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ORIGINAL ARTICLE

Mesenteric arteries responsiveness to acute variations of wall shear stress is impaired in rats with liver cirrhosis

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Abstract

Objective. In liver cirrhosis, excessive splanchnic vasodilation is due to abnormal synthesis of endogenous vasodilators and to decreased sensitivity to vasoconstrictors. The role of mechanical stimuli such as wall shear stress (WSS) on splanchnic circulation remains unclear. The aim of this study was to assess the vasodilation induced by wall shear stress (WSS) and acute changes in blood flow in the mesenteric arteries in an experimental model of liver cirrhosis. ***Materials and Methods.*** The effect of acute changes in intraluminal flow (0, 10, and 20 μl/min) and WSS on the diameter of the mesenteric arteries (diameters <500 μm) of control and cirrhotic rats was assessed, at baseline and after the inhibition of nitric oxide synthase, cyclooxygenase and hemeoxygenase. Concentration–response curves to phenylephrine were also obtained. ***Results.*** In controls, the increase in intraluminal flow led to a significant increase in arterial diameter ($p < 0.05$), while WSS remained stable; the effect was maintained in vessels pre-constricted with phenylephrine, blocked by the exposure to indomethacin and L-NAME and restored by the subsequent addition of chromium mesoporphyrin ($p < 0.05$). In cirrhotic arteries, arterial diameters did not change in response to acute increase in flow, neither at baseline nor after exposure to indomethacin and L-NAME, while WSS increased ($p < 0.01$). Responsiveness to flow was partially restored ($p < 0.05$) after exposure of the arteries to chromium mesoporphyrin in addition to indomethacin and L-NAME. ***Conclusions.*** Arteries from cirrhotic rats showed an abolished responsiveness to acute variations in flow, which exposes the mesenteric endothelium to sudden variations in WSS.

Key Words: *chromium mesoporphyrin, flow-mediated vasodilation, indomethacin, liver cirrhosis, L-NAME, splanchnic vasodilation, wall shear stress*

Introduction

Hemodynamic forces, generated by blood flow pulsatility, exert a crucial influence on the endothelium. Among these mechanical stimuli, the most relevant ones are hydrostatic pressure, circumferential stress (due to intercellular connections) and wall shear stress (WSS) [1]. WSS, the frictional force exerted by blood flow on a vessel wall, is known to induce various phenotypical changes in the endothelium via modification of gene expression, thus promoting cytoskeletal rearrangement, production of growth factors, fibrinolysis factors, adhesion molecules [2], antioxidant agents (cytochrome 1A1 and 1B1, hemeoxygenase [HO]), gap-junctions proteins and elastin [3]. This force has been shown to be tightly connected to flow-mediated vasodilation [4]. Firstly, it has been demonstrated that this phenomenon is mediated by the endothelium, as it cannot be observed after the removal of this cellular layer [5]. A correlation between vasodilation and flow velocity was demonstrated several years ago [6,7], while other studies have highlighted the connection between vasodilation and the increase in flow viscosity [4]. WSS is related to flow viscosity, as well as its velocity, thus this force might be one of the most relevant factors acting on the

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endothelium and impinging on flow-mediated vasodilation [4].

Nitric oxide (NO) is the most relevant mediator of endothelium-dependent vasodilation, and appears to exert a major role in flow and WSS-dependent vasodilation [8]. Other mediators, such as an endothelium-derived hyperpolarizing factor [8], prostacyclin [9] and other molecules produced by cyclooxygenase (COX) [10], epoxieicosatrienoic acids (EETs) [11] and some HO products, which may act via large conductance calcium-activated potassium channels (BK_{Ca}), [12–14] are also involved.

Our study examines the role of WSS in the regulation of the diameter of mesenteric arteries in rats with liver cirrhosis. The development of this condition is characterized by major rearrangements in vascular reactivity. While vascular resistance increases in the liver, mesenteric arteries show a decrease in this parameter, which contributes to maintain and worsen portal hypertension, leading to the development of hyperdynamic circulation [15]. Excessive vascular splanchnic vasodilation is due to abnormal synthesis of endogenous vasodilators [16,17] and to decreased sensitivity to vasoconstrictors [18]. Alterations in endothelial function have been demonstrated in liver cirrhosis [19], including excessive production of NO [20] and prostacyclin [21], and increased expression of HO-1 in splanchnic tissues [22,23]. Mechanical stimuli, such as WSS and increase in blood flow, enhance endothelial nitric oxide synthase (eNOS) activity [24,25]. Based on studies conducted on rat aortas, it has been hypothesized that excessive splanchnic vasodilation, due to NO hyperproduction and enhanced eNOS activity, could be a consequence of the increase in WSS [26,27]. In contrast, studies regarding an earlier stage of portal hypertension, when the decrease in splanchnic resistance and hyperdynamic circulation have not yet taken place [28–30], have led to the conclusion that enhanced eNOS activity precedes the onset of hyperdynamic circulation, thus excluding a causal involvement of WSS [28,30]. More recently, it has been hypothesized that myogenic vasoconstriction of the superior mesenteric artery, taking place a few hours after the onset of portal hypertension, could trigger the increased production of NO [31].

