LIVER DISEASE

Hepatic venous dysregulation contributes to blood volume pooling in cirrhotic rats

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Background and aims: In cirrhosis, despite increased total blood volume, the circulation behaves as if it were volume depleted, a phenomenon termed "decreased effective circulating volume". As the gut/liver veins are the major blood reservoir, this suggests hepatosplanchnic venous pooling. We therefore aimed to elucidate the vasoactive responses of the hepatic veins in cirrhosis.

Methods: Cirrhosis was induced by chronic bile duct ligation in rats. The *in vivo* responses of postsinusoidal venules and sinusoids to vasoactive drugs, 20% haemorrhage, and 20% mannitol (volume expansion) were examined by intravital microscopy. In isolated perfused livers, change in liver weight was measured as an index of the hepatic vascular volume response.

Results: Blood volume was significantly increased in cirrhotic rats. In the cirrhotic hepatic vasculature, constrictive responses to norepinephrine and haemorrhage were blunted compared with controls. In contrast, the dilatory responses to the nitric oxide (NO) donor sodium nitroprusside and volume expansion were enhanced. Both constrictive and dilatory abnormalities were reversed by the NO synthase inhibitor N-nitro-L-arginine methyl ester.

Conclusions: The hepatic sinusoidal and venous bed of cirrhotic rats showed an enhanced dilatory capacity to buffer volume increases but inadequately constricted in response to volume depletion or catecholamines. Both abnormalities may contribute to volume pooling and are mediated by NO.

In patients with cirrhosis, despite augmentation of the plasma and total blood volume, the circulation behaves as if it were volume depleted, a concept termed "decreased effective circulating volume". Most proponents of this concept believe that peripheral arterial vasodilatation is predominantly responsible for this effective hypovolaemia. However, most of the circulating blood/fluid volume is physically in the veins, which are the capacitance vessels of the circulation. Therefore, decreased effective volume implies some degree of venous blood pooling.

Nitric oxide (NO) has been proposed as a primary mediator of peripheral vasodilation. There is evidence that overproduction of NO in the cirrhotic peripheral vasculature not only directly dilates the vessels but also contributes to impaired responsiveness to vasoconstrictors.^{1–4} In contrast, NO activity in the cirrhotic liver appears to be decreased, resulting in hyperresponsiveness of the hepatic vasculature to vasoconstrictor influences.^{5,6}

The splanchnic venous bed, comprised of the hepatic and mesenteric veins, is the major blood volume reservoir in the body. Ordinarily, the splanchnic veins can compensate for blood volume loss by mobilising its reserve.⁷ Therefore, effective hypovolaemia suggests a dysregulation or insufficient mobilisation of the splanchnic venous blood reservoir. As the liver is the largest splanchnic organ, with a large venous reservoir, we felt that any study of the splanchnic venous bed should first focus on the liver. Therefore, we studied the hepatic venous responses to diverse physiological and pharmacological constrictor and dilator stimuli. Our aim was to examine the blood reservoir function of the liver.

MATERIALS AND METHODS

The protocol was approved by the University of Calgary Faculty of Medicine Animal Care Committee and the experimental procedures were carried out in accordance with guidelines established by the Canadian Council on Animal Care.

Animal preparation

Sprague-Dawley rats weighing 200–250 g were used in the experiments. Cirrhosis was induced by bile duct ligation (BDL), as previously described.⁸ Briefly, under halothane anaesthesia, through a midline laparotomy, the common bile duct was doubly ligated with 4-0 silk thread and sectioned between the ligatures. Incisions were closed with silk, and animals were given an intramuscular injection of benzathine penicillin G (30 000 IU) immediately after operation to prevent sepsis. Control rats (sham) underwent exactly the same surgical procedures except for ligation and section of the bile duct. Animals were then kept for 3–4 weeks by which time a body weight of 300–400 g had been attained and an obvious cirrhosis had developed. Food but not water was withdrawn from the cages 12 hours before assay or surgery.

