• *BRIEF REPORTS* •

Disturbance of hepatic and intestinal microcirculation in experimental liver cirrhosis

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Abstract

AIM: To analyze hepatic, mesenteric and mucosal microcirculation and leukocyte-endothelium interaction (LEI) in a rat model with liver cirrhosis.

METHODS: Hepatic cirrhosis was induced in Wistar rats by gavage with carbon tetrachloride, and intravital videomicroscopy was performed in liver, mesentery and small intestine mucosa. Special emphasis is given on microcirculatory and morphometric changes during cirrhotic portal hypertension.

RESULTS: LEI was influenced significantly in the cirrhotic liver but not in the gut. Blood flow measurement showed significant differences among liver, main mesenteric vessels and the mucosa. The results of our study indicate that liver cirrhosis leads to alterations in hepatic and mesenteric blood flow but not in mucosal blood flow.

CONCLUSION: The enhanced inflammatory response in hepatic microvessels may be caused by a decrease of antithrombin III levels and could be responsible for disturbances of organ pathology.

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INTRODUCTION

Liver cirrhosis is recognized as an important risk factor for the development of severe septical complications such as spontaneous bacterial peritonitis and bacteriaemia[1]. The impairment of intestinal microcirculation and mucosal barrier may contribute to an increased intestinal permeability and bacterial translocation[2-4]. Hypoperfusion of the gut mucosa has been implicated as an important mechanism contributing to gut-derived endotoxinemia and bacterieamia in liver cirrhosis. Due to splanchnic arterial vasodilatation and portal hypertension, intestinal capillary pressure is altered and effective arterial blood volume decreases[5].

It is generally recognized that there is a close relationship between the hepatic microcirculation and liver function and structure[6,7]. Recent studies established the important correlation between hepatic microcirculation and the development of liver pathology[8]. Furthermore, it has been described that leukocyte adherence in liver sinusoids is amplified after gut ischemia and reperfusion due to an up-regulation of adhesion molecules[9].

We still lack quantitative data describing hepatic and mesenteric microvascular parameters such as blood flow velocity, volumetric blood flow, leukocyte-endothelial interaction and vessel size within one experimental setting. Currently, intravital videomicroscopy is the most pretentious technique to measure variables of microvascular perfusion *in vivo*. In addition to the measurement of mesenteric blood flow and leukocyte kinetics, we quantified mucosal blood flow in terminal arterioles directly, since the assessment of mucosal blood flow has been simplified[10].

The present study was undertaken to quantitate basic microvascular parameters in normal rat liver and intestine and to investigate changes in these parameters associated with the development of cirrhosis.

MATERIALS AND METHODS

Male Wistar rats weighing 450±47 g at the time of surgery (initial weight 250±5 g) were used in all experiments. They were housed in a controlled environment with a 12-h light: dark cycle and were fed standard rat diet with water ad libitum.

Chronic, progressive hepatic cirrhosis was induced according to the method of Proctor and Chatamra by gavage with carbon tetrachloride (CCl_4)[11]. Animals were given phenobarbital (35 mg/100 mL) in their drinking water to induce the enzyme cytochrome P450, which has been shown to increase rat liver sensitivity to CCl_4[12]. Two weeks later an initial dose of 0.04 mL CCl_4 was given after orogastric intubation and enhanced weekly in steps of 0.04 mL to a maximum dose of 0.4 mL CCl_4. Animals were weighed weekly.

Six control animals without liver cirrhosis were observed for statistical analysis.

Videomicroscopy

Videomicroscopy was performed under general anesthesia. Left carotid artery was cannulated with a polypropylene catheter (B. Braun, Melsungen, FRG) to monitor arterial blood pressure, heart rate and for blood-gas analysis (ABL Radiometer). The right jugular vein was cannulated for drug administration. The abdomen was opened via a median incision. The presence of ascites and macroscopic appearance of liver cirrhosis were noted.

