Role of vasoactive substances and cellular effectors in the pathophysiology of cirrhotic portal hypertension: the past, the present and the future
– Georges Brohée lecture –

Wim Laleman

Unit for Liver, Biliary and Pancreatic Diseases, University Hospital Gasthuisberg, K.U. Leuven, Leuven, Belgium.

Abstract

In the last decade, knowledge regarding mechanisms involved in the pathogenesis of portal hypertension has taken unprecedented levels. However, many aspects still remain to be elucidated. Portal hypertension is primarily caused by an increase in resistance to portal outflow and secondly by an increase in splanchnic blood flow. In a later phase, these changes lead to a hyperdynamic circulation with increased cardiac output and decreased systemic vascular resistance and perfusion pressure. Regional alterations in vasoreactivity (vasodilation and vasoconstriction) play a central role in the pathophysiology of portal hypertension by contributing to increased intrahepatic resistance, hyperdynamic circulation, and expansion of the collateral circulation. Among vasoactive substances activated in portal hypertension, nitric oxide (NO) is considered as the most important vasodilator. Endothelin-1 and cyclooxygenase-derived prostaglandins are the foremost vasoconstrictor factors. The imbalance between the hyperresponsiveness and overproduction of vasoconstrictors and the hyporesponsiveness and impaired production of vasodilators are the main responsible of the increased vascular one in the sinusoidal area of the liver. In addition to an imbalance in vasoactive substances, a major role has been attributed to activated hypercontractile hepatic stellate cells which cause vascular remodelling as an adaptive response to the changed balance in vasoactive substances. The present paper aims to elucidate on available knowledge and novel mechanisms gathered over the last years with regard to cirrhotic portal hypertension and the increased intrahepatic vascular resistance in particular. (Acta gastroenterol. belg., **2009**, 72, **9-16**).

Key words : portal hypertension, nitric oxide synthase, thromboxane A2, increased intrahepatic vascular tone.

Introduction

Portal hypertension (PHT) is characterized by a pathological increase in portal vein pressure as a result of an impediment to portal flow, which in over 90% of cases in Europe is caused by cirrhosis. Most of the lethal complications of cirrhosis, independent of its etiology (alcoholic, viral hepatitis, non-alcoholic fatty liver disease, metabolic, ...), are directly related to the presence of portal hypertension, including (bleeding) gastroesophageal varices, hepatic encephalopathy, ascites, pulmonary and renal dysfunction (1,2). Because of the combined impact of these complications, portal hypertension remains the most important cause of morbidity and mortality in patients with cirrhosis.

Over the last 2 decades, it has become clear that a decrease in portal pressure, obtained by the use of non-selective beta-blockers, is not only protective against the risk of variceal (re)bleeding but is also associated with a long term reduction of developing complications and with an improved long-term survival (3-6). However, in practice, less than half the patients under beta-blokkade are protected from these risks, supporting the overall demand for innovation and expansion of our therapeutic armamentarium (1,5,7,8).

The present paper aims to elucidate on available knowledge and novel mechanisms gathered over the last years with regard to cirrhotic portal hypertension and the increased intrahepatic vascular resistance in particular, since the possibility of pharmacological manipulation of this latter has challenged the paradigm that portal pressure can only be reduced by decreasing splanchnic inflow by means of splanchnic vasoconstrictors.

The fundamentals of experimental cirrhotic portal hypertension : immutable dogmas from the past as a basis for the future

In the normal liver, intrahepatic resistance changes with variations in portal blood flow, thereby keeping portal pressure within normal limits. In cirrhosis however, both intrahepatic resistance and splanchnic blood flow are increased, resulting in increased portal pressure (1,9). The increase in intrahepatic vascular resistance (IHVR) is considered the primary event in cirrhotic PHT. In contrast, splanchnic hyperemia is regarded as a secondary phenomenon which maintains or worsens the portal hypertensive syndrome and gives rise to the hyperdynamic systemic state, which in an attempt to maintain effective circulating volume, is characterized by an increased heart rate, cardiac output, plasma volume but eventually also low overall vascular resistance (1,5,9) (Fig. 1).

The *increased IHVR* in the context of cirrhosis was long considered as an unalterable, passive phenomenon due to extensive fibrosis, thrombosis, nodule formation

Correspondence to : Wim Laleman, M.D., Ph.D., Unit for Liver, Biliary and Pancreatic Diseases, University Hospital Gasthuisberg, K.U. Leuven, 3000 Leuven, Belgium. E-mail : wim.laleman@uz.kuleuven.ac.be

Submission date : 09/01/2009
Acceptance date : 09/01/2009

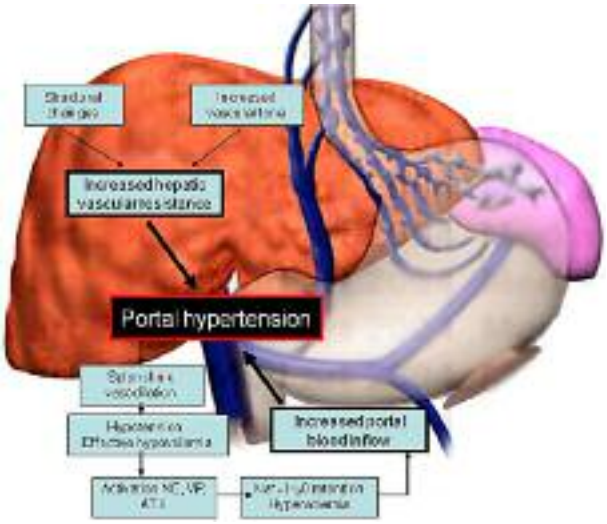

Fig. 1. — Schematic overview of the pathophysiology of PHT. (NE, norepinephrine ; VP, vasopressin ; AT-II, angiotensin-II).

