Heme oxygenase mediates hyporeactivity to phenylephrine in the mesenteric vessels of cirrhotic rats with ascites

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Abbreviations:
CO: carbon monoxide; CrMP: chromium mesoporphyrin; EC(50): molar concentration of phenylephrine causing 50% contraction; eNOS: endothelial nitric oxide synthase; HO: Heme oxygenase; HO-1: inducible heme oxygenase; HO-2: constitutive heme oxygenase; iNOS: inducible nitric oxide synthase; L-NAME: \( N^\text{G} \)-nitro-L-arginine methyl ester; NO: nitric oxide; NOS: nitric oxide synthase; PE: phenylephrine; PSS: polysalin solution.
ABSTRACT

Background & Aims - Heme-oxygenase could play a role in the pathogenesis of arterial vasodilation in cirrhosis. This study was aimed at verifying the role of heme-oxygenase in the hyporesponsiveness to phenylephrine of small mesenteric arteries in rats with CCL\textsubscript{4}-induced cirrhosis, with and without ascites.

Methods - Pressurized small resistance mesenteric arteries were challenged with increasing doses of phenylephrine. Dose-response curves were evaluated in basal condition, after inhibition of heme-oxygenase with chromium-mesoporphyrin, after inhibition of nitric oxide synthase (NOS) with \textit{N}⁢\textit{G}-nitro-L-arginine-methyl-ester (L-NAME), and then after inhibition of both NOS and heme-oxygenase. Heme-oxygenase protein expression was also analyzed.

Results - 26 control rats and 35 rats with cirrhosis (17 with and 18 without ascites) were studied. Response to phenylephrine was lower in nonascitic and ascitic cirrhosis than in controls. Chromium-mesoporphyrin increased the response to phenylephrine only in ascitic cirrhosis (p<0.001). L-NAME increased the response to phenylephrine in controls (p<0.001) and in ascitic and nonascitic cirrhosis (p=0.002, p<0.001, respectively), but the final response in nonascitic cirrhosis was similar to that of control rats, while it remained impaired in ascitic cirrhosis. The addition of chromium-mesoporphyrin to L-NAME improved the response to phenylephrine in ascitic cirrhosis (p<0.01), with final values not different from those of the other two groups. Protein expression of the inducible isoform of heme-oxygenase was increased in the mesenteric vessels of cirrhotic rats.

Conclusion - heme-oxygenase mediates hyporeactivity to phenylephrine in the mesenteric vessels of experimental cirrhosis with ascites. NOS plays a major role only in the first stage of the disease.
INTRODUCTION

Heme oxygenase (HO) is a microsomal enzyme with two main distinct isoforms, namely, inducible (HO-1) and constitutive form (HO-2).[1][2][3] It catalyzes the rate-limiting step in the degradation of heme into biliverdin, carbon monoxide (CO) and free iron.[2][4] CO generated in endothelial and smooth muscle layers of blood vessels by HO, modulates vascular tone, by inducing relaxation of vascular smooth muscle cells by stimulating soluble guanylyl cyclase, opening large-conductance calcium-activated K+ channels and inhibiting the cytochrome P450 dependent monooxygenase system,[1][5] with a decrease in 20-hydroxyeicosatetraenoic acid (20-HETE).[6] which sustains contractile tone by inhibiting potassium channels.

An increased expression of HO-1 has been reported in the mesenteric artery of rats with pre-hepatic portal hypertension [7] and with common bile duct ligation [8] and an increased expression of HO-2 has been reported in rats with CCl₄ cirrhosis.[9] HO inhibition improved pressure response to vasoconstrictors of the mesenteric system evaluated according to McGregor,[10] both in portal hypertensive rats [11] and in CCl₄ cirrhotic rats,[9] and it improved the alterations of systemic hemodynamics of rats with secondary biliary cirrhosis.[8] Therefore, the HO/CO system may play a role in the mesenteric vasodilatation of experimental portal hypertension, but a series of questions has yet to been answered. Indeed, the role of HO in the regulation of small resistance mesenteric arteries has not yet been analyzed, nor has its involvement in the different stages of experimental cirrhosis. Moreover, the relationship between the HO/CO and nitric oxide synthase (NOS)/nitric oxide (NO) systems in cirrhosis deserves further study.