For intravital microscopic investigations a Leitz microscope (Leitz GmbH, Wetzlar, Germany) was used. With different filter blocks in the epiillumination technique, selective visualization of FITC-labeled erythrocytes and rhodamine 6G-stained leukocytes was possible. For contrast enhancement, FITC-labeled albumin was administered intravenously.

Hepatic microcirculation

The hepatic microvascular parameters were measured *in vivo* using the epiillumination microscopy setup described in detail elsewhere[13]. In brief, the left liver lobe was exteriorized onto a specially designed stage and prepared for intravital videomicroscopy. Continuous superfusion with buffered 37 ℃ Ringer's solution was provided. At the beginning of videomicroscopy, FITC-labeled erythrocytes (1 mL/kg BW) and rhodamine 6G-labeled leukocytes (0.05 nmol/kg BW) were injected intravenously. Using a FITC selective filter block, 5 fields of normal liver tissue during a period of 30 s were analyzed. Observations of leukocytes were done using a rhodamine-selective filter block. Vessels with a length of at least 100 μm were registered for a period of 60 s each.

Intestinal microcirculation

In a second step, terminal ileum was exteriorized and placed on a glass slide. Videomicroscopy of mean mesenteric vessels was performed. Subsequently the bowel was opened along the antimesenteric border and fixed at the incision margins. The prepared bowel was bathed and superfused with buffered Ringer's solution. Mesenteric microcirculation was investigated in 10 fields of ileal arteries and corresponding veins following the scheme of hepatic videomicroscopy. Mucosal microcirculation was measured in the main arteriole of 5 single villus. Each vessel was observed for 30 s.

Data interpretation

Measurements were videotaped and off-line analysis was performed by means of an image-processing system (Capimage®, Zeintl GmbH, Heidelberg, Germany)[14]. The red cell blood velocity and vessel diameter were measured using the frame-to-frame method. Leukocyte contact to endothelium was analyzed and described as leukocyte-endothelium interaction (LEI). The definition of the duration of this interaction results in leukocyte rolling and leukocyte sticking. Leukocyte rolling was based on the movement along the endothelial lining that was less than 66% of the red cell blood velocity. Temporary interactions with the endothelium of not more than 30 s duration were also considered as rollers. Leukocyte sticking was defined as the attachment to the vessel endothelium for at least 30 s.

Microvascular blood flow (V_b in nL/min) was calculated using the following equation[15]:

$V_b = \pi \times v_e \times (D/2)^2$. Determinants were erythrocyte velocity (v_e) and vessel diameter (D).

Histology and blood tests

At the end of experiments, liver and small intestine specimens were taken and the sections were stained with hematoxylin and eosin. Blood samples were taken for measurement of serum concentration of GOT, GPT, γ-GT and AP. Prothrombin time and the antithrombin III level were taken for hemostatic analysis.

Statistical analysis

Results were expressed as mean±SD. Statistical analysis was performed using Mann-Whitney U test. Results are considered significant at $P<0.05$.

RESULTS

Cardiorespiratory parameters did not differ between groups throughout the observation period (Table 1).

Table 1 Cardiorespiratory parameters

	Cirrhosis	Controls	P
Mean arterial blood pressure (mmHg)	107±8.4	112±7.3	NS
Heart rate (bpm)	310±9.2	304±8.4	NS
paO$_2$ (mmHg)	89.1±5.3	92.3±4.8	NS

Histological findings

Ascitic rats had microscopic evidence of cirrhosis in all cases. Liver histology revealed extensive deposits of fibrous tissue with regenerative nodules in at least some areas. Foci of necrotic cells were also observed. Sections of ileum showed a higher number of lymph vessels in cirrhotic rats than that in controls.

Intravital observations

The quantitative measurements of microvascular parameters are summarized in Table 2. Blood flow in terminal arterioles of the villus was similar between both the groups (5.3±0.3 *vs* 5.4±0.2 nL/min in control animals, NS). Marked differences were observed in main mesenteric blood flow (135.1±3.5 *vs* 156.5±4.3 nL/min in controls, $P<0.01$). Liver blood flow remained comparable (32.1±0.4 *vs* 31.2±0.6 nL/min in controls, NS), although red blood cell velocity in cirrhosis was reduced significantly (0.93±0.09 *vs* 1.22±0.18 mm/s in controls, $P<0.05$).