In summary, the influence of WSS on splanchnic circulation in liver cirrhosis remains unclear, for it is not known whether an increase in WSS has a role in the onset/progression of these vascular alterations or is their consequence.

The aim of this study was to assess vasodilation induced by acute changes in blood flow and in WSS in mesenteric arteries, in an experimental model of liver cirrhosis. The vessels were exposed to varying flow velocities and flow-mediated vasodilation was evaluated. Subsequently, responses to increasing doses of vasoconstrictors at different flow velocities were also measured. Finally, the potential mediators involved in this phenomenon were investigated by exposing the vessels to inhibitors of NOS, COX, and hemeoxygenase (HO).

Materials and methods

Animals

The study was performed on 32 adult male Wistar rats (body weight, 200–250 g; Charles River Laboratories, Calco, Italy). The experiments were carried out in accordance with the legislation of the Italian pertinent authorities (D.L. 27/01/1992 116), which complies with the European Community guidelines (CEE Directive 86/609) for the care/use of experimental animals. The experimental protocol was approved by the Institutional Animal Care and Use Committee.

Cirrhosis was induced by carbon tetrachloride (CCl_4) inhalation in 14 rats being also administered phenobarbital (0.30 g/l in the drinking water), as fully described elsewhere [22]. Treatment was continued for 14 weeks and the experiment performed a week after the last administration of CCl_4. After CCl_4 treatment completion, livers from all rats showed a finely granulated surface. In our previous experience, this is invariably associated with cirrhosis [22]. Eighteen age-matched animals served as controls.

Isolation and microvessel preparation

Anesthesia was induced by ketamine hydrochloride (100 mg/kg b.wt. i.m.), a midventral laparotomy was performed and a section of the small intestine was removed. As the low splanchnic vascular resistance observed in portal hypertension depends mostly on mesenteric resistance arteries, which are precapillary arteries with diameters <500 μm [32], these vessels were chosen for purposes of analysis.

The clamped section of the small intestine was placed in a chilled oxygenated modified Krebs bicarbonate buffer [physiological salt solution (PSS)] containing 118.5 mM NaCl, 4.7 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 2.8 mM $CaCl_2$, 25 mM $NaHCO_3$, and 11 mM dextrose. Third/fourth-order branches of the superior mesenteric artery (diameter <500 μm, length 1–2 mm) were isolated from the surrounding perivascular tissue, removed from the mesenteric vascular bed, and mounted on glass micropipettes in a water-jacketed perfusion chamber (Living Systems Instrumentation, Burlington, VT) in warmed (37°C), oxygenated (95%

O_2 and 5% CO_2) PSS. The vessels were mounted onto a proximal micropipette connected to a pressure servo controller.

The lumen of the vessel was flushed to remove residual blood, and the end of the vessel mounted onto a distal micropipette connected to a three-way stopcock. The vessel was superfused with PSS (4 ml/min) at 37°C and gassed with 95% O_2 and 5% CO_2. Both ends of the vessels were cannulated, and an intraluminal flow of PSS solution established. After an equilibration period of 30–40 min, the vessels were challenged with the α1-adrenoreceptor agonist phenylephrine (Phe; 1 μM). Arteries were discarded if obviously leaking or failing to constrict by >20% in response to Phe. The endothelium was considered functional based on prompt relaxation to acetylcholine (ACh) (1 μM) after Phe-induced (1 μM) contraction. Only vital vessels with functional endothelium were utilized.

In order to examine arterial reactivity in the presence of intraluminal flow, a peristaltic pump was used. Three different flow velocity steps were chosen: 0, 10, and 20 μl/min (see below); intraluminal pressure was maintained at 80 mmHg throughout the experiment. Vascular diameters were measured by use of a video system consisting of a microscope with a charge-coupled device television camera (Eclipse TS100-F; Nikon, Tokyo, Japan), a television monitor (Ultrak Inc., Lewisville, TX), and a video measuring system (Living Systems Instrumentation).

Preliminary study

Preliminary studies were conducted to evaluate arterial sensitivity to flow in control rats, comparing diameters measured in the absence of flow (flow 0) to those obtained after exposure to different flow velocities. At low flow levels (2 or 5 μl/min), no changes in intraluminal diameter were observed; in contrast, a significant increase in diameter was obtained with a 10 μl/min flow; so this flow velocity was chosen for application to the vessels in the experiment, and qualified as *low flow*. In order to evaluate vasodilation responses to high flow, vessels were exposed to 30–50 μl/min flows, but intraluminal pressure could not be maintained constant, possibly in relation to the size of both the vessels and the cannulas. Finally, a 20 μl/min flow was applied and qualified as *high flow*.