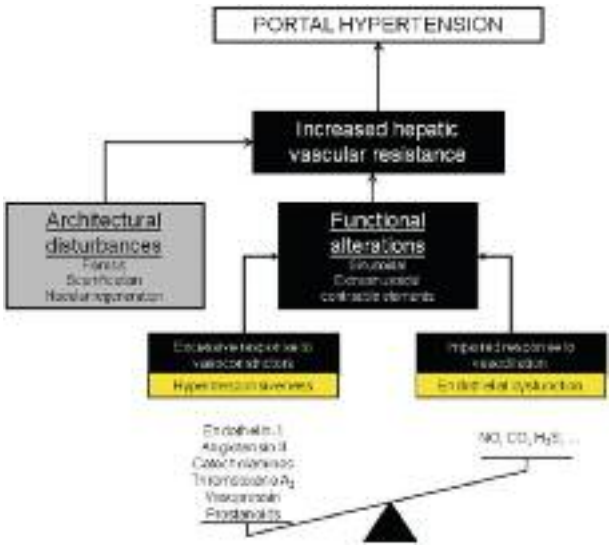

Fig. 2. — Schematic representation of the increased intrahepatic vascular tone.

and collagenization ("capillarization") of the space of Disse. This explains why the first efforts to reduce portal hypertension were primarly aimed at reducing portal inflow (which uptil today in clinical practice is still addressed by the use of non-selective beta-blockers). In 1985, Bhatal and Grossman astonished the hepatological society by demonstrating the existence of an active, dynamic modifiable component as a part of the IHVR in an isolated perfused cirrhotic rat liver (10). By adding different vasodilators (nitroprusside, prostacyclin, cytochalasin, ...), they estimated that increased intrahepatic vascular tone accounted for 30-40% of the total IHVR. As a basis for their findings they postulated that an increased number of a special type of contractile cells in a strategic location within the cirrhotic nodule may modulate, through contraction, the resistance of the hepatic vascular bed to portal inflow.

This hypothesis induced the search for *contractile elements* within the liver and for abnormalities in the mechanisms regulating and driving cell contraction. Although other cellular elements such as vascular smooth muscle cells of the intrahepatic vasculature (i.e. small portal venules in portal areas) may also be involved, many studies have pointed out a dominant role for perisinusoidally located hepatic stellate cells (HSC) (11-15) (Fig. 2). The main arguments herefore are : 1) the fact that the hepatic sinusoid is the narrowest vascular structure within the liver and therefore most eligible as the principal site of blood flow regulation, 2) the anatomical location of hepatic stellate cells, which embrace the sinusoids, and as such provide a favorable arrangement for sinusoidal constriction and for control of sinusoidal vascular tone and blood flow and 3) the fact that hepatic stellate cells – following liver injury – undergo a striking morphological and functional transition to a "myofibroblast-like" phenotype with increased contractile properties besides increased fibrogenetic, immunomodulatory and migratory potential (11-15).

Besides the availability of a cellular hypercontractile effector, an *imbalance in vasoactive substances* is an additional prerequisite to realize an increased intrahepatic vascular tone (Fig. 3). In cirrhosis, there is an overexpression locally and/or systemically of (neuro)humoral *vasoconstrictors*, such as norepinephrine, endothelins, angiotensin II and leukotrienes, leading to an increased vascular tone of the cirrhotic liver as well as to an exaggerated response ("hyperresponsiveness") of the hepatic vascular bed to some of these mediators (1,5,16,17). Of these vasoconstrictors endothelin-1 (ET-1) seems the most potent one. Both hepatic concentration and endothelin receptor expression are increased in human and experimental cirrhosis (18). The ET_A-receptor subtype, present on vascular contractile cells, causes vasoconstriction, while the ET_B-receptor subtype on endothelial cells is believed to cause vasorelaxation by stimulating endothelial nitric oxide synthase (eNOS) (1,16). Furthermore, ET-1 has also been reported to induce a strong pro-fibrogenetic response, emphasizing its role not only in the active dynamic but also in the "passive" intrahepatic resistance (19). Opposed to an intrahepatic excess in vasoconstrictive potential, the intrahepatic (endothelial) production and/or availability of *vasodilators* is inadequate, leading to an impaired intrahepatic endothelium-dependent vasorelaxation capacity or endothelial dysfunction. Nitric oxide (NO), the most renown vasodilator substance, is a reactive, gaseous molecule with a half-life of 3-5 seconds, produced from L-arginine by one of the 3 nitric oxide synthase (NOS)-isoforms. These isoforms are categorized in 2 families of enzymes : endothelial cells (eNOS) and neurons (nNOS) contain a distinct "constitutive" NOS, whereas a wide variety of cells, mainly cells involved in inflammatory

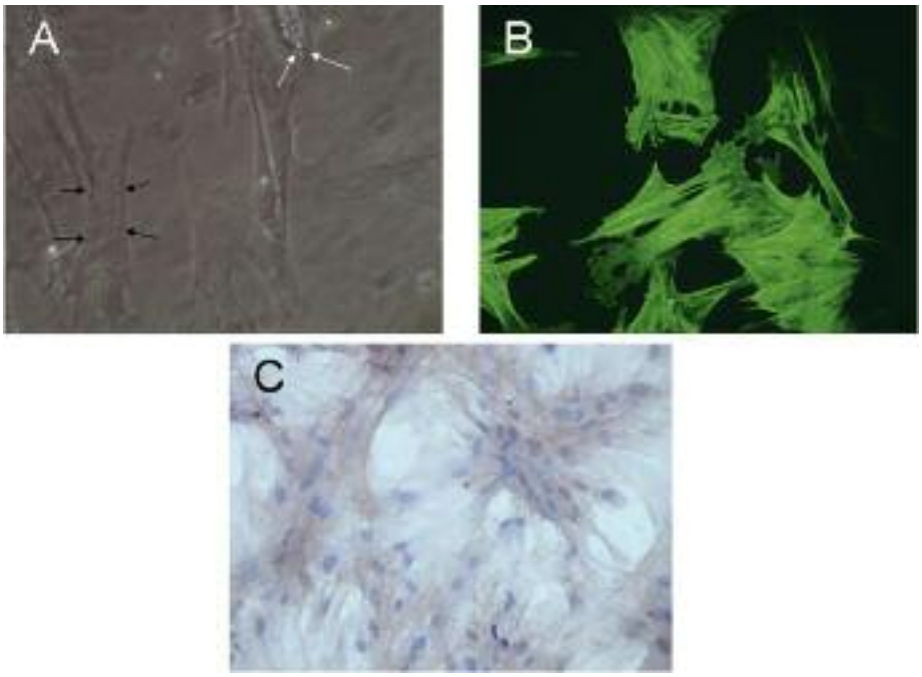

Fig. 3. — "culture-activated" hepatic stellate cells.
Phase-contrast microscopy after 24-48 h of culture on plastic showing elongated stellate shaped cells (black arrows) with the presence of minimal cytoplasmic lipid droplets (white arrows) (original magnification × 200), B. and C. Illustration of the presence of α-smooth muscle actin, a highly-specific marker of HSCs, by a phalloidin-probe, (B) and immunohistochemistry (C), respectively (both original magnification × 200).

