by decreased mean arterial pressure, increased cardiac output and decreased peripheral resistance. Studies using surgically-induced portal hypertensive rats demonstrated that changes in portal pressure are first sensed by the vascular beds of the intestinal microcirculation, followed by arteries in the splanchnic and then systemic circulation [84]. Vasodilator molecules involved in arterial vasodilatation include eNOS-derived NO, carbon monoxide (CO), PGI2, endocannabinoids such as anandamide, adrenomedullin, and endothelium-derived hyperpolarizing factor (EDHF) [28,85,86]. Some controversy surrounds the identity of EDHF in the hepatic system [28]. Candidate molecules include EETs, the monovalent cation K+, components of gap junctions, and hydrogen peroxide.

Concomitant with vasodilation and hyperesponse to vasodilators like ACh, splanchnic and systemic arteries exhibit decreased contractile response to vasoconstrictors.

4.1. Cyclooxygenase-dependent AA metabolites

PGI2, a major product of vascular COX, is formed primarily in ECs in response to both physical and humoral stimuli that also release NO [87–89]. Two major steps control the production of PGI2: (1) the release of AA from phospholipids, through the action of phospholipase (PL)A, C, and/or D [87–89] and (2) the formation of PGH2 from AA after activation of COX activity [87–89]. PGI2 release is modulated by shear stress [90,91] and humoral factors, including pro-inflammatory substances [87–89], and causes relaxation of vascular smooth muscle by activating adenylyl cyclase and increasing cyclic AMP (cAMP). An increased basal release of PGI2 is thought to have a major role in the pathogenesis of vasodilation and vascular hypocontractility associated with portal hypertension [92]. In agreement with this hypothesis, the whole-body production of PGI2 is increased in portal hypertensive animals [93–95]. Portal venous PGI2 levels are increased in portal hypertensive animals and cirrhotic patients, which suggests that mesenteric release of PGI2 may play a role in the development of the splanchnic hyperhemia, collateral circulation, and portal hypertensive gastropathy [90,94]. Initial studies with indomethacin demonstrated a reduction in splanchnic blood flow in portal hypertensive animals concomitant with a significant reduction in circulating PGI2 levels [94,96]. Indomethacin treatment fails to significantly alter splanchnic blood flow in normal animals, suggesting that COX products, and, in particular, PGI2, does not play a major role in regulating superior mesenteric artery blood flow under normotensive conditions [96,97]. In contrast, following the full development of portal hypertension, i.e. after 15 days in portal vein ligated (PVL) rat, COX inhibition significantly decreases circulating PGI2 levels concomitant with a preferential decrease in superior mesenteric artery blood flow. These changes occur despite no increase in the expression of COX-1 within the superior mesenteric artery during the development of PHT, suggesting that constitutive COX-1 overexpression within the splanchnic vasculature may not play an etiological role in this condition. The increased circulating levels of PGI2 present prior to the development of PHT (day 2 and 4 post-ligation) may be due, in part, to (1) decreased metabolism due to portosystemic shunting (2) enhanced agonist-induced COX-1 activity within this bed, or (3) increased COX-1 expression/activity within the systemic circulation in general, as COX-1 expression was increased in the thoracic aorta of portal hypertensive animals after 2 days [96]. PGI2 synthesis stimulated by adrenaline is enhanced in portal hypertension. However, phorbol ester dibutyrate (a protein kinase C [PKC] activator), AA (the substrate for PGI2 synthesis), and the Ca^{2+} ionophore A23187 and thapsigargin (both of which elevate intracellular Ca^{2+}) induced PGI2 release is reduced or unchanged. Hence, there is a specific increase in adrenoceptor-linked PGI2 synthesis in portal hypertensive animals, which may contribute to arterial vasodilation in this experimental PVL model. Agonist-stimulated PGI2 production, in general, may be enhanced because several studies have demonstrated that vasopressor-induced changes in splanchnic blood flow in portal hypertensives and cirrhotics are enhanced after inhibition of COX activity with indomethacin [97–101]. Furthermore, evidence for a role of agonist-induced PGI2 release in PHT also is derived from studies on the beneficial effects of long-term TNF-α inhibition on systemic hemodynamics in PHT. These studies confirmed that TNF-α mediates its effects via NO and COX pathways, and that TNF-α-induced PGI2 and NO production is enhanced in PHT [102].