Hepatic LEI was enhanced in cirrhosis. The number of rolling leukocytes and high-affinity leukocytes raised significantly (4.80±0.90 *vs* 2.33±0.75 roller/100 μm and 1.91±0.28 *vs* 0.5±0.5 sticker/100 μm in controls, $P<0.01$). Analysis of LEI in main mesenteric arterioles showed no differences between the groups (Table 3).

Table 2 Microcirculatory parameters

	Cirrhosis	Controls	P
Vessel diameter (μm)			
Liver	27.86±2.03	23.03±0.62	<0.01
Mesentery	32.06±8.91	33.46±11.66	NS
Mucosa	7.51±0.30	7.20±0.23	NS
Red blood cell velocity (mm/s)			
Liver	0.93±0.09	1.22±0.18	<0.05
Mesentery	2.81±0.49	3.11±0.35	NS
Mucosa	2.03±0.15	2.18±0.16	NS
Volumetric blood flow (nL/min)			
Liver	32.10±0.4	31.20±0.6	NS
Mesentery	135.10±3.5	156.50±4.3	<0.01
Mucosa	5.30±0.3	5.40±0.2	NS

Table 3 Leukocyte–endothelium interaction

	Cirrhosis	Controls	P
Adherent leukocytes (n/100 μm)			
Liver	1.91±0.28	0.50±0.5	<0.01
Mesentery	2.54±1.19	1.62±0.85	NS
Rolling leukocytes (n/100 μm)			
Liver	4.80±0.90	2.33±0.75	<0.01
Mesentery	7.68±3.18	6.88±1.94	NS

Blood tests

Serum enzyme tests revealed significant disturbances of hepatocellular integrity and reduction of blood clotting capacity. Further antithrombin III levels were significantly reduced in cirrhotic animals (108.5±14.59 *vs* 125.0±8.96 IU in controls, *P*<0.05) (Table 4).

Table 4 Blood tests

	Cirrhosis	Controls	P
GOT (U/L)	63.67±10.42	28.67±4.85	<0.01
GPT (U/L)	31.83±4.84	16.67±1.11	<0.01
GGT (U/L)	5.33±1.49	3.17±0.69	<0.05
AP (U/L)	130.50±35.75	56.83±2.67	<0.01
Prothrombin time (%)	75.30±5.4	102.4±2.7	<0.01
Antithrombin III (IU)	108.50±14.59	125.0±8.96	<0.05

DISCUSSION

Liver cirrhosis has been detected to be an important risk factor for mortality in critically ill patients[16]. Its incidence in western countries is rising and represents the most common cause of mortality among the non-malignant digestive diseases[17]. The mechanisms for the increased morbidity and mortality may be immunological, mechanical or pharmacological. Special emphasize is given to hepatic and intestinal microcirculatory disorders. However, it is still not clear how alterations in hepatic microcirculation contribute to the progression of the disease and its sequelae.

In the present study, we have applied the technique of intravital videomicroscopy to quantitate intestinal and hepatic microcirculation and LEI in cirrhotic rats. We used a model of CCl₄-induced cirrhosis. Liver damage is mainly caused by hepatotoxic CCl₄ metabolites produced within the liver. The intensity of the hepatic damage is exaggerated when the microsomal enzyme oxidizing system is previously induced, for instance by the administration of phenobarbital[12]. Induced cirrhosis is associated with marked hemodynamic disturbances. These include changes in both the intrahepatic and splanchnic circulation resulting in portal hypertension[18,19]. Our quantitative studies of hepatic and mesenteric microvascular parameters indicate that cirrhosis leads to a marked arterial vasodilatation. This observation is in accordance with other experimental studies. The pathogenesis of vascular tone is explained by biochemical investigations and disorders of vascular heme oxygenase-1 expression and nitric oxide synthase expression in experimental cirrhosis[20,21].