reactions, are capable of expressing the inducible form (iNOS). In the liver, both eNOS and iNOS can be active. In chronic liver injury, the molecular basis of the intrahepatic NO-deficiency has uniformly been attributed to a decreased activity of the endothelial isoform (eNOS) (20-24).

The present : evolving doctrines

Hepatic stellate cells : the inimitable centipedes of the liver (Fig. 2)

For over 130 years, the hepatic stellate cell, also know as Ito cell, hepatic adipocyte or lipocyte, has surprised and engaged physiologists, pathologists, and hepatologists. The paradigm in liver injury of activation from quiescent vitamin A-rich stellate cells into proliferative, contractile, and fibrogenic myofibroblasts has launched an era of astonishing progress in the understanding of the mechanistic basis of hepatic fibrogenesis and involvement in the increased intrahepatic vascular tone. Furthermore, this simple paradigm has now yielded to a remarkably broad appreciation of this cell's functions not only in liver injury, but also in hepatic development, regeneration, xenobiotic responses, intermediary metabolism, immunoregulation, tumor growth and auxiliary assist in hepatic progenitor cell amplification and differentiation.

Because of their apparent key role in the modulation of hepatic microcirculation (1,5,12,14,15), considerable effort has been made to elucidate the regulation that governs contractile force generation in these cells. The functional and ultrastructural resemblance to pericytes, the expression of smooth muscle proteins (like α-smooth muscle actin and myosin II) (25,26), the expression of L-type voltage operated Ca^{2+}-channels (27) and the fact that agonists that are known to cause contraction in HSCs are associated with increases in intracellular Ca^{2+} (27) fuelled the prevailing belief activated HSCs had a "smooth-muscle cell-like" Ca^{2+}-dependent contraction pattern (12,14,15,26,27). Although persuasive, these arguments were challenged by a lack of direct evidence proving that an increase in intracellular Ca^{2+} indeed mediated force generation, and by an apparent contradiction in terminology, as witnessed in the " smooth-muscle cell-like contraction pattern of a myofibroblast-like activated HSC" (since these latter are considered Ca^{2+}-independent) (28,29). A first support to this premise was suggested by Melton *et al.* (28) who found that increasing $[Ca^{2+}]_i$ by depolarizing the plasma membrane did not induce stellate cell force generation. Moreover, they observed that even superphysiological increases in $[Ca^{2+}]_i$, triggered by the ionophore ionomycin, were capable of stimulating only a small increase in contractile force. Yet the truth seems somewhere in the middle since we demonstrated some later that calcium-dependent

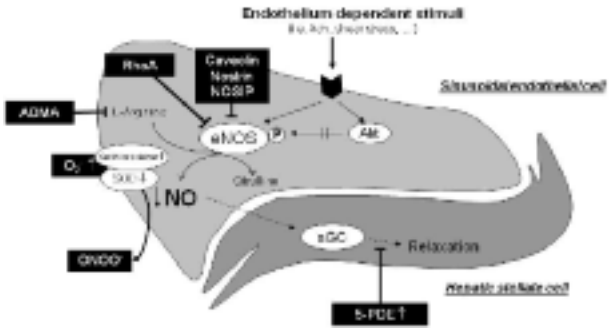

Fig. 4. — Possible mechanisms involved in the decreased intrahepatic NO bioavailability : dysfunction of endothelial NO-synthase (eNOS) in the sinusoidal endothelial cell due to excessive inhibitory activity of caveolin, nostrin, NOSIP or RhoA, defective phosphorylation of eNOS by the serine-threonine kinase Akt, decreased translation of the eNOS protein, competition for its substrate arginine by ADMA or increased degradation of NO to peroxynitrite (due to increased superoxide production) or of its second messenger cyclic guanosine 3',5'-monophosphate (due to upregulated phosphodiesterases). (Ach, acetylcholine ; sGC, soluble guanylate cyclase ; O_2^-, superoxide ; $ONOO^-$, peroxynitrite ; 5-PDE, 5-phosphodiesterase ; SOD, superoxide dismutase).

pathways (which result in increased phosphorylation of the regulatory light chain of myosin (rMLC)) are nonetheless indispensable for HSC contraction but, like Melton *et al*. (28), we could document that this pathway was not the presumed main means to cause contraction (29). We substantiated a more important role for Ca^{2+}- independent pathways, which appeared to involve the RhoA and protein kinase C-signalling-pathway. Even more interesting, was the comparison with vascular smooth muscle cells, in which gradual elevation of intracellular Ca^{2+} ultimately led to a similarly efficient contraction as obtained after agonist stimulation, and with cardiac myofibroblasts, which – like other types of myofibroblasts – appeared Ca^{2+}- insensitive. Clearly, these observations refute any comparison for HSCs with smooth muscle cells and myofibroblasts, making terms like "smooth muscle cell like" and "myofibroblast like" irrelevant and inappropriate, and as such no longer acceptable (18). These in vitro findings, the involvement of both Ca^{2+}-dependent and -independent pathways, were shown of relevance in vivo since inhibition of these pathways attenuated the increased IHVR. Future drug-design, targeting this component, should therefore consider the amentioned pathways.