Determination of plasma and total blood volumes

Plasma volume was measured using Evans blue dye dilution.⁹ Through a jugular vein catheter, 0.2 ml of Evans blue solution (3 mg) was injected and followed by a 0.15 ml flush of physiological saline to clear the dye from the catheter. Five minutes later, 2.0 ml of blood were withdrawn from the femoral artery catheter. Packed cell volume (PCV) was determined in duplicate and the blood sample was centrifuged. A plasma aliquot of 0.2 ml was diluted in distilled water to 2.0 ml and absorbance of the solution was read by a spectrophotometer at a wavelength of 600 nm. Plasma volume was calculated by the formula:

plasma volume (ml) = $A_{\text{standard}}/A_{\text{sample}} \times 10$
where the standard is 3 mg of Evans blue in 10 ml of plasma diluted 10 times.

Abbreviations: BDL, bile duct ligated; L-NAME, N-nitro-L-arginine methyl ester; NE, norepinephrine; NO, nitric oxide; NOS, nitric oxide synthase; SNP, sodium nitroprusside; PCV, packed cell volume; IVC, inferior vena cava

Total blood volume was determined from the equation:
 $\text{blood volume (ml)} = \text{plasma volume} \times 100/100 - (\text{PCV} \times 0.95)$
 where 0.95 is the correction factor to obtain true cell percentage (total body PCV).

Liver weight responses to vasoactive drugs

A change in liver weight was used as an index for change in liver volume in response to the catecholamine vasoconstrictor norepinephrine (NE) or the NO donor vasodilator sodium nitroprusside (SNP). When the hepatic vasculature constricts, it squeezes more volume out of the liver and weight decreases; conversely, if the vasculature dilates, the liver will accommodate more volume and weight increases. Although this reasoning appears logical, the validity of this approach to indirectly estimate hepatic vascular volume changes was first confirmed by a series of pilot studies showing reproducibility of the responses and the expected response to several vasoconstrictor and vasodilator drugs.

Surgical procedure

An isolated perfused liver preparation was used in the experiment, based on methods described previously,¹⁰ with modifications. BDL or sham rats were anaesthetised with sodium pentobarbitone (50 mg/kg intraperitoneally). A midline incision was made and the liver was exposed. The left phrenic and gastric and right adrenal veins were ligated. In sham rats, the common bile duct was also ligated. After the animal was injected intravenously with heparinised saline, the portal vein was cannulated with PE-90 polyethylene catheter, and the hepatic artery was ligated at the same time. After ligating the infrahepatic inferior vena cava (IVC), the liver was flushed with 15 ml of Krebs-Bulbring buffer. Then the suprahepatic IVC was cannulated with PE-240 polyethylene catheter and this was used as the outlet of the perfusion. The liver was excised and placed into a container containing Krebs-Bulbring buffer that was kept at 37°C with an electronic aquarium heater (Tronic, Montreal, Canada). The container sat on a balance, and the change in liver weight was directly read from the balance.

Liver perfusion

The isolated liver was perfused with Krebs-Bulbring buffer via the portal vein at a constant flow rate of 16 ml/min through a peristaltic pump (Harvard Apparatus, Boston, USA). Krebs-Bulbring buffer (pH 7.4, 37°C) contained (mM): NaCl 133; KCl 4.7; NaH₂PO₄ 1.35; NaHCO₃ 20.0; MgSO₄ 0.61; glucose 7.8; and CaCl₂ 2.52, and was oxygenated with a 95% O₂/5% CO₂ mixture. The outflow was through an open suprahepatic IVC cannulation. Both cannulae in the portal vein and suprahepatic IVC were fixed by a stand to avoid movement during drug injection. After a 20 minute steady state period, the drugs were administered.

Drug administration

NE bitartrate (Sigma, St Louis, Missouri, USA) was freshly dissolved in 0.1 mM of ascorbic acid and diluted with Krebs-Bulbring buffer to a dose range of 10⁻⁹–10⁻⁷ M. SNP (Sigma) was freshly dissolved in Krebs-Bulbring buffer and diluted to a dose range of 10⁻⁵–10⁻³ M and wrapped in aluminium foil to avoid light. Each freshly prepared NE or SNP was used once only on the experimental day. Drugs were administered as a 1 ml bolus injection over one minute through the portal vein via a three way stopper, at 15 minute intervals between doses. Duration of each injection was one minute, and liver weight was immediately read when the injection was finished.