In this study, presence of liver cirrhosis had no effect on villus blood flow measured in the terminal, central villus arteriole. To our knowledge, there is no other study where the effects of cirrhosis and portal hypertension on villus blood flow were derived directly from data obtained on the level of single villus arterioles. The disturbance of mucosal blood flow may contribute to an impairment of the intestinal barrier function. It has been described that alterations of the mucosal permeability in cirrhosis are related to the degree of liver failure and to the progression of the liver disease[4].

However, the impact of liver cirrhosis on hepatic microcirculation is more evident. LEI is enhanced significantly and may explain organ disturbances. LEI itself is modulated by a variety of adhesion molecules expressed on the surface of leukocytes and endothelial cells. These adhesion molecules mediate the decrease in leukocyte rolling and the increase in leukocyte adherence and migration.

In summary, this study demonstrates that in the gut mucosa, cirrhosis may not induce disturbances in the villus microcirculation, despite altered levels of mesenteric blood flow.

Experimental liver cirrhosis is associated with a marked increase of liver enzymes and a decrease of antithrombin III levels and prothrombin time. Antithrombin III is one of the most important physiological inhibitors of coagulation. It is synthesized in liver parenchymal cells. Moreover, it has been described that AT III has anti-inflammatory actions in experimental sepsis and hepatic ischemia-reperfusion[22,23]. It could be presumed that in cirrhosis a drop of the antithrombin III level leads to hemostatic disorders similar to disseminated intravasal coagulation (DIC) and inflammatory response in the microcirculatory units, even if clinical observations did not emphasize those findings[24,25]. In our study, hemostatic dysfunction could be measured and LEI in liver parenchyma and mesenteric vessels was enhanced significantly.

We conclude that experimental liver cirrhosis leads to disturbances in hepatic and mesenteric but not in mucosal microcirculation. Although not proven by our experiments, lower antithrombin III levels as found in our study could potentially maintain or aggravate hemostatic disorders and alterations in hepatic inflammatory response to portal hypertension associated with a deterioration of liver function.

REFERENCES

1   **Borzio M,** Salerno F, Piantoni L, Cazzaniga M, Angeli P,

Bissoli F, Boccia S, Colloredo-Mels G, Corigliano P, Fornaciari G, Marenco G, Pistara R, Salvagnini M, Sangiovanni A. Bacterial infection in patients with advanced cirrhosis: a multicentre prospective study. *Dig Liver Dis* 2001; **33:** 41-48

2   **Llovet JM,** Bartoli R, March F, Planas R, Vinado B, Cabre E, Arnal J, Coll P, Ausina V, Gassull MA. Translocated intestinal bacteria cause spontaneous bacterial peritonitis in cirrhotic rats: molecular epidemiologic evidence. *J Hepatol* 1998; **28:** 307-313

3   **Parlesak A,** Schafer C, Schutz T, Bode JC, Bode C. Increased intestinal permeability to macromolecules and endotoxemia in patients with chronic alcohol abuse in different stages of alcohol-induced liver disease. *J Hepatol* 2000; **32:** 742-747

4   **Pascual S,** Such J, Esteban A, Zapater P, Casellas JA, Aparicio JR, Girona E, Gutierrez A, Carnices F, Palazon JM, Sola-Vera J, Perez-Mateo M. Intestinal permeability is increased in patients with advanced cirrhosis. *Hepatogastroenterology* 2003; **50:** 1482-1486

5   **Arroyo V.** Pathophysiology, diagnosis and treatment of ascites in cirrhosis. *Ann Hepatol* 2002; **1:** 72-79

6   **Varin F,** Huet PM. Hepatic microcirculation in the perfused cirrhotic rat liver. *J Clin Invest* 1985; **76:** 1904-1912

7   **Shibayama Y.** On the pathogenesis of portal hypertension in cirrhosis of the liver. *Liver* 1988; **8:** 95-99