Aim of the study was to investigate the role of HO in the regulation of small resistance mesenteric arteries in cirrhosis. The effect of the HO inhibitor chromium mesoporphyrin (CrMP) on phenylephrine (PE)-induced contraction of small resistance mesenteric arteries was evaluated in rats with experimental cirrhosis with and without ascites. As the vasodilating effect of both NO and CO is mediated, at least in part, by the same mechanisms,[12] the effect of CrMP was also evaluated after NOS inhibition with N⁵-nitro-L-arginine-methyl-ester (L-NAME). The expression of HO and NOS isoforms was also evaluated, both in the main trunk of the mesenteric artery and in the small resistance mesenteric arteries.

MATERIALS AND METHODS

The study was performed on 61 adult male Wistar-Kyoto rats (Charles River, Calco, Italia); body weight 200-225 g. Cirrhosis was induced with the CCl₄ inhalation method in 35 rats drinking phenobarbital (0.30 g/L in the drinking water) following a method described elsewhere.[13] Treatment was followed for 10-16 weeks, and animals were free of phenobarbital for the last week before the experiment.[14] The protocols were approved by the Institutional Animal Care and Use Committee. Under anesthesia with ketamine hydrochloride (100 mg/kg body wt intramuscularly), a midventral laparotomy was performed and a section of small intestine was removed. The presence of ascites was confirmed by visual examination at laparotomy. After laparotomy, cirrhotic rats were classified as cirrhosis with or without ascites. The rats were then killed with an over dosage of ketamine. Age-matched animals were used as untreated controls. Two protocols were implemented.

Protocol 1: Evaluation of small mesenteric arteries response to phenylephrine in CCl₄ cirrhotic rats.

Isolated Microvessel Preparation.
The clamped section of the small intestine was placed in a chilled oxygenated modified Krebs bicarbonate buffer (polysaline solution: PSS) containing 118.5 mM NaCl, 4.7 mM KCl, 1.2 mM KH₂PO₄, 2.8 mM CaCl₂, 25 mM NaHCO₃ and 11 mM dextrose. Third/fourth-order branches of the superior mesenteric artery (170-350 µm in diameter, 1-2 mm in length), were isolated from surrounding perivascular tissue, removed from the mesenteric vascular bed and mounted on glass micropipettes in a water-jacked perfusion chamber (Living Systems Instrumentation, Burlington, VT, USA) in warmed (37°C), oxygenated (95% O₂ and 5% CO₂) PSS. The vessels were mounted on a proximal micropipette connected to a pressure servo controller.[15] Subsequently, the lumen of the vessel was flushed to remove residual blood and the end of the vessel was mounted on a micropipette connected to a three-way stopcock. After the stopcock was closed, the intraluminal pressure was allowed to increase slowly until it reached 80 mmHg. The vessel was superfused with PSS (4 ml/min) at 37°C gassed with 95% O₂ and 5% CO₂ for a 45 min period of equilibration.[15] Intraluminal pressure was maintained at 80 mmHg during the experiment. After the equilibration period, the vessels were challenged with a submaximal dose of PE, an alpha₁-adrenoreceptor agonist (10⁻⁶ M). An artery was considered unacceptable for experimentation if it demonstrated leaks or failed to constrict to >20% to PE. The presence of a functional endothelium was determined on the basis of relaxation evoked by acetylcholine (10⁻⁶ M), in the vessel precontracted with PE (10⁻⁶ M). Arteries with less than 60% relaxation of PE induced contractions were discarded.

The effect of increasing doses of PE was evaluated as changes in internal diameter of the vessel.

Evaluation of hemodynamic effect of the inhibition of HO and/or NOS on the response to phenylephrine of small mesenteric arteries.