Intravital microscopy

Surgical procedure

Hepatic vascular microcirculation was observed using intravital microscopy, as previously reported,¹¹ with modifications. Animals were anaesthetised with sodium pentobarbitone (50 mg/kg intraperitoneally) and placed in the supine position on a heating pad with rectal temperature maintained at 37°C. The right jugular vein was cannulated with PE-50 polyethylene catheter for drug administration and anaesthesia maintenance. In the haemorrhage experiment, a PE-50 polyethylene catheter was inserted into the left femoral artery and served as the route of blood withdrawal. Both catheters were filled with heparinised saline. After that, a midline incision was made and the liver was exposed. The skin and muscles were removed close to the costal margin, and the hepatoforn ligament was released. Rats were positioned on their left side and the left lobe of the liver was gently placed onto an adjustable stage. By doing so, the lower surface of the liver was situated horizontal to the microscope. The use of an adjustable stage avoided mechanical obstruction of microvessels and minimised respiratory movement of the lobe. The exposed area of the left liver lobe was immediately covered with a glass coverslide and continually superfused with 37°C normal saline solution to avoid temperature fluctuation and drying of the tissue by ambient air. All other exposed areas were covered with saline soaked cotton gauze.

Measurement of vessel diameters

The diameter changes of postsinusoidal venules and sinusoids in response to stimuli were observed using a Mikron IV500L intravital microscope system (Mikron, San Marcos, California, USA) with a 100 W mercury lamp (FluoArc for N HBO103, Carl Zeiss, Germany) attached to a fluorescence illuminator equipped with Blue filter blocks (excitation 450–490 nm; emission >520 nm; Carl Zeiss). With the use of a 20×/0.50 W objective lens (Achromplan, water immersion; Zeiss, Don Mills, Ontario, Canada) and a ×10 eyepiece, the image captured by a Pieper charge coupled device video camera (MTI VE1000SIT, Michigan City, Indiana, USA) was displayed on a video monitor and transferred to a videocassette recorder for offline evaluation. Contrast enhancement for visualisation of the hepatic microvasculature was achieved by intravenous injection of 5% fluorescein isothiocyanate labelled dextran (molecular weight 150 000; 0.1 ml/100 g body weight; Sigma). A separate postsinusoidal venule and 8–10 draining sinusoids were located. Changes in the diameters of postsinusoidal venules and sinusoids in response to stimuli were calculated by the difference between pre- and post-stimuli. For each animal, three sinusoids were observed and the average was used for the results. After a 20 minute steady state, the experiments were started.

Figure 1 Effects of norepinephrine (NE) on liver weight in bile duct ligated (BDL) or sham operated rats. Data are expressed as mean (SEM) of six animals. **p* < 0.05 compared with the corresponding sham groups.

Figure 2 Effects of sodium nitroprusside (SNP) on liver weight in bile duct ligated (BDL) or sham operated rats. Data are expressed as mean (SEM) of six animals. * $p < 0.05$ compared with the corresponding sham groups.

Protocol design

NE (10^{-10} – 10^{-8} M), SNP (10^{-6} – 10^{-4} M), or the β_2 adrenergic agonist terbutaline (10^{-8} – 10^{-6} M) were given as a bolus injection through the jugular vein in a volume of 0.1 ml/100 g body weight. Acute haemorrhage was produced by withdrawing 12 ml/kg body weight (approximately 20% of the blood volume) through a motorised withdrawal pump at a rate of 2 ml/min. Acute volume expansion was achieved by an intravenous infusion of mannitol 20% solution at a dose of 1 ml/100 g body weight. The role of NO in mediating the hepatic vascular response to acute haemorrhage or mannitol were evaluated using the NO synthase inhibitor N-nitro-L-arginine methyl ester (L-NAME), given as 15 mg/kg intravenous bolus administration 20 minutes before the challenges.

Statistical analysis

Data are presented as mean (SEM). Differences between groups were analysed by one way analysis of variance followed by post hoc comparisons with Bonferroni's correction. Statistical significance was set at $p < 0.05$.

RESULTS

Plasma and total blood volumes

A significant 21% increase in plasma volume (BDL 4.59 (0.16) ml/100 g *v* sham 3.78 (0.21) ml/100 g) and 16% elevation in total blood volume (BDL 7.79 (0.26) ml/100 g *v* sham 6.69 (0.33) ml/100 g) were observed in BDL animals. There was no significant difference in packed cell volume (BDL 43.2 (0.9)% *v* sham 45.7 (1.2)%).