Evaluation of flow-mediated vasodilation at different intraluminal flow velocities

After a vessel was mounted onto the micropipettes in the perfusion chamber and after an equilibration period of approximately 30–40 min, intraluminal diameter was measured at flow 0. Then intraluminal flow was established and, after an equilibration period of 15 min, intraluminal diameter was measured at low flow, and, after 15 further minutes, at high flow. The experiment was performed on vessels from both control and cirrhotic rats.

Evaluation of vasoconstriction in response to Phe, in the absence and presence of intraluminal flow (10 and 20 μl/min)

Responses to increasing doses of Phe (10^{-8} to 10^{-4} M) were determined in arteries superfused with PSS. Phe was added to the bath and cumulative dose–response curves were generated, at 2–3 min intervals between doses. Vascular diameters were measured 1–3 min after the addition of Phe. Concentration–response curves were determined in the absence and in the presence of flow (10 and 20 μl/min). After each dose–response test, tissues were washed with fresh PSS for at least 20 min. The experiment was performed on vessels from both control and cirrhotic rats.

Effect of the administration of NOS, COX and HO inhibitors on the response to Phe, in the absence and presence of intraluminal flow (10 and 20 μl/min)

Concentration–response curves to increasing doses of Phe were obtained after the administration of the inhibitors of NOS, COX, and HO. Inhibitors were added to freshly prepared PSS, and 20–30 min drug-tissue contact was allowed before re-testing the response to Phe on each vessel. At first, the NOS inhibitor NG-nitro-L-arginine-methyl-ester (L-NAME) (1 mM) and COX inhibitor indomethacin (Indo) (2.8 μM) were added and the response to increasing doses of Phe was tested in the absence of flow (0 μl/min) and at two different flow levels (low flow, 10 μl/min, and high flow, 20 μl/min). Then the HO inhibitor CrMP (chromium mesoporphyrin) (15 μM) was administered to arteries pre-treated with Indo and L-NAME, and concentration–response curves obtained both in the absence and in the presence of low and high flow. The experiment was performed with both control and cirrhotic animals.

Evaluation of shear stress levels

WSS was calculated according to the formula: $wss = 4Q\eta/\pi r^3$, where Q = flow (10 or 20 μl/min); η = fluid viscosity (0.01 Poise for PSS solution), r = vessel radium. WSS values obtained at different

flow velocities, at different contraction levels, and in the presence of the inhibitors of NOS, COX and HO were compared. In the absence of flow (flow 0) WSS value corresponds to 0, thus shear stress values were obtained only after exposure to low/high flow.

Chemicals

CrMP was obtained from Porphyrin Products (Logan, UT, USA). All other chemicals were obtained from Sigma Chemical (St. Louis, MO, USA). Phe and L-NAME were dissolved in deionized water and diluted with PSS. CrMP was dissolved in a solution of 50 mM $NaCO_3$.

Data analysis

Data are expressed as mean ± SE. The effect of flow on vessel diameter was analyzed as percentage change in intraluminal diameter. Concentration–response curves derived from each vessel were fitted separately to a logistic function by nonlinear regression. The concentration–response curves from controls and treated groups were compared using the Friedman test. The remaining data were analyzed by Wilcoxon matched pairs test for paired observations, and Mann–Whitney test for unpaired observations. The *n* values indicate the number of experiments performed or animals utilized. The null hypothesis was rejected at $p < 0.05$.

Results

All rats treated with CCl_4 included in the study had macronodular or micronodular cirrhosis. Some developed mild ascites. Control rats had no apparent alterations in liver appearance. At the time of study, no difference in body weight between cirrhotic (570 ± 24 g) and control rats (575 ± 20 g) was observed.

Evaluation of flow-mediated vasodilation in arteries from control rats before and after vasoconstriction, at baseline and after the exposure to Indo, L-NAME, and CrMP

At baseline, the presence of intraluminal flow (10 µl/min) led to a relevant increase in arterial diameter (+ 16.6 ± 7.0%, $p < 0.05$). A further increase in intraluminal flow (20 µl/min) led to a further, significant increase in arterial diameter (+ 41.3 ± 12.1%, $p < 0.05$) ($n = 7$) (Figure 1). This effect was blunted by the exposure to the inhibitors Indo and L-NAME (p = NS) ($n = 8$) (Figure 1). Vasodilation response to the increase in intraluminal flow was restored by the addition of CrMP ($p < 0.05$) ($n = 5$) (Figure 1).