Responses to PE (10⁻⁸ to 10⁻⁴ M) were determined in PSS containing vehicles for the inhibitors tested. Inhibitors were added to freshly prepared PSS, and 20 to 30-min drug-tissue contact time was allowed before retesting the response to the agonist PE in the same vessel. PE was added to the bath (extraluminal application), and cumulative dose-response curves were generated, with 2 to 3 min between doses. After each dose-response test, the tissues were washed with fresh PSS for at least 20 min. Vascular diameters were measured 1 to 3 min after the addition of PE to the bath with the use of a video system composed of a microscope with a CCD television camera (Eclipse TS100-F, Nikon, Tokyo, Japan), a television monitor (Ultrak Inc., Lewisville, Tx, USA) and a video measuring system (Systems Instrumentation, Burlington, VT, USA). Both in control and in cirrhotic rats (ascitic and nonascitic), dose-response curves to PE were evaluated: a) before and after 20 min superfusion with the HO inhibitor CrMP (15 µM); b) before and after 30 min superfusion with the NOS inhibitor L-NAME (1 mM); c) after a further 20 min CrMP (15 µM) plus L-NAME (1mM) superfusion in rats already evaluated after L-NAME superfusion alone. In each artery only one experiment was performed.

Chemicals

CrMP was obtained from Porphyrin Products (Logan, UT, USA). All other chemicals were obtained from Sigma Chemical (Saint Louis, MO, USA). PE and L-NAME were dissolved in deionized water and diluted with PSS. CrMP was dissolved in a solution of 50 mM NaCO₃.
Protocol 2: Western blot analysis of HO-1, HO-2, endothelial NOS (eNOS) and inducible NOS (iNOS) protein expression in mesenteric arteries of CCl₄ cirrhotic rats.

Standard techniques were used to evaluate protein expression. After removal of veins and adipose tissue, small mesenteric arteries (30-40 arteries with diameter <500µm) and the main trunk of the mesenteric artery were separately collected for every rat after residual blood had been removed, snap frozen in liquid N₂ and stored at -80°C until analyzed. The vessels were homogenized in urea lysis buffer. Protein extracts were assayed for protein content using the BCA protein assay kit (Pierce, Rockford, IL, USA). Sodium dodecyl sulphate - polyacrilammide gel elettrophoresis (SDS-PAGE) and immunoblotting were performed on 50µg of total protein extracts. HO-1, HO-2, iNOS and eNOS protein expression were detected using polyclonal rabbit anti - HO-2, iNOS and eNOS antibodies (StressGen Biotechnologies Corp., Victoria, BC, Canada) and a monoclonal murine antibody against HO-1 (StressGen Biotechnologies Corp., Victoria, BC, Canada). The secondary antibodies, anti-rabbit and anti-mouse conjugated to horseradish peroxidase, respectively, were diluted 1:1,000 in Phosphate-buffered saline containing 2% nonfat dry milk. Antigenic detection was visualized by standard ECL-enhanced chemiluminescence (Amersham, Arlington Heights, IL, USA) with exposure to X-ray film. Recombinant soluble protein rat Hsp-32 (StressGen Biotechnologies Corp., Victoria, BC, Canada) was used as the positive control for HO-1, recombinant human HO-2 (StressGen Biotechnologies Corp., Victoria, BC, Canada) was used as the positive control for HO-2, synthetic peptide corresponding to amino acids 1131-1144 of mouse macrophage NOS (US Biological, Swampscott, MA, USA) was used as the positive control for iNOS, and synthetic peptide corresponding to amino acids 596-610 of human eNOS (Chemicon International, Temecula, CA, USA) was used as the positive control for eNOS. Protein expression was determined by densitometric analysis using the VersaDoc Imaging System (Bio-Rad Laboratories, Hercules, CA, USA). After stripping, the blots were assayed for β-actin content as standardization of sample loading. The quantitative densitometric values of each protein of interest were normalized to β-actin and displayed in histograms.

Data Analysis

Data are expressed as mean±SE. All responses were measured as percentage of contraction (i.e., reduction in vessel diameter relative to baseline diameter before addition of agonist or antagonist). Concentration-response data derived from each vessel were fitted separately to a logistic function by non linear regression and EC(50) (molar concentration of PE causing 50% contraction) was calculated and expressed as -log [M]. A two-way ANOVA was used to compare dose-response curves between controls and treated groups. Other data were analyzed by a one-way ANOVA or Student’s t test for paired or unpaired observations when appropriate. The null hypothesis was rejected at p<0.05.