Effects of vasoactive drugs on liver weight

Baseline liver weights were 12.67 (0.47) g in sham controls and 21.79 (0.79) g in BDL rats. NE (10^{-9} – 10^{-7} M) dose dependently decreased liver weight in sham and BDL rats but the response in the latter group was attenuated (fig 1). In contrast, liver weight increased after injection of SNP (10^{-5} – 10^{-3} M) in sham and BDL animals in a dose dependent manner. The responses in BDL rats were all significantly enhanced compared with sham controls (fig 2).

Effects of vasoactive drugs

Figure 3 shows the basal diameters of postsinusoidal venules (fig 3A) and sinusoids (fig 3B) in sham and BDL groups prior to any stimuli. Both sites in the BDL rats were significantly dilated compared with sham controls.

NE (10^{-10} – 10^{-8} M) constricted the postsinusoidal venules and sinusoids dose dependently in both BDL and sham animals. However, in BDL rats, a significant blunting of constrictive responses was evident (fig 4A, B).

Figure 3 Basal diameters in bile duct ligated (BDL) and sham operated rats. Data are expressed as mean (SEM) of six animals. * $p < 0.05$ compared with the corresponding sham groups.

Figure 4 Effect of norepinephrine (NE) on diameters of the postsinusoidal venules (A) and sinusoids (B) in bile duct ligated (BDL) and sham operated rats. Data are expressed as mean (SEM) of six animals. * $p < 0.05$, ** $p < 0.01$ compared with the corresponding sham groups.

Treatment with incremental doses (10^{-7} – 10^{-5} M) of SNP dose dependently dilated the postsinusoidal venules and sinusoids in sham and BDL rats. However, vasodilatory responses were significantly more pronounced in cirrhotic rats (fig 5A, B).

To determine if the enhanced venodilation to SNP was specific to this NO generating drug, or a reflection of a

Figure 5 Effect of sodium nitroprusside (SNP) on diameters of postsinusoidal venules (A) and sinusoids (B) in bile duct ligated (BDL) and sham operated rats. Data are expressed as mean (SEM) of six animals. * $p < 0.05$, ** $p < 0.01$ compared with the corresponding sham groups.

Figure 6 Effect of the β_2 adrenoceptor agonist terbutaline on diameters of the postsinusoidal venules (A) and sinusoids (B) in bile duct ligated (BDL) and sham operated rats. Data are expressed as mean (SEM) of six animals. * $p < 0.05$, ** $p < 0.01$ compared with the corresponding sham groups.

generalised enhancement to all vasodilators, the β_2 adrenergic agonist terbutaline (10^{-8} – 10^{-6} M) was used. This drug dilated postsinusoidal venules and sinusoids in both BDL and sham rats, but responses in the former group were attenuated (fig 6A, 6B).

Effects of volume manipulation with or without L-NAME

Postsinusoidal venules and sinusoids constricted in response to a 20% haemorrhage in both sham and BDL animals. However, the constrictive responses were blunted in BDL rats compared with sham controls (fig 7A, 7B). In contrast, both sites dilated to a mannitol induced volume expansion in sham and BDL rats, with a more pronounced response in the latter group (fig 8A, B).

For the L-NAME experiments, equivolumic normal saline was used as a control. Normal saline did not affect hepatic vascular diameters 20 minutes after its administration, either in the haemorrhage or volume expansion experiments. L-NAME treatment did not materially affect hepatic vascular responses in sham controls. Specifically, L-NAME did not affect basal diameters or response to volume challenge in sham controls in either the haemorrhage or mannitol experiments (fig 7A, B; fig 8A, B).

However, L-NAME administration reduced basal diameters of sinusoids and venules in BDL rats in both the haemorrhage and mannitol experiments (fig 7A, B; fig 8A, B). In the haemorrhage experiment, at this new baseline, postsinusoidal venules and sinusoids in BDL rats significantly constricted with haemorrhage challenge (fig 7A, 7B). In other words, L-NAME pretreatment restored the constrictive responsiveness to haemorrhage in cirrhotic rats.

Postsinusoidal venules and sinusoids in BDL rats dilated following mannitol injection, and these responses were accentuated compared with sham controls. L-NAME treatment constricted the venules and sinusoids in cirrhotic rats

and also reversed the enhanced dilatory responsiveness to mannitol (fig 8A, B).

DISCUSSION

We found abnormal responses of the hepatic postsinusoidal venules and sinusoids to vasoactive stimuli in cirrhotic liver, manifesting as a blunted reaction to constrictive adrenergic drugs/stimuli, and enhanced vasodilation to NO and volume expansion.