As to the etiology of the enhanced PGI2 release, shear stress is known to induce COX-2 expression in vascular ECs [91]. Elevated PGI2 production by shear stress is mediated by increased AA release and a combination of increased expression of COXs and PGI2 synthase [103,104] and strain-induced stimulation of PGI2 synthase activity [105]. Results in portal hypertensive and/or cirrhotic rats are controversial. Tsugawa et al. have shown that in portal hypertensive rats, the selective COX-2 inhibitor NS-398 improves PHT [106]. However, according to M.C. Hou et al., there are no detectable levels of COX-2 expression throughout the progression of PHT, at least within the superior mesenteric artery and thoracic aorta of these animals, while the baseline expression of COX-1 is significantly enhanced in portal vein ligated (PVL) rats [96].

Evidence from temporal studies in the superior mesenteric artery of portal hypertensive rats suggests that the change in PGI2 release precedes the development of a hyperdynamic circulation within this vascular bed [96], implying that hyperdynamic circulation alone is not solely responsible for the initial increase in PGI2 release within the splanchnic bed [96]. Hence, the initial increase in portal pressure without the development of a hyperdynamic circulation in the periphery may account for the initial increase in the release of PGI2 within the portal venous bed. Indeed, ECs exposed to pulse pressure and shear stress overexpress both COX-1 [107] and COX-2 [90], and, therefore, may account for this early increase in PGI2 levels. These results suggest that generation of endothelial vasodilator prostanoids, from COX-1 and COX-2 isoforms, accounts for the increased mesenteric blood flow in portal hypertension.

Despite the preferential pressor response to eNOS and COX blockade, or a combination of both [97], superior mesenteric artery blood flow still remains significantly elevated in PHT, implying that in addition to PGI2 and NO, other vasoactive substances are involved.

4.2. Lipoxygenase-dependent AA metabolites

Few reports evaluate the role of lipoxygenase products in systemic arterial vascular bed. LTs are reported to be involved in ACh induced vasoconstriction of rat aorta [108] while 5-HETE inhibits PGI2 production in coronary artery ECs [109]. 5-LO products are also reported to be involved in atherosclerosis development by favouring monocytes activation and adhesion to ECs [110]. LOs products have various vasoactive properties including vasoconstriction and vasodilatation [111,112] but no evidence of arachidonate 5- lipoxygenation by endothelial cells has appeared. In contrast, the 12 and 15 LO pathways are important in vasorelaxation in various blood vessels in animal models and humans [111–114].

15 LO products hyperpolarize and relax the underlying smooth muscle cells by activating calcium-activated potassium channels of large conductance BK(Ca) channels [115]. 15 LO products (15S-HPETE) have been demonstrated to be mediators of COX- and NO-independent ACh induced relaxation in rats aorta, and in various mice arteries. The relaxation is inhibited by high extracellular K+ and eliminated by endothelium removal [11,116]. Thus, they have been proposed as endothelium derived hyperpolarizing factor (EDHF).

As shown by Chawengsub et al., 15S-HPETE is further metabolized by hydroperoxide isomerise to HEETA and then by soluble Epoxide Hydrolase to trihydroxyeicosatrienoic acids (THETA). HEETA and THETA induce vascular relaxations by activation of smooth muscle calcium-activated, small-conductance K(+) channels causing hyperpolarization and relaxation. [117]. The same author reported that THETAs mediate ACh-induced relaxations in the rabbit aorta [118]. An increase in 15-LO results in increased synthesis of its products 15-hydroxy-11,12-epoxyeicosatrienoic acid (15H11,12 EETA)15-H-11,12-EETA (HEETA) and 11,12,15-THETA, and increased membrane hyperpolarization with enhanced contribution to relaxation. 15-LO expression is regulated by

transcriptional, translational, and epigenetic mechanisms. Thus, the 15-LO pathway represents the first example of an inducible EDHF [119]. In other arteries, the 12-LO metabolite 12-HETE is synthesized by the vascular endothelium and relaxes smooth muscle by large-conductance, calcium-activated K(+) channel activation [117]. Another mechanism proposed to explain the role of 12/15-LO in arterial endothelial dysfunction is the involvement of 12/15 metabolites in the regulation of endothelial gap-junktions and adhesion molecules expression.