Nitric Oxide : the good, the bad and the ugly

A next crucial element in the increased hepatic vascular tone is the insufficient intrahepatic production and/or availability of vasodilators, with NO considered as most relevant agent (Fig. 4). NOS-dysfunction was initially ascribed to merely diminished translation, which could be partially restored by means of adenoviral-mediated eNOS-gene-transfer (20,30). However, over the years to come additional posttranslational defects have been discovered (31-34). Posttranslational modifications are normally efficiently complemented by multiple protein-protein interactions that help regulate eNOS activity with respect to time and space. These processes encompass for instance binding of chaperone hsp90 to eNOS which may mediate vascular endothelial growth factor-induced eNOS phosphorylation by promoting the interaction between eNOS and Akt (Fig. 4). At the plasma membrane, eNOS is complexed to and inhibited by the master components of caveolae, i.e., caveolin-1 in endothelial cells. Impaired serine-threonine kinase Akt activation of eNOS and increased interaction of eNOS with the inhibitory protein caveolin-1 have been confirmed in hepatic endothelial dysfunction (31,32) (Fig. 3). More recently, the complexity of the protein network governing eNOS activity and trafficking was further highlighted by the identification of the eNOS-interacting protein (NOSIP), which binds to the oxygenase domain of eNOS (33), and of the defective protein Nostrin (34) (Fig. 4).

In addition to these molecular discoveries, we documented a novel humoral compound influencing NOS dysfunction : ADMA or asymmetric dimethylarginine (35) (Fig. 4). ADMA acts as an imposter since it mimics L-arginine, which is the sole substrate of eNOS, and decreases as such by competition NO generation at its catalytic site. ADMA as well as its vasoinactive stereoisomer, the symmetric dimethylarginine (SDMA), are synthesized by enzymatic methylation of L-arginine residues in proteins and released during proteolysis. In contrast to SDMA, which is entirely eliminated by renal excretion, ADMA is mainly metabolized to citrulline and dimethylamine by the liver enzyme dimethylarginine dimethylaminohydrolase DDAH. Consequently, it was suggested that impaired liver function led to increased plasma levels of ADMA. We could indeed document elevated plasma ADMA levels in cirrhotic rats, but these appeared increased only in biliary cirrhotic and not in toxic cirrhotic rats. Hemodynamically, we proved the ability of ADMA to cause an impaired endothelium-dependent vasodilator reaction in the isolated in-situ perfused normal rat liver (by measuring the vasorelaxing response to acetylcholine, which couples to its endothelial muscarinic M_3 receptor and stimulates eNOS to produce the vasodilatory NO). When ADMA was preincubated in biliary cirrhotic rats the pre-existing endothelial dysfunction was further aggravated and resulted in paradoxical vasoconstriction, which would seem evident in an environment where vasoconstrictors are highly upregulated and an already dysfunctional eNOS were to be further impeded or even fully inhibited. In contrast, in toxic cirrhotic rats the decreased vasorelaxing capacity was not further impaired, which might further support the hypothesis of a decreased/dysfunctional eNOS enzyme level as the major causative factor and suggest

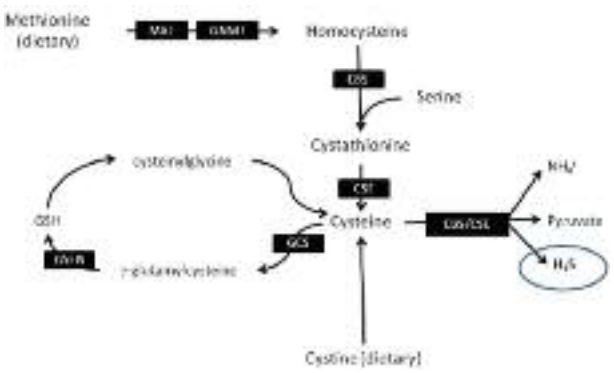

Fig. 5. — Endogenous pathways of hydrogen sulfide production. Methionine, an essential amino acid, is obtained by dietary intake and transported to the liver. Methionine adenosyltransferase (MAT) catalyzes the ATP-dependent of conversion of methionine to S-adenosylmethionine. S-adenosylmethionine is converted to S-adenosylhomocysteine by the glycine N-methyltransferase (GNMT) and then hydrolyzed to homocysteine. CBS (cystathionine-b-synthase), which is regulated by S-adenosylmethionine, catalyzes the production of cystathionine by adding serine to homocysteine. Cystathionine can then be converted to cysteine via CSE (cystathionine-γ-lyase). Cysteine, which can also be produced via reduction of dietary cystine, can then be converted to ammonium, pyruvate, and H_2S via the actions of either CSE or CBS. GSH, glutathione ; GSHS, glutathione synthetase ; GCS, γ-glutamylcysteine synthetase.

that the pathophysiology of PHT might differ depending on the etiology of cirrhosis. On the other hand, clinical data suggest that advanced hepatocellular damage combined with/without hepatic inflammation are the main determinants of elevated ADMA concentrations (36,37). This might hint that ADMA is a secondary event, of relevance only in more advanced, decompensated stages of cirrhosis. Furthermore, as ADMA opposes NOS activity, which in the systemic circulation attains unsurpassed levels (cfr. hyperdynamic circulation), one might also question whether the increase of ADMA is really a pathological event and not rather an inappropriate (and deleterious) attempt to maintain effective circulating volume and homeostasis. Further studies are therefore needed to determine the exact role, importance, and biological fate of ADMA as this might represent an important factor in more advanced stages of cirrhosis.

An important next target with regard to NOS dysfunction is the the RhoA/Rho-kinase pathway, which was already shown to be involved in the hypercontractility of hepatic stellate cells (29,38) (Fig. 4 & 5). 3-Hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (better known as statins) were recently found to inhibit synthesis of isoprenoids, which are necessary for membrane translocation and activation of small GTPases, like RhoA and Ras. Activated RhoA leads to Rho-kinase activation and NO synthase inhibition. In biliary cirrhotic rats we could document that atorvastatin inhibited hepatic RhoA/Rhokinase signaling and activated the NO/Protein kinase G-pathway. This inhibition translated in decreased hepatic stellate cell contraction, increased phosphorylated eNOS and subsequent elevated levels of NO-metabolites. This in turn decreased the intrahepatic resistance, resulting in decreased portal pressure. Meanwhile, a small pilot randomized-control clinical study with simvastatin confirmed these experimental findings since simvastatin reduced portal hypertension (-8.3% [18.8 ± 7.2 to 17.3 ± 4.7] vs 1.6% [20.0 ± 3.8 to 19.4 ± 4.4] 1.6% ($P = 0.003$)) in cirrhotic patients by decreasing hepatic vascular resistance (39). Moreover, tolerability and safety of simvastatin was excellent and the effect of simvastatin was independent of that of beta-blockers. Confirmation in a large randomized-controlled trial is awaited before implementing this interesting strategy into clinical practice.