In cirrhosis, because of the intense mesenteric vasodilatation, the gut veins may sequester even more blood volume in patients with cirrhosis. Kiszka-Kanowitz *et al*, using a radioisotopic method, showed that 25% of total blood volume was sequestered in the splanchnic vessels of cirrhotic patients compared with 18% in healthy controls.¹² Although we did not directly quantify the extent of such sequestration in our cirrhotic rats, the increased baseline diameters of both the sinusoidal and postsinusoidal diameters suggest that a similar phenomenon occurs, at least in hepatic veins, in this experimental animal model. These results concur with previous studies that documented basal vasodilatation of the sinusoids and postsinusoidal venules in CCl₄ cirrhotic rats.^{11–13} To our knowledge, the hepatic microvasculature has not previously been examined by intravital microscopy in the BDL cirrhotic rat model. The BDL rat model also shows another of the features of human cirrhosis, expansion of the plasma and total blood volumes. Therefore, we believe that our results can be extrapolated, albeit cautiously, to the human condition of cirrhosis.

The present results suggest that two mechanisms underlie hepatic volume sequestration. Firstly, the capacitance of the liver veins and sinusoids is increased to accommodate the extra volume. Moreover, the dilatory response to accommodate further volume expansion or other dilatory stimuli is enhanced. Secondly, intrahepatic volume cannot be

Figure 7 Effect of N-nitro-L-arginine methyl ester (L-NAME) and acute haemorrhage on diameters of post-sinusoidal venules (A) and sinusoids (B) in sham and bile duct ligated (BDL) rats. Data are expressed as mean (SEM) of six animals. Percentages are values at 40 minutes compared with values at 20 minutes. * $p < 0.05$ compared with 20 minutes in corresponding groups.

adequately mobilised by vasoconstrictive stimuli such as haemorrhage or catecholamines. It is curious that both abnormalities seem to be mediated by NO.

This is surprising given that several previous studies have demonstrated a deficiency of intrahepatic NO, which contributes to enhanced constrictive influences on hepatic stellate cells, leading to portal hypertension. Moreover, Loureiro-Silva *et al* recently demonstrated that the relative deficiency of NO action in the liver, as judged by pharmacological methods, is most pronounced in the sinusoids and post-sinusoidal venules.¹⁴ Shah *et al* found that endothelial nitric oxide synthase (NOS) activity is impaired by caveolin-1 overexpression in vascular endothelial and hepatic stellate cells in the BDL rat liver.¹⁵ How can our results be reconciled with these previous studies? Several points must be noted. Firstly, we only measured vessel diameter, not flow or pressure. It is entirely possible for sinusoidal and post-sinusoidal venous pressures to be elevated despite dilated vessels. Resistance to blood flow through vessels is dependent not only on luminal diameter, but also on flow volumes and vascular architecture (distorted tortuous vessels produce turbulent rather than laminar flow), as well as blood

Figure 8 Effect of N-nitro-L-arginine methyl ester (L-NAME) and mannitol (volume expansion) on diameters of post-sinusoidal venules (A) and sinusoids (B) in sham and bile duct ligated (BDL) rats. Data are expressed as mean (SEM) of six animals. Percentages are values at 40 minutes compared with values at 20 minutes. * $p < 0.05$ compared with 20 minutes in BDL with normal saline group.

viscosity. Secondly, a significant intrahepatic resistance site may be pre-sinusoidal. Finally, a relative or absolute deficiency of intrahepatic NO generation is by no means incontrovertibly demonstrated. Several animal and human studies in cirrhosis show that hepatic NO production is increased. Expression of NOS mRNA and protein in cirrhotic rat livers are increased, and NOS enzymatic activity is elevated.¹⁵⁻¹⁷ Directly measured NO levels are increased in the hepatic vein of patients with advanced cirrhosis,¹⁸ and levels of nitrate/nitrite, the byproducts of NO metabolism, are also increased in the hepatic venous blood of cirrhotic patients.¹⁹ All of these data indicate increased hepatic NO generation.