After pre-constricting the vessel with a high dose of Phe (10^{-4} M), an increased flow induced an increase in arterial diameter ($p < 0.05$) ($n = 7$) (Figure 1); this effect was higher than that observed in the absence of pre-constriction (+ 102.7 ± 26.6% vs. + 41.3 ± 12.1%, $p = 0.05$); arterial reactivity was maintained after exposure to Indo and L-NAME ($p < 0.01$) ($n = 8$) (Figure 1), and also after exposure to CrMP ($p < 0.05$) ($n = 5$) (Figure 1).

Considerable differences were observed between the concentration–response curves to Phe in relation to no, low, and high flow ($p < 0.01$) (Figure 2). Pretreatment with Indo and L-NAME abolished the Phe-related changes at different flow levels (p = NS) (Figure 2), while the addition of CrMP restored the sensitivity to Phe at high flow ($p < 0.01$) (Figure 2).

Evaluation of flow-mediated vasodilation in arteries from cirrhotic rats before and after vasoconstriction, at baseline and after the exposure to Indo, L-NAME, and CrMP

No significant differences were observed between the diameters of cirrhotic arteries exposed to different flow levels, neither at baseline ($n = 7$) (Figure 1), nor after exposure to Indo and L-NAME ($n = 6$) (Figure 1) or CrMP ($n = 6$) (p = NS) (Figure 1).

The administration of a vasoconstrictor (Phe 10^{-4} M) did not affect the sensitivity to flow at baseline ($n = 7$) (Figure 1) or after the exposure to Indo + L-NAME ($n = 6$) (p = NS), or after the addition of CrMP ($n = 6$) (p = NS) (Figure 1).

Concentration–response curves to Phe were also compared: no relevant differences were observed between the three curves obtained in conditions of no/low/high flow at baseline (Figure 2) or after the exposure to the inhibitors Indo+L-NAME (p = NS) (Figure 2). In contrast, after the exposure to Indo+L-NAME+CrMP, a significant modification was detected between curves obtained in conditions of no/low/high flow ($p < 0.01$) (Figure 2).

Evaluation of shear stress levels

In control arteries at baseline, WSS remained stable despite the increase in flow (0.66 ± 0.22 vs. 0.70 ± 0.20 dyne/cm^2; p = NS). In control arteries exposed to the inhibitors, the increase in flow from 10 to 20 µl/min led to an increase in WSS (from 0.88 ± 0.19 to 1.45 ± 0.27 dyne/cm^2, $p < 0.05$ after pretreatment with Indo + L-NAME; from 0.81 ± 0.12 to 1.32 ± 0.17 dyne/cm^2; $p < 0.05$ after the addition of CrMP) (Figure 3). After vasoconstriction with Phe (10^{-4} M), the increase in flow was not followed by an increase in WSS, neither at baseline