Besides a decreased production of NO, there might also be an increased degradation of NO, which might either be caused by increased degradation of secondary messengers of NO or NO itself (40-46) (Fig. 4). In proof of the first possibility, Loureiro-Silva *et al.* (40) documented an increased degradation of cyclic guanosine 3',5'-monophosphate, the second messenger of NO, by means of increased phosphodiesterase-activity. Sildafenil, a well-known phosphodiesterase-inhibitor, was shown to largely correct this factor in an isolated liver perfusion model. Its use in practice, however, is hampered by the induction of systemic hypotension (41). On the other hand, enhanced superoxide levels, either by augmented production by increased activity of xanthine-oxidase, eNOS-uncoupling and potential other sources or either by decreased clearance by superoxide dismutase, lead to the formation of hyperreactive peroxynitrite with diminished bioavailability of NO as a result (42,43). Strategies pursuing this premise have been shown hopeful in correcting the portal hypertensive syndrome in animal studies by administrating tetrahydrobiopterin (an essential cofactor for NOS) (44) or by adenoviral vector-mediated gene-transfer of extracellular superoxide dismutase (45). In cirrhotic paients, a small patient-controlled series showed that administration of high doses ascorbic acid (3 g intravenously) improved intrahepatic endothelial dysfunction which blunted the postprandial increase in portal pressure (46). These results encourage the performance of further studies testing antioxidants or scavengers of superoxide as adjunctive therapy in the treatment of portal hypertension.

Novel intrahepatic vasodilatory molecules : "it's a gas, gas, gas"

Carbon monoxide (CO), a gaseous molecule released from physiological degradation of haem by heme-oxygenases is a well-known perfusion regulator in several vascular territories. In the liver, the role of this vascular mediator remained largely enigmatic. In a recent study, we were able to show decreased expression and activity

of both heme-oxygenase-isoenzymes in cirrhotic rats (47). Topographic evaluation of the source of CO in the liver led to the finding of extrasinusoidally haemodynamically relevant CO production in normal liver (i.e. heme-oxygenase-2), while intrasinusoidally located HO-1 predominantly regulated the microcirculation in cirrhotic livers. Upon reconstitution of CO by means of a CO-donor or induction of hemeoxygenases by means of hemin, we could attenuate HSC contraction and the increased intrahepatic vascular resistance (47).

Defective generation (by reduced expression/function of cystathionine-synthase and cystathionine-lyase) of hydrogen sulfide (H_2S), the end product of homocysteine/L-cysteine-metabolism, is considered an additional NO-independent lacking key molecule in the intrahepatic endothelial dysfunction associated with portal hypertension (48). Recently, a "methionine-connection" was proposed since reduced expression/function of cystathionine-synthase and cystathionine-lyase not only leads to deficient H_2S production, but also to accumulation of homocysteine (Fig. 5). In a recent study by Distrutti *et al.* (49), experimental perfusion of normal livers with homocysteine impaired NO formation and intrahepatic vascular relaxation induced by acetylcholine (7.3% ± 3.0% versus 26% ± 2.7% ; $P < 0.0001$). In toxic cirrhotic rats a greater percentage of increments in perfusion pressure in response to shear stress, and intrahepatic resistance to incremental increases in flow was further enhanced by homocysteine. In normal hyperhomocysteinemic and cirrhotic rat livers, endothelial dysfunction caused by homocysteine was reversed by perfusion of the livers with sodium sulfide. These findings suggest a potential role for homocysteine in the dysregulated liver microcirculation and that the "methionine-connection" plays a double foul play in the increased intrahepatic vascular tone.

Thromboxane A_2 : the unmasked villain

Activation of G-protein-coupled receptors, such as those for (nor)epinephrine, vasopressin and endothelin-1, promote the release of arachnidonic acid for the biosynthesis of vasoactive-derived prostaglandins and thromboxanes, which might suggest that the phenomenon of hyperresponsiveness could emerge through prostanoids. The result of our studies in a rat model of thioacetamide-induced cirrhosis strongly supported a role for the prostanoid thromboxane A_2 (TXA_2) (22,50). First of all, we recorded increased levels of 11-dehydro-TXB_2 (a stable non-renal- nor thrombocyte-derived metabolite of thromboxane A_2) in the serum of cirrhotic rats as well as increased TXB_2-levels in the experiments illustrating intrahepatic endothelial dysfunction. Secondly, cyclooxygenase (COX)-inhibition attenuated the hyperresponsiveness to methoxamine and improved the impaired intrahepatic endothelial vascular relaxation. Moreover, in this latter condition, the hemodynamic improvement was dose-dependent and was paralleled by a dose-dependent decrease in TXB_2-levels. The importance of this observation was further emphasized by the finding of an increased expression of TXA_2-synthase in the TAA cirrhotic rat liver compared to normal liver. Thirdly, we proved that TXA_2 causes intrahepatic vasoconstriction. *In vitro*, we documented that U44619, a stable TXA_2-analogue, led to HSC contraction, which was dose-dependently decreased following pre-incubation with SQ29,548, a TXA_2-receptor antagonist. *In the perfused liver*, preincubation with SQ29548, a TXA_2-receptor antagonist, significantly improved endothelial dysfunction and attenuated the hyperresponse to methoxamine, further proving the involvement of TXA_2. Moreover, we could prove an interaction between NOS and COX since the correcting effect of flurbiprofen on both the phenomenon of endothelial dysfunction and hyperresponsiveness was markedly attenuated when L-NAME, a well-known NOS-inhibitor, was preincubated. A possible explanation was suggested by Ashton *et al.* (51,52) who documented an interaction between COX-metabolites and eNOS in human umbilical vein endothelial cells. Increased COX-activity in these cells led to increased TXA_2 production, which in turn caused down-regulation of Akt, a serine-threonine kinase and and previously mentioned co-factor of eNOS, causing dysfunction of this latter enzyme. Based on this insight, a translational strategy was tested in cirrhotic rats using nitroflurbiprofen, an NO-releasing cyclooxygenase inhibitor (50). This drug improved portal hypertension without major adverse effects in thioacetamide-induced cirrhotic rats by attenuating intrahepatic vascular resistance, endothelial dysfunction, and hepatic hyperreactivity to vasoconstrictors. Clearly, this strategy is to be further investigated.