However, our results showing an enhanced venous response to NO, by both in vitro and in vivo methods, are more consistent with a baseline NO deficiency. In other words, excessive basal NO generation should have led to a blunted effect of exogenous NO administration whereas we observed the opposite. In this respect, our results agree with several previous studies. Bhathal and Grossman²⁰ and Marteau and colleagues²¹ found a more pronounced SNP induced decrease in intrahepatic vascular resistance in

isolated perfused cirrhotic livers compared with control livers. Similarly, Kakumitsu *et al* reported that the NO precursor L-arginine vasorelaxed the portal circulation to a greater extent in cirrhotic patients than healthy controls.²² The mechanisms underlying the increased responsiveness to NO donors in the cirrhotic liver remain unclear. The sensitivity of rat aortic ring segments to SNP is augmented after removal of endothelium, which implies that a deficit of endogenous NO production increases the sensitivity of the vasculature to exogenous NO.²³ Moncada *et al* showed that SNP enhances vasorelaxation and cGMP production in both endothelium denuded and endothelium intact aortic rings treated with NOS inhibitors.²⁴ They suggested that this supersensitivity to SNP following removal or blockade of basal NO release is due to a feedback upregulation of the NO receptor, soluble guanylate cyclase.

We found that acute volume expansion by mannitol produced an accentuated sinusoidal and venous dilation in cirrhotic rats, even though the vessels were already dilated at baseline. Thus the cirrhotic vasculature shows an enhanced volume capacity. If a similar situation is extrapolated to the patient with cirrhosis, this may explain, in part, why simply volume expanding patients is ineffective in augmenting the "effective circulating volume" (that is, generally does not increase perfusion pressure to vital organs such as the kidneys). The extra volume is simply sequestered in the venous reservoirs. Although NO seems to mediate this effect, we also examined another vasodilator, the β_2 adrenergic agonist terbutaline, to determine the specificity of this enhanced dilatory effect in cirrhosis. The blunted dilatory response to terbutaline indicates that the enhanced dilation with SNP and mannitol is not part of a generalised supersensitivity to all dilatory stimuli.

L-NAME did not affect either the basal diameters or dilatory response to mannitol in control rats. This suggests that NO does not play a major role in the tonic vasoregulation of sinusoids and postsinusoidal venules, or in the hepatic response to volume expansion, in the normal hepatic microcirculation.

Normally, venous blood and volume reserve can be mobilised by neurohumoral pathways in case of need, such as volume depletion or haemorrhage. The major neurohumoral mechanism is via sympathetic activation. Thus we tested the response to both haemorrhage and NE. The blunted hepatic microcirculatory responses to both of these constrictive stimuli in our cirrhotic rats indicate a significantly abnormal regulation of the liver blood volume reservoir. In other words, in cirrhosis, not only is there significant hepatic volume pooling, but also an inability to efficiently mobilise this reserve volume when needed. Both of these abnormalities would contribute to blood pooling and ultimately a decreased effective circulating volume.

To our knowledge, the only previous examination of the hepatic vascular capacitance in a model of liver disease is the study of Schafer and colleagues.²⁵ In a 14 day BDL cat model, they determined that hepatic vascular compliance was unchanged compared with controls but similar to our results; the constrictive response to haemorrhage was impaired. However, the BDL cat seems more appropriate as a model of subacute biliary obstruction as these animals do not develop portal hypertension or significant hepatic fibrosis.²⁵

The observation that L-NAME pretreatment restores the ability of the liver microcirculation to constrict appropriately to haemorrhage strongly suggests that NO also mediates this abnormality in the cirrhotic liver. Again, the lack of significant change in the haemorrhage response of control animals pretreated with L-NAME suggests that NO plays little if any role in the constrictive response to stress in the normal hepatic microcirculation.