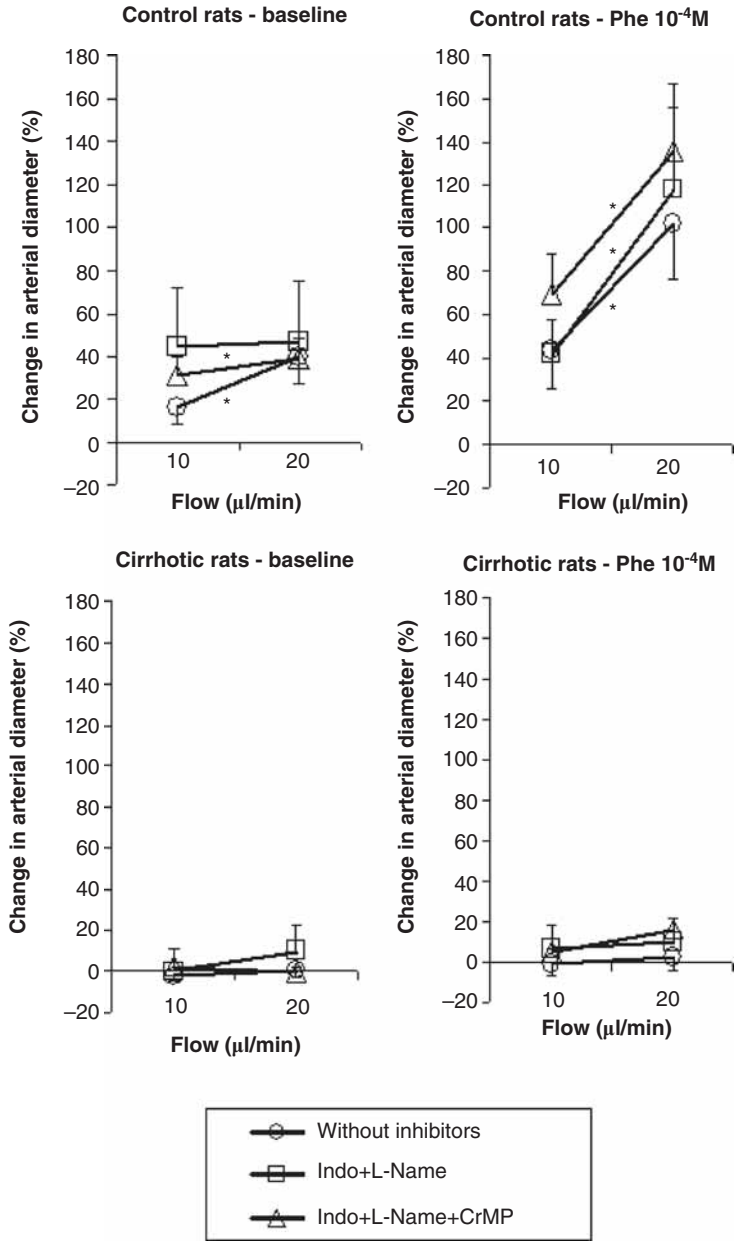

Figure 1. Effect of the increase in blood flow on the diameter of mesenteric arterial vessels before and after pre-constriction with a high dose of phenylephrine (Phe) (10^{-4} M). Open circle: baseline; open square: Indo+L-NAME; open triangle: after addition of CrMP. In control rats, the increase in intraluminal flow led to a significant increase in arterial diameter ($p < 0.05$). This effect was blunted by the exposure to Indo and L-NAME and was restored by the addition of CrMP ($p < 0.05$). After pre-constriction with Phe, the increase in flow induced an increase in arterial diameter ($p < 0.05$), higher than that observed without pre-constriction ($p = 0.05$); arterial reactivity was maintained after the exposure to Indo and L-NAME ($p < 0.05$) and also after exposure to CrMP ($p < 0.05$). In cirrhotic rats, no significant differences were observed between arterial diameters exposed to different flow levels, neither before, nor after the exposure to Indo and L-NAME, nor after the addition of CrMP. Pre-constriction with Phe did not affect the sensitivity to flow neither before nor after the exposure to Indo+L-NAME, and after the addition of CrMP. *Significant increase in arterial diameter ($p < 0.05$).

nor after the exposure to Indo + L-NAME; or after the addition of CrMP (p = NS) (Figure 3).

In the mesenteric arteries of cirrhotic rats, the increase in flow resulted in an increase in WSS both at baseline (from 0.23 ± 0.04 to 0.42 ± 0.09 dyne/cm^2, $p < 0.01$) and after exposure to the inhibitors (from 0.40 ± 0.08 to 0.61 ± 0.11, $p < 0.05$, after the administration of Indo+L-NAME; from