12-15LO metabolites are known to be involved in ACh-induced arterial relaxation in mesenteric arteries and may be involved in the increase in splanchnic blood flow of portal hypertension [120,121]. Moreover transgenic mice that overexpress 15-LO and rabbits infected with adenovirus containing cDNA for human 15-LO have an increased ACh- and AA-mediated relaxation in mesenteric arteries as compared to control animals [120,121]. Because of the increased response to ACh [122] in cirrhosis, a role of 15LO metabolites in the pathological vasodilatation of arterial splanchnic vessels in liver cirrhosis can be hypothesized.

4.3. Cytochrome P450-dependent AA metabolites

In blood vessels from various species, including humans, endothelium-dependent relaxations are partially or totally resistant to inhibitors of NOS and COX, and are observed without an increase in the intracellular level of cyclic nucleotides in the vascular MCs [123,98,124].

Endothelial-dependent relaxation of vascular smooth muscle cells evoked by a number of agonists, including cholinomimetics, is often accompanied by an increase in the membrane potential (repolarization and/or hyperpolarization), caused by an endothelial-derived hyperpolarizing factor (EDHF), which is distinct from NO and PGI2. In large conducting arteries, EDHF may provide a secondary system to NO and PGI2, but in small resistance arteries (100–300 microns), it appears to be a major determinant of vascular calibre under normal conditions, and may therefore be of primary importance in the regulation of vascular resistance [125]. In the combined presence of NOS and COX inhibitors, pulse pressure-induced release of EDHF is endothelial-dependent and proportional to the amplitude of the pressure.

Studies from a number of laboratories indicate that EETs, cytochrome P450-dependent AA metabolites, mediate a portion of the endothelium-dependent relaxations to ACh, bradykinin, shear stress and cyclic stretch in some, but not all, arteries. In this regard, they cause hyperpolarization and relaxation of SMCs and function as EDHFs. Their mechanism of action and role as EDHFs varies with the vascular bed and species. When NO is reduced, the CYP and EET-mediated pathway sustains endothelium-dependent dilation. Enhancing the production of vascular EETs or inhibiting the degradation of the EETs may represent a new therapeutic approach to correct endothelial dysfunction and cardiovascular diseases [126].

In the isolated, perfused rat mesenteric bed, a cytochrome P450-linked metabolite of AA is released as EDHF in response to ACh and histamine, in as much as the PLA2 inhibitor oleyloxyethyl phosphorylcholine blocks vasodilation and also significantly inhibits K+ channel activity. In the case of ACh, relaxation requires transfer of a factor or factors from the endothelium to smooth muscle via gap junctions, whereas A23187 permits release directly into the extracellular space [127,128].

EDHF has been shown to play an important role in shear stress-induced endothelium-dependent relaxations, where K^+ channels, especially Ca^{2+}-activated K^+ channels, appear to be involved [129]. The enhanced endothelium-dependent relaxation to high doses of ACh in cirrhotic animals is only partially reversed using a NOS and/or COX inhibitor [130–132]. It is highly likely that mechanical- and/or hormonal-stimulated EDHF release plays a modulatary role in dictating the vascular responsiveness of hyperhemic vessels in PHT and cirrhosis.