The future : getting closer to the Holy Grail

Portal hypertension is a dynamic multifactorial syndrome in which regional alterations in vasoreactivity (vasodilation and vasoconstriction) play a central role. Various vasoactive substances are involved, some in different roles depending on the vascular territory. These factors contribute to an increased intrahepatic resistance, hyperdynamic circulation, and expansion of the collateral circulation. Adresssing all these issues is therefore not a straightforward assignment.

A possible solution could be to eliminate all excessive vasoactive agents. However, although this approach delivered proof-of-principle that a humoral factor is crucial in the portal hypertensive syndrome, this approach is invasive, costly and most of all short-lived with regard to effect (53).

From a pharmacological point of view, a perfect drug in the treatment of cirrhotic portal hypertension, should involve a drug that decreases IHVR while maintaining or enhancing hepatic blood flow. Furthermore, the vasodilatory effect of this drug should be limited to the hepatic microcirculation to prevent a worsening in

splanchnic/systemic vasodilatation. If this drug additionally also would be able of decreasing hepatic fibrosis and improving liver function, this drug would be invaluable in clinical practice.

To achieve this goal, the future in managing portal hypertension and its complications resides in data obtained in the past enhanced by newly obtained findings, which in a translational manner might help to fine-tune and goal-direct therapy. Furthermore, cross pollination from other disciplines, like for example oncology where signalling pathways have been dissected and exploited to a much larger extent, will become inevitable.

From a clinical perspective and after further investigation, the most promising concepts for the near future involve the use of statins, anti-oxidants, NO-donating cycloxygenase-inhibitors, ...

Financial support

W. Laleman was supported part by a grant of the Research Fund of the Catholic University of Leuven and in part by the National Fund of Scientific Research Belgium (FWO).

Acknowledgement

In the process of writing the doctoral thesis which forms the basis for this paper, the author was (and still is) indebted to his (meanwhile become) colleagues Prof. Dr. Frederik Nevens, Prof. Dr. Johan Fevery, Prof. Dr. Chris Verslype, Prof. Dr. David Cassiman, Prof. Dr. Sing Hiem Yap and Prof. Dr. Werner Van Steenbergen for their continious support. Additional gratitude is in place for Dr. Jonel Trebicka (University of Bonn, Germany), Prof. Dr. Juan-Carlos Garcia-Pagan (Hospital Clinic, Barcelona), Mr. Marcel Zeegers, Mrs Ingrid Vander Elst and Ms Lien Van Landeghem.

