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Endothelin-1 is involved in hepatic sinusoidal vasoconstriction after ischemia and reperfusion

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Abstract Endothelin-1 (ET-1), a vasoactive peptide, causes a significant rise in portal vein pressure, which is most likely a result of severe vasoconstriction in the liver. In this study, the effect of ET-1 on sinusoidal vasoconstriction in the liver after ischemia and reperfusion was directly investigated using intravital microscopy. In anesthetized female Sprague Dawley rats (200–250 g) ischemia of the median and left liver lobes was induced for 90 min by temporary ligation of the left pedicle. After declamping and a 90-min reperfusion period, the livers were exposed for intravital microscopy. Using a Nikon MM-11 fluorescence microscope (545 nm, 330x), a CCD camera (Cohu FK 6990), and a SVHS video recording unit, the hepatic microcirculation was directly investigated. Besides sham groups, two ischemia groups were studied, receiving ET-1 antiserum (anti-ET-1; 0.5 ml; Peptide Inst., Osaka, Japan) or NaCl 0.9% (0.5 ml) 5 min prior to reperfusion of the liver ($n = 6$/group). Following a transient drop in the mean arterial blood pressure in the anti-ET-1-treated groups, comparable systemic hemodynamic conditions among the four groups were noted during intravital microscopic assessment at the end of the 90-min reperfusion period. Reduction in the sinusoidal diameters during postischemic reperfusion (7.7 ± 0.5 µm) was prevented by anti-ET-1 treatment (9.6 ± 0.25 µm; $P < 0{,}01$; mean ± SEM) back to control values (9.6 ± 0.32 µm), while most of other microcirculatory parameters did not show significant differences. The results supported further the role of ET-1 in dysregulation of the sinusoidal vascular tone in the liver, e.g., after ischemia and reperfusion.

Key words Endothelin-1
Ischemia · Reperfusion · Liver
Sinusoidal vasoconstriction

Introduction

Reperfusion of the liver following warm and cold ischemia of the liver is generally associated with severe microcirculatory disturbances including sinusoidal vasoconstriction [1], nonparenchymal cell injury [2, 9], Kupffer cell activation [18, 20], and leukocyte adhesion [10, 19] that leads ultimately to parenchymal cell injury. While direct and indirect leukocyte-mediated liver injury during the reperfusion period seems to be largely regulated by macrophage-derived mediators [12] such as tumor necrosis factor (TNF) [3, 4], the regulation of sinusoidal

perfusion is not yet clearly understood. There is evidence that sinusoidal vascular tone is regulated by sinusoidal endothelial cells (SEC) in synergy with network-like contractile filaments of Ito cells surrounding the SEC on the abluminal side [21]. It is of interest, that in vitro Ito cell contractions are regulated by mediators released by Kupffer cells, e.g., prostaglandin E_2 [6]. Furthermore, there is significant evidence that endothelin-1 (ET-1), as contracting factor, and nitric oxide (NO), as endothelium-relaxing factor, are involved in the regulation of the hepatic vascular tone [14–17]. In this respect, it seems of importance that plasma ET-1 levels have been reported to significantly increase after orthotopic liver transplantation [8] and warm ischemia of the liver [5]. Taking these considerations into account, the aim of this study was to determine whether ET-1 accounts for sinusoidal vasoconstriction following warm ischemia of the liver.

Materials and methods

Female Sprague-Dawley rats weighing (200–250 g) were anesthetized with ether and subsequently with pentobarbital sodium (50 mg/kg 30 min after reperfusion). The carotid artery and jugular vein were canulated for determination of mean arterial blood pressure (MABP), and infusion of Ringers acetate (3 ml/h), respectively. Ischemia of the left and median liver lobes was induced after laparotomy and ligation of the left pedicle of the liver hilus using an atraumatic vessel loop. Following 90 min of ischemia, the abdomen, which was temporarily closed, was opened again and restoration of blood flow to the liver was allowed by release of the vessel loop. Following a 90-min reperfusion period, the liver was exposed under an intravital microscope (Nikon MM-11, 545 nm filter, opt. magn. 330x) that was connected via a CCD-camera (Pieper, FK 6990, Schwerte, D) and a time date generator (VTG 33, FOR-A Co, Tokyo) to a SVHS video recording system (Panasonic NV FS 1 HQ). Using acridine orange as fluorescence dye (1 µmol/kg), the hepatic microciruculation and leukocyte endothelium interactions were investigated and evaluated as earlier described [10–12, 19]. Additionally, blood samples were taken at the end of baseline, ischemia, and reperfusion periods for determination of acid-base state. Four groups were investigated in a blinded protocol as indicated in Table 1. In the sham-operated groups (S), all procedures were performed as in the ischemia groups (I) except temporary ligation of the left pedicle of the liver hilus. Four minutes prior to declamping, animals received either 0.5 ml NaCl 0.9% (N) or 0.5 ml anti-endothelin-1 antiserum (AS) that was diluted 1:30 with 10 m*M* phosphate-buffered saline IV. Endothelin-1 antiserum was obtained from Peptide Institute Inc. (Osaka, Japan) and was shown to have a 100% crossreactivity with human/rat endothelin-1. Moreover, it was shown to block effectively the effect of endothelin in other rat models [5, 15].