8   **Ekataksin W,** Kaneda K. Liver microvascular architecture: an insight into the pathophysiology of portal hypertension. *Semin Liver Dis* 1999; **19:** 359-382

9   **Horie Y,** Wolf R, Miyasaka M, Anderson DC, Granger DN. Leukocyte adhesion and hepatic microvascular responses to intestinal ischemia/reperfusion in rats. *Gastroenterology* 1996; **111:** 666-673

10   **Ruh J,** Ryschich E, Secchi A, Gebhard MM, Glaser F, Klar E, Herfarth C. Measurement of blood flow in the main arteriole of the villi in rat small intestine with FITC-labeled erythrocytes. *Microvasc Res* 1998; **56:** 62-69

11   **Proctor E,** Chatamra K. Standardized micronodular cirrhosis in the rat. *Eur Surg Res* 1984; **16:** 182-186

12   **McLean EK,** McLean AE, Sutton PM. Instant cirrhosis: An improved method for producing cirrhosis of the liver in rats by simultaneous administration of carbon tetrachloride and phenobarbitone. *Br J Exp Pathol* 1969; **50:** 502-506

13   **Menger MD,** Marzi I, Messmer K. *In vivo* fluorescence microscopy for quantitative analysis of the hepatic microcirculation in hamsters and rats. *Eur Surg Res* 1991; **23:** 158-169

14   **Zeintl H,** Sack FU, Intaglietta M, Messmer K. Computer assisted leukocyte adhesion measurement in intravital microscopy. *Int J Microcirc Clin Exp* 1989; **8:** 293-302

15   **Gross JF,** Aroesty J. Mathematical models of capillary flow: a critical review. *Biorheology* 1972; **9:** 225-264

16   **Foreman MG,** Mannino DM, Moss M. Cirrhosis as a risk factor for sepsis and death: analysis of the National Hospital Discharge Survey. *Chest* 2003; **124:** 1016-1020

17   **Shellman RG,** Fulkerson WJ, DeLong E, Piantadosi CA. Prognosis of patients with cirrhosis and chronic liver disease admitted to the medical intensive care unit. *Crit Care Med* 1988; **16:** 671-678

18   **Vorobioff J,** Bredfeldt JE, Groszmann RJ. Increased blood flow through the portal system in cirrhotic rats. *Gastroenterology* 1984; **87:** 1120-1126

19   **Sherman IA,** Pappas SC, Fisher MM. Hepatic microvascular changes associated with development of liver fibrosis and cirrhosis. *Am J Physiol* 1990; **258:** H460-H465

20   **Chen YC,** Gines P, Yang J, Summer SN, Falk S, Russell NS, Schrier RW. Increased vascular heme oxygenase-1 expression contributes to arterial vasodilation in experimental cirrhosis in rats. *Hepatology* 2004; **39:** 1075-1087

21   **Bhimani EK,** Serracino-Inglott F, Sarela AI, Batten JJ, Mathie RT. Hepatic and mesenteric nitric oxide synthase expression in a rat model of $CCl_{(4)}$-induced cirrhosis. *J Surg Res* 2003; **113:** 172-178

22   **Okajima K,** Uchiba M. The anti-inflammatory properties of antithrombin III: new therapeutic implications. *Semin Thromb Hemost* 1998; **24:** 27-32

23   **Maksan SM,** Maksan MO, Gebhard MM, Herfarth C, Klar E. Reduction of hepatic reperfusion injury by antithrombin III and aprotinin. *Transpl Int* 2000; **13** Suppl 1: S562-S564

24   **Ben-Ari Z,** Osman E, Hutton RA, Burroughs AK. Disseminated intravascular coagulation in liver cirrhosis: fact or fiction? *Am J Gastroenterol* 1999; **94:** 2977-2982

25   **Scherer R,** Kabatnik M, Erhard J, Peters J. The influence of antithrombin III (AT III) substitution to supranormal activities on systemic procoagulant turnover in patients with end-stage chronic liver disease. *Intensive Care Med* 1997; **23:** 1150-1158

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