References

1. LALEMAN W., LANDEGHEM L., WILMER A., FEVERY J., NEVENS F. Portal hypertension : from pathophysiology to clinical practice. *Liver Int*., 2005, **25** : 1079-1090.
2. WONGCHARATRAWEE S., GROSZMANN R.J. Diagnosing portal hypertension. *Baillieres Best. Pract. Res. Clin. Gastroenterol*., 2000, **14** : 881-894.
3. LEBREC D., POYNARD T., HILLON P., BENHAMOU J.P. Propanolol for the prevention of recurrent gastrointestinal bleeding in patients with cirrhosis : a controlled study. *N. Engl. J. Med*., 1981, **305** : 1371-1374.
4. PASCAL J.P., CALES P. Propanolol in the prevention of first upper gastrointestinal tract hemorrhage in patients with cirrhosis of the liver and esophageal varices. *N. Engl. J. Med*., 1987, **317** : 856-861.
5. LALEMAN W., NEVENS F. Cirrhotic portal hypertension : current and future medical therapy for primary and secondary prevention of variceal bleeding. *Minerva Med*., 2006, **97** : 325-345.
6. ABRALDES J.G., TARANTINO I., TURNES J., GARCIA-PAGAN J.C., RODES J., BOSCH J. Hemodynamic response to pharmacological treatment of portal hypertension and long-term prognosis of cirrhosis. *Hepatology*, 2003, **37** : 902-908.
7. FEU F., GARCIA-PAGAN J.C., BOSCH J., LUCA A., TERES J., ESCORSELL A., RODES J. Relation between portal pressure response to pharmacotherapy and risk of recurrent variceal haemorrhage in patients with cirrhosis. *Lancet*, 1995, **346** : 1056-1059.
8. BOSCH J., GARCIA-PAGAN J.C. Complications of cirrhosis. I. Portal hypertension. *J. Hepatol*., 2000, **32** (Suppl 1) : 141-156.
9. BOSCH J., GROSZMANN R.J. (eds.) : Portal hypertension : pathophysiology and treatment. Oxford : Blackwell, 1994.
10. BHATAL P.S., GROSSMAN H.J. Reduction of the increased intrahepatic vascular resistance of the isolated perfused cirrhotic rat liver by vasodilators. *J. Hepatol*., 1985, **1** : 325-337.
11. ROCKEY D.C., HOUSSET C.N., FRIEDMAN S.L. Activation-dependent contractility of rat hepatic lipocytes in culture and in vivo. *J. Clin. Invest*., 1993, **92** : 1795-1804.
12. FRIEDMAN S.L. Seminars in medicine of the Beth Israel Hospital, Boston. The cellular basis of hepatic fibrosis. Mechanisms and treatment strategies. *N. Engl. J. Med*., 1993, **328** : 1828-1835.
13. CASSIMAN D., ROSKAMS T. Beauty is in the eye of the beholder : emerging concepts and pitfalls in hepatic stellate cell research. *J. Hepatol*., 2002, **37** : 527-535.
14. REYNAERT H., THOMPSON M.G., THOMAS T., GEERTS A. Hepatic stellate cells : role in microcirculation and pathophysiology of portal hypertension. *Gut*, 2002, **50** : 571-581.
15. REYNAERT H., URBAIN D., GEERTS A. Regulation of sinusoidal perfusion in portal hypertension. *Anat. Rec. (Hoboken)*, 2008, **291** : 693-698.
16. ROCKEY D.C. Vascular mediators in the injured liver. *Hepatology*, 2003, **37** : 4-12.
17. MÖLLER S., BENDTSEN F., HENRIKSEN JH. Vasoactive substances in the circulatory dysfunction of cirrhosis. *Scand. J. Clin. Lab. Invest*., 2001, **61** : 421-430.
18. GHANDI C.R., SPROAT L.A., SUBBOTIN V.M. Increased hepatic endothelin-1 levels and endothelin receptor density in cirrhotic rats. *Life Sci*., 1996, **58** : 55-62.
19. ROCKEY D.C., CHUNG J.J. Endothelin antagonism in experimental hepatic fibrosis. Implications for endothelin in the pathogenesis of wound healing. *J. Clin. Invest*., 1996, **98** : 1381-1388.
20. VAN DE CASTEELE M., OMASTA A., JANSSENS S., ROSKAMS T., DESMET V., NEVENS F., FEVERY J. In vivo gene transfer of endothelial nitric oxide synthase decreases portal pressure in anaesthetised carbon tetrachloride cirrhotic rats. *Gut*, 2002, **51** : 440-445.
21. ROCKEY D.C., CHUNG J.J. Reduced nitric oxide production by endothelial cells in cirrhotic rat liver : endothelial dysfunction in portal hypertension. *Gastroenterology*, 1998, **114** : 344-351.
22. LALEMAN W., VANDER ELST I., ZEEGERS M., SERVAES R., LIBBRECHT L., ROSKAMS T., FEVERY J., NEVENS F. A stable model of cirrhotic portal hypertension in the rat : thioacetamide revisited. *Eur. J. Clin. Invest*., 2006, **36** : 242-249.
23. HARTLEB M., MICHIELSEN P.P., DZIURKOWSKA-MAREK A. The role of nitric oxide in portal hypertensive systemic and portal vascular pathology. *Acta Gastroenterol. Belg*., 1997, **60** : 222-232.
24. WIEST R., GROSZMANN R.J. The paradox of nitric oxide in cirrhosis and portal hypertension : Too much, not enough. *Hepatology*, 2002, **35** : 478-491.
25. SAAB S., TAM S.P., TRAN B.N., MELTON A.C., TANGKIJVANICH P., WONG H., YEE H.F. Jr. Myosin mediates contractile force generation by hepatic stellate cells in response to endothelin-1. *J. Biomed. Sci*., 2002, **9** : 607-612.
26. ROCKEY D.C., WEISIGER R.A. Endothelin induced contractility of stellate cells from normal and cirrhotic rat liver : implications for regulation of portal pressure and resistance. *Hepatology*, 1996, **24** : 233-240.
27. PINZANI M., FAILLI P., RUOCCO C., CASINI A., MILANI S., BALDI E., GIOTTI A., GENTILIN P. Fat-storing cells as liver-specific pericytes : spatial dynamics of agonist-stimulated intracellular calcium transients. *J. Clin. Invest*., 1992, **90** : 642-646.
28. MELTON A.C., DATTA A., YEE H.F. Jr. [Ca2+]i-independent contractile force generation by rat hepatic stellate cells in response to endothelin-1. *Am. J. Physiol. Gastrointest. Liver Physiol*., 2006, **290** : G7-13.
29. LALEMAN W., VAN LANDEGHEM L., SEVERI T., VANDER ELST I., ZEEGERS M., BISSCHOPS R., VAN PELT J., ROSKAMS T., CASSIMAN D., FEVERY J., NEVENS F. Both Ca2+ -dependent and -independent pathways are involved in rat hepatic stellate cell contraction and intrahepatic hyperresponsiveness to methoxamine. *Am. J. Physiol. Gastrointest. Liver Physiol*., 2007, **292** : G556-564.
30. YU Q., SHAO R., QIAN H.S., GEORGE S.E., ROCKEY D.C. Gene transfer of the neuronal NO synthase isoform to cirrhotic rat liver ameliorates portal hypertension. *J. Clin. Invest*., 2000, **105** : 741-748.
31. SHAH V.J., TORUNER M., HADDAD F., CADELINA G., PAPAPETROPOULOS A., CHOO K., SESSA W.C., GROSZMANN R.J. Impaired endothelial nitric oxide synthase activity associated with enhanced caveolin binding in experimental cirrhosis in the rat. *Gastroenterology*, 1999, **117** :