RESULTS

All rats treated with CCl₄ included in the study had macronodular or micronodular cirrhosis. In 17 out of 35 cirrhotic rats the presence of ascites was confirmed by visual examination at laparotomy. Control rats had no appreciable alteration in liver appearance. Duration of treatment (CCl₄-inhalation) was 12±1 wk in rats without ascites and 15±1 wk in ascitic rats. At the time of the study no difference in body weight between cirrhotic (nonascitic rats: 549±14 g, ascitic rats: 532±14 g) and control rats (539±17 g) was observed.

Protocol 1: Hemodynamic study.
Baseline results:

Mesenteric vascular response to PE was blunted in cirrhotic rats, both in ascitic and in nonascitic animals (p<0.001, two-way ANOVA) (Fig. 1). The response of the ascitic group was not significantly lower than that of nonascitic animals. EC(50) was 6.14±0.12 –log[M] in controls (n. 26) vs 5.49±0.11 –log[M] in cirrhotics (n. 35), p<0.001. Analyzing separately ascitic (n. 17) and nonascitic (n. 18) rats, EC(50) was lower in controls rats both in respect of nonascitic rats (5.62±0.18 –log[M], p=0.014), and of ascitic rats (5.35±0.14 –log[M], p<0.001). Among cirrhotic rats, EC(50) was not different between ascitic and non ascitic rats (p: NS).

Effect of CrMP:

CrMP did not modify the dose-response curve to PE in control rats (n. 9) (p: NS, two-way ANOVA); EC(50) to PE was 6.17±0.17 –log[M] before and 6.17±0.12 –log[M] after CrMP (p: NS).

On the contrary, a significant decrease in EC(50) was evident in cirrhotic rats after CrMP (n. 14): from 5.71±0.09 –log[M] to 6.03±0.12 –log[M], p=0.010. However, analyzing separately ascitic (n. 6) and nonascitic (n. 8) rats, we found that CrMP produced a leftward displacement in the concentration-response curve to PE only in ascitic rats (p<0.001, two-way ANOVA) (Fig. 2). EC(50) to PE changed from 5.75±0.22 –log[M] to 5.96±0.18 –log[M] (p: NS) in cirrhotic rats without ascites, while it decreased significantly from 5.64±0.13 –log[M] to 6.12±0.18 –log[M] (p=0.010) in rats with cirrhosis and ascites.

Effect of L-NAME:

L-NAME caused an increase in the vascular response to PE both in control rats (n. 14) and in cirrhotic rats, with (n. 7) or without (n. 6) ascites, as demonstrated by the leftward shift of the dose-response curves (p<0.001, p=0.002, p<0.001, respectively, two-way ANOVA) (Fig. 3) and by the significant decrease in EC(50): from 6.10±0.18 –log[M] to 6.47±0.17 –log[M] (p=0.010) in control rats; from 5.68±0.22 –log[M] to 6.45±0.18 –log[M] (p=0.009) in nonascitic rats and from 5.45±0.15 –log[M] to 5.83±0.19 –log[M] (p=0.008) in ascitic rats. However, after L-NAME, EC(50) to PE was similar in controls and in nonascitic cirrhotic rats (p: NS), while in ascitic cirrhotic rats it remained significantly higher in respect both of controls (p=0.030) and of nonascitic cirrhotic rats (p=0.027).

Effect of the addition of CrMP to L-NAME:

In control rats (n. 5) and in rats with cirrhosis without ascites (n. 6), the addition of CrMP to L-NAME did not modify the dose-response curve to PE obtained after L-NAME administration (p: N.S., two-way ANOVA). EC(50) changed from 6.32±0.15 –log[M] to 6.33±0.16 –log[M] (p: NS) in control rats and from 6.27±0.16 –log[M] to 6.32±0.13 –log[M] (p: NS) in cirrhotic rats without ascites. On the contrary, in rats with cirrhosis and ascites (n. 6) the addition of CrMP to L-NAME caused a significant leftward shift of the dose-response curve to PE in respect of the curve obtained after L-NAME alone (p<0.01 in respect of the L-NAME curve; p<0.001 in respect of the baseline curve; two-way ANOVA) (Fig. 4). In ascitic rats, the EC(50) decreased from 5.65±0.21 –log[M] after L-NAME to 6.32±0.21 –log[M] after L-NAME+CrMP (p=0.05). After inhibiting both NOS and HO, vascular response to PE was similar in control and in cirrhotic rats, both with and without ascites (p: NS).