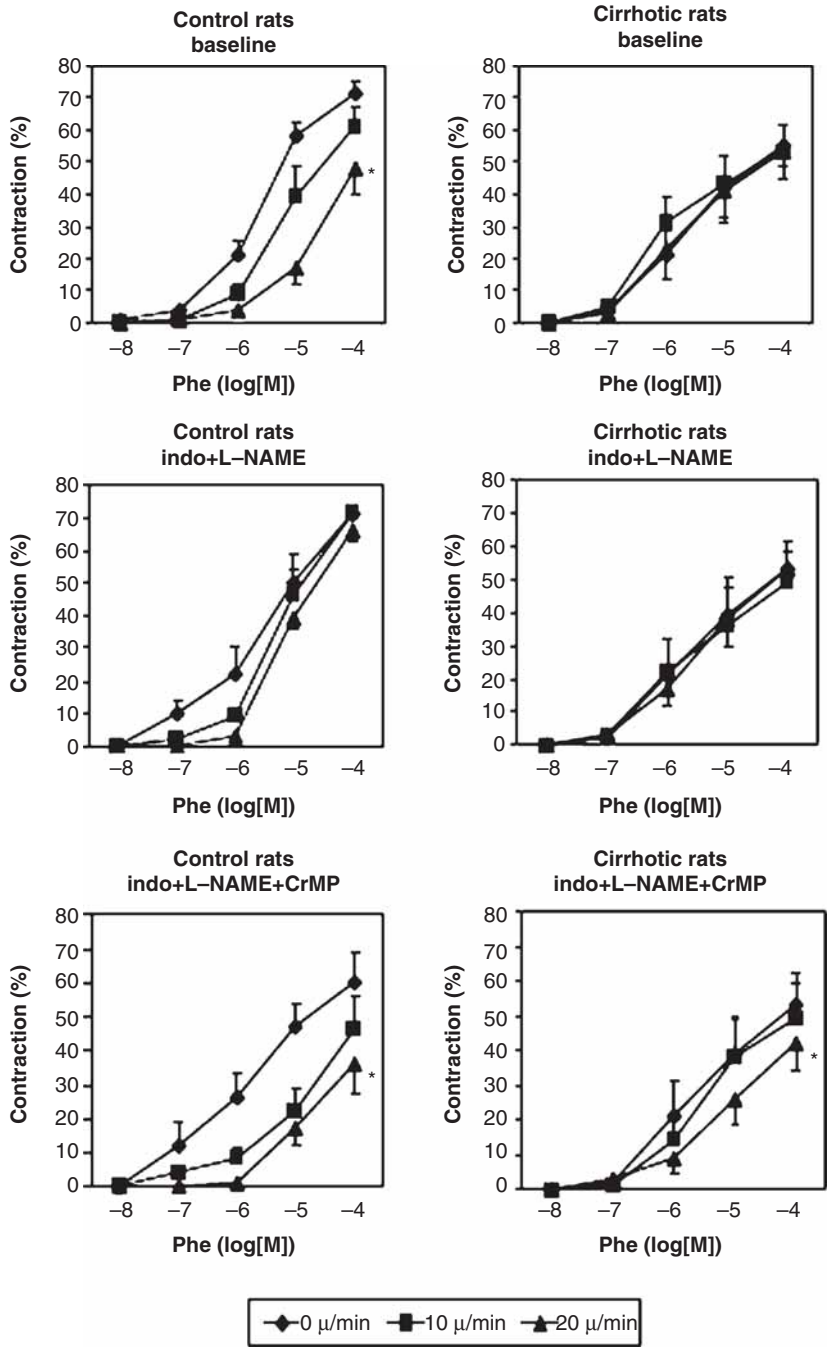

Figure 2. Concentration–response curves to phenylephrine (Phe) of resistance mesenteric arteries. In control rats, a significant difference was observed between the curves obtained in the absence of flow, after exposure to low and high flow ($p < 0.01$); exposure to Indo and L-NAME abolished these differences (p = NS), while the addition of CrMP restored the sensitivity to Phe at different flow levels ($p < 0.01$). In cirrhotic rats, no relevant differences were observed between the three curves, neither before nor after the exposure to the inhibitors Indo + L-NAME (p = NS). In contrast, after the addition of CrMP, a significant decrease in sensitivity to Phe with high flow was detected ($p < 0.01$). *Significantly different from the concentration–response curve obtained in the absence of flow ($p < 0.05$).

0.27 ± 0.06 to 0.74 ± 0.27, $p < 0.01$, after the addition of CrMP). In cirrhotic arteries pre-constricted with Phe (10^{-4} M), the increase in flow was associated with a slight increase in WSS at baseline (from 7.2 ± 2.5 to 13.6 ± 4.8 dyne/cm^2, $p = 0.06$), while after the exposure to Indo+ L-NAME and after the addition of CrMP, there was an increase which did not reach significance, possibly in relation to the high variability of the results (Figure 3).