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REFERENCES

- Garcia-Estan J, Ortiz MC, Lee SS. Nitric oxide and renal and cardiac dysfunction in cirrhosis. *Clin Sci* 2002;102:213-22.
- Claria J, Jimenez W, Ros J, *et al*. Increased nitric oxide-dependent vasorelaxation in aortic rings of cirrhotic rats with ascites. *Hepatology* 1994;20:1615-21.
- Castro A, Jimenez W, Claria J, *et al*. Impaired responsiveness to angiotensin II in experimental cirrhosis: role of nitric oxide. *Hepatology* 1993;18:367-72.
- Sieber CC, Lopez-Talavera JC, Groszmann RJ. Role of nitric oxide in the in vitro splanchnic vascular hyporeactivity in ascitic cirrhotic rats. *Gastroenterology* 1993;104:1750-4.
- Rockey DC, Chung JJ. Reduced nitric oxide production by endothelial cells in cirrhotic rat liver: endothelial dysfunction in portal hypertension. *Gastroenterology* 1998;114:344-51.
- Gupta TK, Toruner M, Chung MK, *et al*. Endothelial dysfunction and decreased production of nitric oxide in the intrahepatic microcirculation of cirrhotic rats. *Hepatology* 1998;28:926-31.
- Price HL, Deutsch S, Marshall BE, *et al*. Hemodynamic and metabolic effects of hemorrhage in man, with particular reference to the splanchnic circulation. *Circ Res* 1966;18:469-74.
- Lee SS, Girod C, Braillon A, *et al*. Hemodynamic characterization of chronic bile duct-ligated rats: effect of pentobarbital sodium. *Am J Physiol* 1986;251:G176-80.
- Bianchi M, Bellini G, Hesson H, *et al*. Body fluid volumes in the spontaneously hypertensive rat. *Clin Sci (Lond)* 1981;61:685-91.
- Yang W, Benjamin IS, Alexander B. Localisation of hepatic vascular resistance sites in the isolated dual-perfused rat liver. *Eur J Pharmacol* 1999;364:13-21.
- Vollmar B, Siegmund S, Menger MD. An intravital fluorescence microscopic study of hepatic microvascular and cellular derangements in developing cirrhosis in rats. *Hepatology* 1998;27:1544-53.
- Kiszka-Kanowitz M, Henriksen JH, Moller S, *et al*. Blood volume distribution in patients with cirrhosis: aspects of the dual-head gamma-camera technique. *J Hepatol* 2001;35:605-12.
- Sherman IA, Pappas SC, Fisher MM. Hepatic microvascular changes associated with development of liver fibrosis and cirrhosis. *Am J Physiol* 1990;258:H460-5.
- Loureiro-Silva MR, Cadelina GW, Groszmann RJ. Deficit in nitric oxide production in cirrhotic rat livers is located in the sinusoidal and postsinusoidal areas. *Am J Physiol Gastrointest Liver Physiol* 2003;284:G567-74.
- Shah V, Cao S, Hendrickson H, *et al*. Regulation of hepatic eNOS by caveolin and calmodulin after bile duct ligation in rats. *Am J Physiol Gastrointest Liver Physiol* 2001;280:G1209-16.
- Yokomori H, Oda M, Ogi M, *et al*. Enhanced expression of endothelial nitric oxide synthase and caveolin-1 in human cirrhosis. *Liver* 2002;22:150-8.
- Wei CL, Khoo HE, Lee KH, *et al*. Differential expression and localization of nitric oxide synthases in cirrhotic livers of bile duct-ligated rats. *Nitric Oxide* 2002;7:91-102.
- Battista S, Bar F, Mengozzi G, *et al*. Hyperdynamic circulation in patients with cirrhosis: direct measurement of nitric oxide levels in hepatic and portal veins. *J Hepatol* 1997;26:75-80.
- Shijo H, Yokoyama M, Ota K, *et al*. Nitrate kinetics in patients with compensated cirrhosis: correlation with hemodynamics. *Am J Gastroenterol* 1996;91:2190-4.
- Bhathal PS, Grossman HJ. Reduction of the increased portal vascular resistance of the isolated perfused cirrhotic rat liver by vasodilators. *J Hepatol* 1985;1:325-37.
- Marleau P, Ballet F, Chazouilleres O, *et al*. Effect of vasodilators on hepatic microcirculation in cirrhosis: a study in the isolated perfused rat liver. *Hepatology* 1989;9:820-3.
- Kakumitsu S, Shijo H, Yokoyama M, *et al*. Effects of L-arginine on the systemic, mesenteric, and hepatic circulation in patients with cirrhosis. *Hepatology* 1998;27:377-82.
- Shirasaki Y, Su C. Endothelium removal augments vasodilation by sodium nitroprusside and sodium nitrite. *Eur J Pharmacol* 1985;114:93-6.
- Moncada S, Rees DD, Schulz R, *et al*. Development and mechanism of a specific supersensitivity to nitrovasodilators after inhibition of vascular nitric oxide synthesis in vivo. *Proc Natl Acad Sci U S A* 1991;88:2166-70.
- Schafer J, d'Almeida MS, Weisman H, *et al*. Hepatic blood volume responses and compliance in cats with long-term bile duct ligation. *Hepatology* 1993;18:969-77.