Protocol 2: Western Blot analysis.
In mesenteric arteries of control rats, eNOS and HO-2 protein expression were evident, while iNOS protein expression was absent and that of HO-1 was low. eNOS protein expression was greater in the small mesenteric arteries in respect of the main trunk of the artery (p=0.006), while HO-2 protein was equally expressed in the main trunk of the artery and in the small vessels (Fig. 5,6). In cirrhosis without ascites, there was an increase in protein expression of eNOS (particularly in the main trunk of the mesenteric artery) and of HO-1 (particularly in the small vessels) (p=0.039). In rats with cirrhosis and ascites, HO-1 protein expression further increased in respect of rats without ascites in the main trunk of the artery, while, surprisingly, eNOS protein expression decreased in respect of cirrhotic rats without ascites (Fig. 5,6).

In cirrhosis, a very weak iNOS expression was detected in only one sample out of six nonascitic rats and in only one sample out of five rats with ascites (Fig. 6). HO-2 protein expression was not modified in cirrhosis (Fig. 6).

**DISCUSSION**

This study provides information on the influence of HO on small mesenteric arteries response to PE in experimental cirrhosis. The salient conclusion derived from the study is that an overexpression of HO participates in the decreased mesenteric response to PE only in the advanced stage of the disease, while NOS overexpression mainly participates in the first stage of the disease.

Vascular resistance is more dependent on small rather than large vessels [16] and there is evidence that small and large arteries have different physiological regulatory systems.[16] Low splanchic vascular resistance observed in portal hypertension depends mostly on mesenteric resistance arteries, which are pre-capillary resistance arteries with diameters less than 500 µm.[16] Therefore, we explored vascular response and protein expression directly in small resistance mesenteric arteries of less than 500 µm. The no-flow model was chosen to avoid interference by the shear stress phenomenon.

Small resistance mesenteric arteries of cirrhotic rats were hyporesponsive to PE. This result is in keeping with previous studies, which have demonstrated a blunted pressure response of perfused superior mesenteric arterial bed to KCl in cirrhotic rats with ascites [14] and to methoxamine in portal hypertensive rats [17], and an impaired response to PE and KCl in mesenteric resistance arterial rings from portal hypertensive rats.[18] Hyporesponsiveness was present both in ascitic and nonascitic rats, highlighting that there is a decreased response of the mesenteric artery to vasoconstrictors from the first stage of the disease.

To evaluate the role of HO on mesenteric response to PE, we analyzed the effect of CrMP, a potent non selective HO inhibitor with no significant effect on NOS activity.[19] HO inhibition did not modify the vascular response to PE in control rats, in keeping with the lack of effect of HO-inhibitors on perfusion pressure of mesenteric arterial bed of controls rats.[9] On the contrary, HO inhibition improved vascular response in cirrhotic rats, as already suggested by Fernandez et al.,[11] who studied portal hypertensive rats, and by Sacerdoti et al.,[9] who studied cirrhotic rats, both using the McGregor preparation.[10] But in our study, the improvement was evident only in ascitic rats. A higher HO-1 protein expression was evident in cirrhotic rats, particularly in those with ascites.