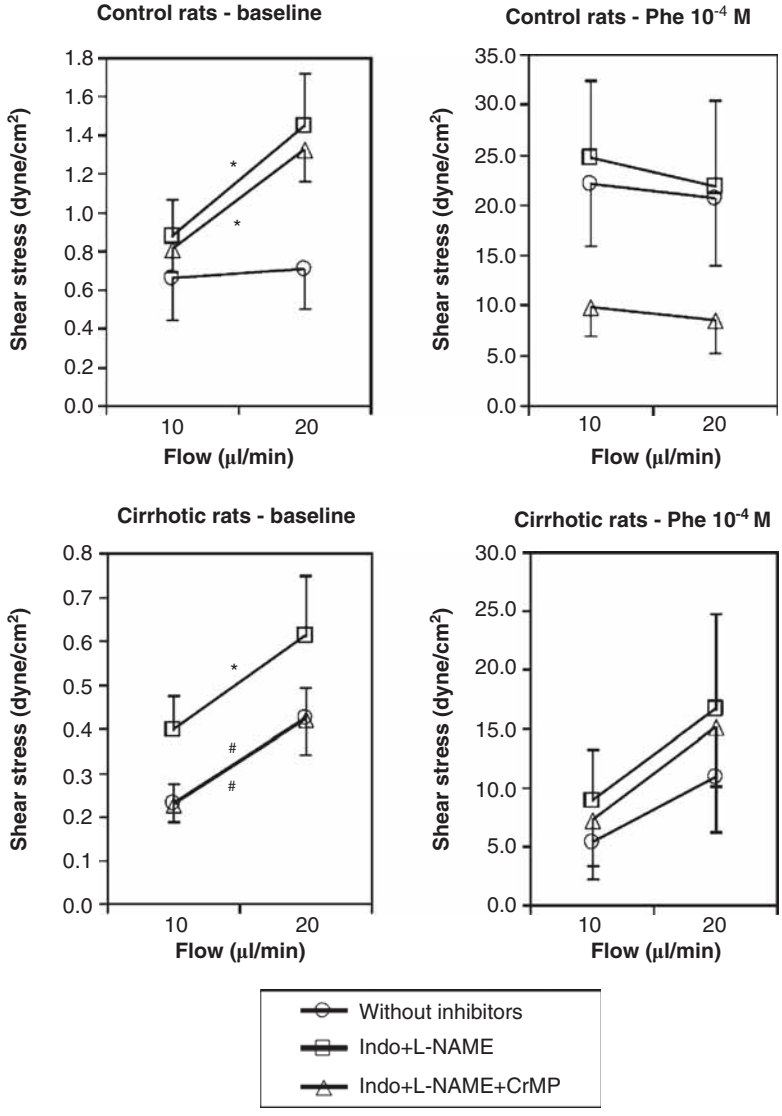

Figure 3. Effect of the increase in blood flow on wall shear stress in mesenteric arterial vessels, before and after pre-constriction with a high dose of phenylephrine (Phe) (10^{-4} M). Open circle: baseline condition; open square: Indo+L-NAME; open triangle: CrMP. In control rats, the increase in intraluminal flow did not affect wall shear stress (p = NS); in contrast, after the exposure to Indo+L-NAME and the addition of CrMP, the increase in flow was followed by a significant increase in wall shear stress ($p < 0.05$). After pre-constriction, wall shear stress was not modified by the increase in flow, neither at baseline nor after Indo+L-NAME or CrMP (p = NS). In cirrhotic rats, the increase in flow was followed by a significant increase in wall shear stress at baseline ($p < 0.01$), after Indo+L-NAME ($p < 0.05$) and after the addition of CrMP ($p < 0.01$). *Significant increase in wall shear stress ($p < 0.05$). #Significant increase in wall shear stress ($p < 0.01$).

Discussion

These data demonstrate that in cirrhotic rats mesenteric arteries are unable to compensate for an increase in WSS with an increase in vascular diameter.

In control arteries, the increase in flow caused vasodilation, which was more obvious in vessels pre-constricted with Phe, and led to a decrease in contractile response to Phe. At baseline, the increase in flow caused a mild increase in diameter, without affecting WSS. After the administration of high doses of vasoconstrictor, vasodilation induced by flow was more pronounced, while WSS remained constant. Thus, mesenteric vessels showed higher sensitivity to flow after a contractile stimulation. This could be explained by the fact that mesenteric arteries have a limited range of self-regulation compared to renal, cerebral, cremasteric, and muscular vessels [33,34]. Pre-constriction of the arteries allowed unmasking of flow-mediated vasodilation [33,34].

In mesenteric arteries from control rats, an increase in flow volume probably leads to an initial increase in

WSS, with consequent vasodilation. On constant flow rates, vasodilation is followed by a decrease in flow velocity, and then in WSS, which returns to baseline values [7]. Therefore, the constant WSS observed in our experiment after an increase in flow, even after high contractile stimuli, appears to be related to the significant vasodilation.

After the administration of Indo (COX inhibitor) and L-NAME (NOS inhibitor), the increase in flow did not produce an increase in arterial diameter, with no relevant differences between concentration–response curves to Phe. This result confirms that NO and prostanoids play a crucial role in flow- and shear stress-related vasodilation [8,35,36]. Once NO and prostanoids production was abolished, flow and shear stress did not produce vasodilation. However, after a contractile stimulus (Phe 10^{-4} M), a restored sensitivity to flow was observed. Therefore, other vasodilatory factors may play a role in pre-constricted arteries. Alternatively, the existence of an "escape" mechanism to NOS and COX inhibitors can be postulated, implying that the inhibition of the production of NO and prostanoids could be incomplete, and not sufficient in a stress condition such as that of potent vasoconstriction. Indeed, low levels of NO have been detected even after arteries were exposed to the inhibitors L-NAME or nitro-L-arginine (L-NNA) [37,38], while other studies have highlighted that some precursors of NO can be stored in tissues [39,40].