Very few data exist on the role of EETs in the pathophysiology of portal hypertension. We have shown that 8-9-, 11-12-, 14-15-EETs levels are more than doubled in plasma from cirrhotic patients with portal hypertension [74], and the increased nitric oxide/PGI2-independent vasodilation of mesenteric arterial circulation in experimental cirrhosis is because of, at least in part, hyperreactivity to 11,12-EET through an increased expression of myoendothelial gap junctions (Figs. 2 and 3). In cirrhotic rats with increased response to ACh vasodilatation of mesenteric small arteries (<300 μm diameter) is blunted by EET inhibition with miconazole only in cirrhotic animals. 18a-glycyrrhetinic acid (18α-GA), a putative gap-junctions uncoupler, blunts the response to ACh more in cirrhotic than in control rats. Concentration-response curves to 11,12-EET show an increased endothelium-dependent vasodilating response in cirrhotic rats; the big conductance calcium-activated potassium channels (BK_{Ca}) inhibitor Iberiotoxin (25 nM) blocks the response in normal rats but not in cirrhotic rats, while 18α-GA blunts the response in cirrhotic rats but not in control rats, and there is an increased mRNA and protein expression of connexin-40 and -43 in cirrhotic arteries. Thus, the increased nitric oxide/PGI2-independent vasodilation of mesenteric arterial circulation in cirrhosis is because of, at least in part, hyperreactivity to increased 11,12-EET through an increased expression of myoendothelial gap junctions. We have also shown that 11,12-EET increases heme-oxygenase-1 (HO-1) expression and activity in endothelial cells [133], that 11,12-EET rat mesenteric vasodilator action is mediated via an increase in HO activity and an activation of calcium-activated potassium channels [134], and that an increased sensitivity to the heme-oxygenase/carbon monoxide relaxant effect participates to the endothelium-dependent mesenteric vasodilatation in cirrhotic rats with ascites, through BK_{Ca} α subunits, which are more expressed in cirrhosis [135]. Thus, vasodilatation in the mesenteric circulation of cirrhotic portal hypertension is caused not only by the increase in NO/PGI2 but also by the activation of the EET/HO/Ca-activated K channels/gap junctions system.

Concerning the peripheral vasodilatation, characterized by vasodilatation and/or impaired reactivity to pressor agents, inhibition of epoxygenase with miconazole in cirrhotic patients causes a higher decrease in skin blood flow, compared to normal subjects, and the effect is evident also after NO inhibition with l-NG-Nitroarginine methyl ester (l-NAME). Thus, increased EETs participate also in the peripheral vasodilatation of cirrhotic portal hypertension.

5. Extrahepatic collateral vessel formation

Porto-systemic collaterals (or shunts) develop through the opening of vessels in response to an increase in portal pressure [136,137]. The mesenteric vascular bed produces various angiogenic factors, such as VEGF [138–140] and placental growth factor [141] which stimulate angiogenesis and promote the formation of portosystemic collaterals. Using a vascular corrosion technique, it was demonstrated that collaterals are formed in the splanchnic circulation in portal hypertensive mice by both sprouting and intussusceptive angiogenesis [142]. These collaterals cause serious complications such as variceal bleeding and hepatic encephalopathy [2125]. Incubation with indomethacin significantly potentiates the response of collaterals to vasopressors such as vasopressin, which suggests a functional role for PGs as mediators in the regulation of the portal-systemic collateral circulation in portal hypertensive rats [89].

A reduction of portal systemic collaterals would be clinically relevant. As previously discussed, increased EETs may stimulate collaterals formation by increasing VEGF. Thus inhibition of epoxygenase may be effective in reducing portal systemic collaterals.

6. Conclusions

AA metabolites through the three pathways, COX, LO, and P450 monooxygenase and epoxygenase, are involved in endothelial dysfunction of portal hypertension. Increased TxA2 production by LSEC via increased COX-1 activity/expression, increased LTs, increased EETs (vasoconstrictors of the portal circulation), represent a major component in the increased portal resistance, in the decreased portal response to vasodilators and in the hyper-response to vasoconstrictors. Increased PGI2 via COX-1 and COX-2 overexpression, and increased EETs/HO-1/K channels/gap junctions (EDHF system) play a major role in mesenteric vasodilatation, hyporeactivity to vasoconstrictors, and hypereactivity to vasodilators. EETs, which are mediators of liver regeneration after hepatectomy and of angiogenesis, may play a role in the development of regenerative nodules and collateral circulation, through stimulation of VEGF inside the liver and in the portal circulation. Pharmacological manipulation of AA metabolites may be beneficial for cirrhotic portal hypertension.

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