1222-1228.
32. MORALES-RUIZ M., CEJUDO-MARTIN P., FERNANDEZ-VARO G., TUGUES S., ROS J., ANGELI P., RIVERA F., ARROYO V., RODES J., SESSA W.C., JIMENEZ W. Transduction of the liver with activated Akt normalizes portal pressure in cirrhotic rats. *Gastroenterology*, 2003, **125** : 522-531.
33. KÖNIG P., DEDIO J., MÜLLER-ESTERL W., KUMMER W. Distribution of the novel eNOS-interacting protein NOSIP in the liver, pancreas, and gastrointestinal tract of the rat. *Gastroenterology*, 2002, **123** : 314-324.
34. MOOKERJEE R.P., WIESENTHAL A., ICKING A., HODGES S.J., DAVIES N.A., SCHILLING K., SEN S.,WILLIAMS R., NOVELLI M., MÜLLER-ESTERL W., JALAN R. Increased gene and protein expression of the novel eNOS regulatory protein NOSTRIN and a variant in alcoholic hepatitis. *Gastroenterology*, 2007, **132** : 2533-2541.
35. LALEMAN W., OMASTA A., VAN DE CASTEELE M., ZEEGERS M., VANDER ELST I., VAN LANDEGHEM L., SEVERI T., VAN PELT J., ROSKAMS T., FEVERY J., NEVENS F. A role for asymmetric dimethylarginine in the pathophysiology of portal hypertension in rats with biliary cirrhosis. *Hepatology*, 2005, **42** : 1382-1390.
36. LLUCH P., TORONDEL B., MEDINA P., SEGARRA G., DEL OLMO J.A., SERRA M.A., RODRIGO J.M. Plasma concentrations of nitric oxide and asymmetric dimethylarginine in human alcoholic cirrhosis. *J. Hepatol*., 2004, **41** : 55-59.
37. MOOKERJEE R.P., MALAKI M., DAVIES N.A., HODGES S.J., DALTON R.N., TURNER C., SEN S., WILLIAMS R., LEIPER J., VALLANCE P., JALAN R. Increasing dimethylarginine levels are associated with adverse clinical outcome in severe alcoholic hepatitis. *Hepatology*, 2007, **45** : 62-71.
38. TREBICKA J., HENNENBERG M., LALEMAN W., SHELEST N., BIECKER E., SCHEPKE M., NEVENS F., SAUERBRUCH T., HELLER J. Atorvastatin lowers portal pressure in cirrhotic rats by inhibition of RhoA/Rho-kinase and activation of endothelial nitric oxide synthase. *Hepatology*, 2007, **46** : 242-253.
39. ABRALDES JG., ALBILLOS A., TURNES J., R. BANARES., R. GONZALEZ., M. GONZALEZ., GARCIA-PAGAN J.C., BOSCH J. Hemodynamic effects and safety of one-months simvastatin treatment in patients with cirrhosis and portal hypertension : a double-blind, randomized, placebo-controlled multicenter trial. *J. Hepatol*., 2008, **48** : S25.
40. LOUREIRO-SILVA M.R., IWAKIRI Y., ABRALDES J.G., HAQ O., GROSZMANN R.J. Increased phosphodiesterase-5 expression is involved in the decreased vasodilator response to nitric oxide in cirrhotic rat livers. *J. Hepatol*., 2006, **44** : 886-893.
41. COLLE I., DE VRIESE A.S., VAN VLIERBERGHE H., LAMEIRE N.H., DEVOS M. Systemic and splanchnic haemodynamic effects of sildenafil in an in vivo animal model of cirrhosis support for a risk in cirrhotic patients. *Liver Int*., 2004, **24** : 63-68
42. RODRÍGUEZ-VILARRUPLA A., BOSCH J., GARCÍA-PAGÁN J.C. Potential role of antioxidants in the treatment of portal hypertension. *J. Hepatol*., 2007, **46** : 193-197.
43. VAN DE CASTEELE M., VAN PELT J.F., NEVENS F., FEVERY J., REICHEN J. Low NO bioavailability in CCl4 cirrhotic rat livers might result from low NO synthesis combined with decreased superoxide dismutase activity allowing superoxide-mediated NO breakdown : a comparison of two portal hypertensive rat models with healthy controls. *Comp. Hepatol*., 2003, **2** : 2.
44. MATEI V., RODRÍGUEZ-VILARRUPLA A., DEULOFEU R., GARCÍA-CALDERÓ H., FERNÁNDEZ M., BOSCH J., GARCIA-PAGÁN J.C. Three-day tetrahydrobiopterin therapy increases in vivo hepatic NOS activity and reduces portal pressure in CCl4 cirrhotic rats. *J. Hepatol*., 2008, **49** : 192-197.
45. LAVIÑA B., GRACIA-SANCHO J., RODRÍGUEZ-VILARRUPLA A., CHU Y., HEISTAD D.D., BOSCH J.,GARCÍA-PAGÁN J.C. Superoxide dismutase gene transfer reduces portal pressure in CCl4 cirrhotic rats with portal hypertension. *Gut*, 2009, **58** : 118-125.
46. HERNÁNDEZ-GUERRA M., GARCÍA-PAGÁN J.C., TURNES J., BELLOT P., DEULOFEU R., ABRALDES J.G., BOSCH J. Ascorbic acid improves the intrahepatic endothelial dysfunction of patients with cirrhosis and portal hypertension. *Hepatology*, 2006, **43** : 485-491.
47. VAN LANDEGHEM L., LALEMAN W., VANDER ELST I., ZEEGERS M., VAN PELT J., CASSIMAN D., NEVENS F. Carbon monoxide produced by intrasinusoidally located haem-oxygenase-1 regulates the vascular tone in cirrhotic rat liver. *Liver Int*., 2008 Sep 15 [Epub ahead of print].
48. FIORUCCI S., ANTONELLI E., MENCARELLI A., ORLANDI S., RENGA B., RIZZO G., DISTRUTTI E., SHAH V., MORELLI A. The third gas : H2S regulates perfusion pressure in both the isolated and perfused normal rat liver and in cirrhosis. *Hepatology*, 2005, **42** : 539-548.
49. DISTRUTTI E., MENCARELLI A., SANTUCCI L., RENGA B., ORLANDI S., DONINI A., SHAH V., FIORUCCI S. The methionine connection : homocysteine and hydrogen sulfide exert opposite effects on hepatic microcirculation in rats. *Hepatology*, 2008, **47** : 659-667.
50. LALEMAN W., VAN LANDEGHEM L., VAN DER ELST I., ZEEGERS M., FEVERY J., NEVENS F. Nitroflurbiprofen, a nitric oxide-releasing cyclooxygenase inhibitor, improves cirrhotic portal hypertension in rats. *Gastroenterology*, 2007, **132** : 709-719.
51. ASHTON A.W., WARE J.A. Thromboxane A_2 receptor signaling inhibits vascular endothelial growth factor-induced endothelial cell differentiation and migration. *Circ. Res*., 2004, **95** : 372-379.
52. GAO Y., YOKOTA R., TANG S., ASHTON A.W., WARE J.A. Reversal of angiogenesis in vitro, induction of apoptosis, and inhibition of AKT phosphorylation in endothelial cells by thromboxane A_2. *Circ. Res*., 2000, **87** : 739-745.
53. LALEMAN W., WILMER A., EVENEPOEL P., ELST IV., ZEEGERS M., ZAMAN Z., VERSLYPE C., FEVERY J., NEVENS F. Effect of the molecular adsorbent recirculating system and Prometheus devices on systemic haemodynamics and vasoactive agents in patients with acute-on-chronic alcoholic liver failure. *Crit. Care*, 2006, **10** (4) : R108.