Results

As indicated in Table 1, administration of ET-1 antiserum resulted in a transient drop in the mean arterial blood pressure (MABP) in sham and ischemia groups. However, MABP recovered within 20 min so that at the time of intravital microscopy comparable MABP values were observed.

Metabolic parameters of acid base state did not reveal differences between ischemia or sham groups (Table 1). Assessment of the hepatic microcirculation indicated that over 90% of the sinusoids were perfused in all groups. Sinusoidal diameters, determined at the borderline from midzonal to pericentral region in five lobules of each experiment demonstrated a significant narrowing of sinusoidal width in the ischemia group (group I/N; Fig. 1). By administration of the ET-1 antiserum, sinusoidal width significantly increased as illustrated in Fig. 1 (I/AS group). Consequently, sinusoidal blood flow as

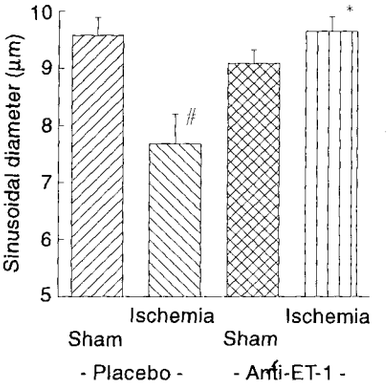

Fig. 1 Widths of liver sinusoids at the border of the midzonal and pericentral azinus. Diameters were determined at a specimen/monitor ratio of 1050 using a computerized image analysis system [12]. Mean ± SEM; # $P < 0.01$ versus sham/NaCl; * $P < 0.01$ versus ischemia/NaCl; ANOVA

Table 1 Experimental groups and systemic parameters. Mean arterial blood pressure (MABP) and pH are given as mean ± SEM (t90, End of ischemic period; t110, 20 min after reperfusion; t180, 90 min after reperfusion prior to microscopy)

Group	*n*	MABP (t90)	MABP (t110)	MABP (t180)	pH (t180)
Sham/NaCl (S/N)	4	92.0 ± 3.7	88.0 ± 3.3	101.5 ± 4.3	7.38 ± 0.05
Sham/anti-ET-1 (S/AS)	6	95.0 ± 3.2	87.8 ± 3.6	92.5 ± 5.4	7.34 ± 0.03
Ischemia/NaCl (I/N)	6	97.3 ± 5.1	85.3 ± 2.2	86.3 ± 5.5	7.33 ± 0.04
Ischemia/anti-ET-1 (S/AS)	6	84.2 ± 5.3	70.6 ± 4.6	70.2 ± 8.6	7.35 ± 8.6

estimated using the flow velocity of labeled circulating leukocytes and sinusoidal diameters indicated was higher in the I/AS group ($55201 \pm 11145\ \mu m^3$) when compared to the I/N group ($44414 \pm 7473\ \mu m^3$). In the S/AS group ($33105 \pm 4384\ \mu m^3$), sinusoidal blood flow was reduced in contrast to the S/N group ($45317 \pm 5218\ \mu m^3$). In respect to leukocyte-endothelium interactions, the substantial increase in permanent adherent leukocytes after ischemia/reperfusion (S/N $10.9 \pm 0.6\%$; I/N $40.6 \pm 5.6\%$, $P < 0.01$) was not affected by ET-1 antiserum (I/AS $41.4 \pm 3.9\%$). (Parenchymal cell injury to the liver, as indicated by serum SGOT/SGPT values obtained at 90 min of reperfusion demonstrated??.)

Discussion

Postischemic reperfusion of the liver results in severe microcirculatory disturbances with subsequent local liver injury or even remote organ injury, e. g., to the lung [3, 4]. It has been suggested that mediators of activated Kupffer cells cause severe microcirculatory disturbances in the hepatic microcirculation, e. g., after liver transplantation [12, 13]. In this context, it has been suggested recently that endothelium-derived contracting factors, such as endothelin-1 or -3 [7], may contribute to liver injury [5, 15]. Therefore, the aim of this study was to evaluate whether ET-1 contributes to postischemic microvascular disturbances in liver sinusoids, e. g., by contraction of sinusoids. Using an intravital microscopic approach, we demonstrated that severe constriction of liver sinusoids took place during postischemic reperfusion. This was most likely not due to simple cell swelling, as studies with hypertonic solutions have suggested [1], but rather a mediator-dependent effect, partly involving prostacyclin (unpublished observation). The results of this study clearly showed that narrowing of hepatic sinusoids after ischemia could be prevented by blocking the effect of ET-1 by giving an ET-1 antiserum just prior to declamping. This effect of ET-1 antiserum was related in this study to the microvascular perfusion, e. g., prevention of vasoconstriction and increase in volumetric blood flow. ET-1 antiserum had no effect on pathological leukocyte adhesion in this protocol. However, upregulation of adhesion molecules on leukocytes by endothelin has recently been demonstrated. Thus, only the microvascular perfusion but not the inflammatory hepatic response in liver sinusoids was attenuated by anti-endothelin-1 after warm ischemia of the liver. Further studies are needed to identify the regulatory mechanisms involved in the release of endothelins after liver ischemia and possible improvement in graft functions by an endothelin antagonist. Therefore, the relevance of ET-3 [7] and of systemic hemodynamic effects with secondary impact on hepatic blood flow after anti-ET antiserum administration should be carefully investigated.

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