NOS inhibition caused an increase in the mesenteric response to PE both in control rats and in cirrhotic rats, according to the study of Sieber et al.[14] who verified the pressure response to KCl in the perfused mesenteric arterial bed. However, it is of particular interest that in our study L-NAME completely reversed mesenteric hyporesponsiveness to PE in compensated cirrhosis, but was not so effective in ascitic cirrhosis. Similar results were recently obtained in our laboratory analyzing the splanchic haemodynamics of cirrhotic rats in vivo by a perivascular ultrasonic flow probe applied to the main trunk of the mesenteric artery [20]. In this paper, L-NAME decreased blood flow and increased resistance in the superior mesenteric artery in cirrhotic rats, but the effect
was much less intense in rats with ascites. These data suggest that NOS activation is the main factor responsible for mesenteric vasodilation in the first stage, but it is not the only factor in the advanced stage of the disease. This hypothesis is in agreement with the findings of Forrest et al.,[21] who reported that L-NAME improved heart rate and systemic arterial pressure in compensated but not in decompensated cirrhotic patients. These authors hypothesized that in the advanced stage of the disease, NO plays a minor role in the pathogenesis of hyperdynamic circulation, overcome by other vasoactive systems. Our analysis of NOS protein expression supports this interpretation. Indeed, eNOS protein expression in small mesenteric arteries was increased in cirrhosis without ascites, according to other studies that analyzed eNOS protein expression in the superior mesenteric artery vascular bed of portal hypertensive rats [22][23] and in the proximal 1 cm of the main trunk of the same artery of cirrhotic rats, both with and without ascites.[24]

But, surprisingly, in the advanced stage of the disease (ascitic phase) mesenteric eNOS protein expression was not increased, according to the studies of Morales-Ruiz et al.[25] who analyzed the mesenteric arterial bed of cirrhotic rats with ascites.

Mesenteric iNOS expression was absent in control rats and almost negligible in cirrhotic rats. Therefore, a very low expression of iNOS could not be excluded in cirrhotic animals, even though such a low levels are probably not significant.

Considering that: a) CO and NO cause smooth muscle cells relaxation interplaying on the same mechanisms, and b) NO seems to be primarily responsible for mesenteric hyporesponsiveness to vasoconstrictors in cirrhosis, we also decided to evaluate the effect of CrMP in rats previously treated with L-NAME. In control rats and in cirrhotic rats without ascites, the addition of CrMP to L-NAME did not further increase vascular response in respect of the effect of L-NAME alone, while in cirrhotic rats with ascites HO inhibition was effective in improving mesenteric response to PE. By inhibiting both NOS and HO, mesenteric response to PE was similar in control rats and in cirrhotic rats, with and without ascites. Therefore, in the advanced stage of experimental cirrhosis, an increased expression of HO and production of CO could participate in the maintaining and worsening of mesenteric vasodilation. This hypothesis is indirectly supported by the finding of an increased CO concentration in the exhaled air and blood carboxyhemoglobin (COHb) reported in human cirrhosis, particularly in patients with ascites.[26]

The analysis of HO and NOS protein expression provided some interesting data. First of all, the contemporary analysis of the constitutive and inducible forms of the two enzymes in the two different stages of the evolution of cirrhosis (nonascitic and ascitic), allowed us to show that the expression varies with the progression of the disease. In particular, in the ascitic phase of the disease, a higher expression of HO-1 and a lower expression of eNOS were evident. Secondly, analyzing protein expression separately in the small resistance branches and in the main trunk of the mesenteric artery, we were able to discover that HO and NOS expression is different in the two districts. This result emphasizes the importance of analyzing selectively the small resistance arteries when the aim is to evaluate the regulation of mesenteric resistance. An increased expression of HO-1 has also been reported in mesenteric arteries of rats with pre-hepatic portal hypertension [7] and with common bile duct ligation.[8] On the contrary, only an increased expression of HO-2 has been reported in the mesentery of Sprague-Dawley rats with CCl₄ cirrhosis by Sacerdoti et al.[9] The different result may be explained by the different site of protein expression analysis and by the different stage of cirrhosis. The different experimental model (Sprague-Dawley instead of Wistar-Kyoto rats) may also have played a role.

The different expression of eNOS and HO-1 in mesenteric vessels of rats with and without ascites may be explained by the relationships existing between the two systems. Indeed, NO is known to induce the expression of HO-1 [12] [27][28][29] leading to the formation of endogenous CO.[27] [29] Increased levels of HO, in turn, have been shown to decrease NO concentration.[3] The mechanisms by which the induction of HO-1 impair local NO generation have been identified as follows: competitive consumption of NADPH between the two enzyme systems, degradation of the prosthetic heme required for assembly of NOS, and CO binding to the NOS heme.[30][31]
Hence, a role for HO/CO system in the mesenteric hyporesponsiveness to PE in experimental cirrhosis can be hypothesized. In the first stage of the disease, an increase in eNOS expression has been demonstrated,[24] responsible for the early mesenteric hyporesponsiveness to vasoconstrictors. The chronic increase in NO level might induce HO-1 expression, together with other mechanisms, such as the high level of oxidative stress, glucagon, and angiotensin II.[28] [32] An increasing role of HO/CO system may therefore become evident in the advanced stage of the disease, and the interfering action of HO on NOS might contribute in shifting the balance towards the HO system. Indeed, activation of HO-1 may lead to a deficiency in intracellular heme required as a coenzyme for NOS.[30]