After the exposure to CrMP (HO inhibitor) of vessels previously treated with Indo and L-NAME, sensitivity to flow was restored, which was confirmed by both comparisons between dose–response curves to Phe, and by the changes in arterial diameters. The presence of a vasodilation inhibitor may be postulated: this factor could act after the exposure to indo and L-NAME, and then be blocked after the addition of CrMP. It could be a product of HO activity, such as carbon monoxide (CO), the role of which in vascular reactivity regulation has not been fully clarified. Evidence has been found of both vasodilatory [41–44] and vasoconstricting properties for this molecule [45–47]. Most of the data regarding the vasoconstricting action of CO rely on the hypothesis that this activity is carried out through NOS inhibition. This does not help explaining our results, because in our setting NOS activity had been blocked by L-NAME. Another possible explanation for the CO action can be found in a study by Thom et al. [48], who observed that endothelial cells exposed to CO undergo oxidative stress damage. As reactive oxygen species (ROS) can promote vasoconstriction and interfere with vasodilation [49], it has been hypothesized that CO-related vasoconstriction may take place through the production of ROS. In an experiment performed with rat renal interlobular arteries, exposure to CO stimulated the production of superoxide anion and caused vasoconstriction, which could be prevented by the addition of antioxidant molecules [50]. ROS seem to play a controversial role in resistance arteries: production of these molecules has also been connected to flow-mediated vasodilation and remodeling [51,52].

The natural history of liver cirrhosis encompasses the gradual onset of splanchnic vasodilation and hyperdynamic circulation [16]; it would therefore be reasonable to expect that an increased sensitivity to flow and shear stress could contribute. However, in our study, the increase in flow and shear stress in cirrhotic arteries did not lead to relevant variations of arterial diameter. As a consequence, the increase in flow produced an increase in WSS. We can therefore assume that the increase in WSS did not lead to an increase in arterial diameter.

While in control rats the endothelium of the mesenteric arteries is protected from sudden increases in WSS thanks to an increase in vascular diameter, in cirrhotic mesenteric arteries the endothelium is exposed to the potentially noxious effect of acute increases in WSS, because vessels seem unable to expand their diameter. This phenomenon cannot be ascribed to preexisting vasodilation of cirrhotic arteries, because it persists even after arterial preconstriction with Phe.

Decreased vasoconstricting response in mesenteric arteries of experimental cirrhosis has already been demonstrated [53,54], and it has also been related to bacterial translocation [55]. This phenomenon does not seem to be associated with acute variations in flow: examining our results, the acute change in flow did not modify concentration–response curves to Phe in cirrhotic arteries, neither did the exposure to Indo and L-NAME. In contrast, the subsequent administration of CrMP partially restored sensitivity to flow and shear stress. A remarkable increase in HO-1 (the inducible form of HO) expression in splanchnic arteries has already been demonstrated in rats with liver cirrhosis [23], particularly when liver disease is decompensated [54]. As for control arteries, one of the products of HO, probably CO, may affect arterial sensitivity to acute modifications in flow and shear stress, promoting the production of ROS [50]. This seems to contradict the protective and antioxidant role usually attributed to HO [56,57]. However, it is known that some HO products, such as iron and CO, can also favor oxidative reactions, and consequently regulate enzyme activity [58].

It has therefore been suggested that the antioxidant properties of HO may be limited to a specific and probably narrow threshold of overexpression

which, if exceeded, can lead to harmful and pro-oxidant effects [57,59].

An alteration in endothelial activity has been demonstrated in cirrhotic rats, consisting mostly in excessive production of vasodilatory molecules [16,19]. We can hypothesize that the impairment observed in reactivity to acute variations in flow may be attributed to a pre-existent overexpression of endothelial vasodilatory factors, which cannot be further stimulated by flow-related mechanisms. Endothelial dysfunction could also reduce sensitivity to flow-related signals.

A study in portal vein ligated rats showed a structural remodeling in mesenteric arteries, which appeared to have decreased mechanical resistance properties, diminished vascular stiffness and structural changes in the internal elastic lamina of the arterial wall [60]. It seems reasonable to hypothesize that endothelial dysfunction and structural modifications of the mesenteric arteries in liver cirrhosis could lead to impairment in responsiveness to flow. On the other hand, the lack of sensitivity to acute variations in flow in cirrhotic arteries, which exposes arterial walls to WSS-induced damage, may contribute to endothelial dysfunction in this condition. The role exerted by vascular reactivity to variations in flow in splanchnic vasodilation and hyperdynamic circulation requires further investigation; a comparison between different models of experimental cirrhosis (such as CCl4-induced and bile duct ligation-induced cirrhosis) might support the results.

In conclusion, WSS can modify vasoconstrictor responses to Phe in the mesenteric arteries of control rats. This seems mainly due to an NO- and prostanoid-mediated mechanism. Arteries from cirrhotic rats showed an abolished responsiveness to acute variations in flow, which results in exposure of the mesenteric endothelium to sudden variations in WSS, which, in turn, may impair endothelial function and participate in the structural remodeling of the vessel walls. One of the products of HO could be responsible for the impairment in arterial responsiveness to acute variations in flow. This result challenges the hypothesis that shear stress plays a major role in promoting and maintaining mesenteric vasodilation in cirrhotic rats.

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