Further studies are necessary to confirm our results. Indeed, the pathophysiological significance of our findings in isolated vessels will receive added value if confirmed by measuring CO levels in mesenteric circulation and by in vivo experiments assessing the effect of CrMP in the mesenteric circulation of cirrhotic rats with ascites.

In conclusion, HO plays a role in the mesenteric hyporesponsiveness of cirrhotic rats. In early stages of cirrhosis, the NO/NOS system plays a major role in splanchnic vasodilation, whereas in the late stages HO-1 derived CO seems to mediate further aggravation.

The Authors declare no competing interests.

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Figure legends

Figure 1. Dose-response curves to phenylephrine (PE) of small resistance mesenteric arteries. *= significantly different (p<0.01) from the other two curves.

Figure 2. Effect of heme oxygenase inhibition with chromium mesoporphyrin (CrMP) on mesenteric vascular response to phenylephrine (PE). Panel A: Control rats (n. 9). Panel B: cirrhotic rats without ascites (n. 8). Panel C: cirrhotic rats with ascites (n. 6).

Figure 3. Effect of nitric oxide synthase inhibition with N\textsuperscript{G}-nitro-L-arginine-methyl-ester (L-NAME) on mesenteric vascular response to phenylephrine (PE). Panel A: Control rats (n. 14). Panel B: cirrhotic rats without ascites (n. 6). Panel C: cirrhotic rats with ascites (n. 7).

Figure 4. Effect of heme oxygenase inhibition with chromium mesoporphyrin (CrMP) on mesenteric vascular response to phenylephrine (PE), in arteries treated with nitric oxide synthase inhibition with N\textsuperscript{G}-nitro-L-arginine-methyl-ester (L-NAME). Panel A: Control rats (n. 5). Panel B: cirrhotic rats without ascites (n. 6). Panel C: cirrhotic rats with ascites (n. 6), *= significantly higher (p<0.01) in respect of baseline and of L-NAME alone.

Figure 5. Western blot analysis of HO-1, HO-2, iNOS and eNOS in the main trunk of the mesenteric artery and in the small resistance mesenteric vessels of controls rats and of rats with and without ascites. The reported blots are representative of 4 to 7 experiments. Examples of β-actin expression, analyzed as index of the adequacy of sample loading, are also displayed.

Figure 6. Quantitative densitometric evaluation, normalized to β-actin, of iNOS, eNOS, HO-1 e HO-2 in the main trunk of the mesenteric artery and in the small resistance mesenteric vessels of control rats and of rats with and without ascites. White columns: protein expression in the main trunk of the mesenteric artery; black columns: protein expression in the small resistance mesenteric arteries.

Results shown are from 4-7 experiments. *= p=0.039 in respect of control rats; **= p=0.027 in respect of control rats; #= p=0.007 in respect of control rats; §= p=0.006 in respect of the main trunk of the artery.
REFERENCES


Contraction (%) vs. PE (log[M])

- Control rats
- Cirrhotic rats without ascites
- Cirrhotic rats with ascites

p<0.001 two-way ANOVA

* Indicate significant differences.
Cirrhotic rats with ascites  Nonascitic cirrhotic rats  Control rats  Positive Controls
small main vessels trunk small main vessels trunk small main vessels trunk

HO-1
HO-2
β-actin

Control rats:
Nonascitic cirrhotic rats:
Cirrhotic rats with ascites:

Positive Controls:

42 kDa
30 kDa
34.4 kDa

eNOS iNOS β-actin

140 kDa
130 kDa
42 kDa