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Quantitative analysis of the microcirculation of xenogeneic haemoperfused rat livers by intravital microscopy

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Abstract Livers from male Sprague-Dawley rats were perfused with heparinised, unmodified isogeneic rat blood ($n = 6$) or xenogeneic human blood. The microcirculation of these livers, as the primary manifestation of hyperacute xenogeneic rejection, was directly observed and quantified by using fluorescence videomicroscopy. Bile flow and enzyme release of the isogeneic perfused livers were in the physiological range, whereas bile flow was significantly reduced and enzyme release increased during xenogeneic perfusion. In contrast to an almost physiological acinar (90.4 %) and sinusoidal (93.6 %) perfusion rate in the isogeneic group, a rapid breakdown of microcirculation with an acinar perfusion index of 47.5 % and a sinusoidal perfusion rate of 67.1 % were found in the xenogeneic group. This direct quantification of microcirculatory parameters is a step forward towards sensitive and early characterisation of the severity of the xenogeneic rejection of the liver.

Key words Xenograft · Microcirculation · Liver · Isolated perfusion · Rat

Introduction

Like most other immunological disease mechanisms, xenograft rejection is thought to manifest primarily at the level of the microcirculatory unit [1]. Despite extensive histological studies [2, 3] of hyperacute xenogeneic rejection (HXR), little is known about microhaemodynamic alterations during HXR. The purpose of this study was the direct observation and quantification, using intravital microscopy (IVM), of the microcirculation of rat livers perfused by human blood. This technique permits the dynamic investigation of the hepatic microcirculation in vital organs after staining the blood with different fluorescent dyes.

Materials and methods

Rat liver perfusion

Male Sprague-Dawley rats (250–300 g) were anaesthetised by intraperitoneal injection of pentobarbital sodium (60 mg/kg). After midline abdominal and subcostal incisions, the livers were prepared in situ to avoid relevant trauma or ischaemia. Livers were perfused using a modified technique of Miller et al. [4]. Briefly, the hepatic artery was ligated, and the common bile duct and portal vein were cannulated. Immediately after cannulation of the portal vein, the livers were flushed with 30 ml of cold lactated Ringer's solution and the rats were killed by exsanguination. The perfusion circuit was closed by cannulating the suprahepatic inferior caval vein and ligating the infrahepatic part of the inferior caval vein. After a total cold ischaemic time of less than 4 min, the livers were reperfused for 45 min at a constant flow rate of 1.15 ± 0.1 ml/min per gram of liver. The perfusate consisted of 35 ml heparinised (10 IU/ml) fresh isogeneic rat blood or xenogeneic human blood diluted to a haematocrit of 30 ± 2 % with hydroxyethylstarch. The temperature, pH and portal pressure were monitored continuously during the course of the perfusion.

Four different groups were established: two perfusion groups (isogeneic or xenogeneic perfusion of a rat liver, $n = 6$) and two

Table 1 Microcirculatory parameters (mean ± SEM) during isogeneic and xenogeneic in situ perfusion of rat livers, assessed by intravital fluorescence microscopy

	Isogeneic perfusion (rat)	Xenogeneic perfusion (human)
Index of acinar perfusion (45 min)	90.4 ± 1.0 %	47.5 ± 2.1 %*
Sinusoidal perfusion		
Periportal	92.2 ± 1.0 %	59.0 ± 0.3 %*
Midzonal	92.1 ± 0.8 %	66.1 ± 0.3 %*
Pericentral	96.6 ± 0.7 %	76.1 ± 0.3 %*
Mean	93.6 ± 0.3 %	67.1 ± 0.3 %*
Adherent WBC in perfused sinusoids [n/lobule]		
Periportal	21.0 ± 1.2	9.3 ± 1.3
Midzonal	25.1 ± 1.0	7.3 ± 1.9
Pericentral	11.8 ± 1.1	3.8 ± 0.9
Mean	57.9 ± 1.5	20.4 ± 3.5
Sinusoidal diameter of perfused sinusoids (mean of the three liver zones)		
0–15 min	6.1 ± 0.1	6.7 ± 0.1*
15–45 min	6.5 ± 0.1	8.3 ± 0.3*
30–45 min	6.5 ± 0.1	9.2 ± 0.2*
Postsinusoidal venules		
WBC velocity [μm/sec]	1196 ± 75	890 ± 128*
Adherent WBC [n/mm^2]	122 ± 27	55 ± 13*

* $P < 0.01$ (Mann-Whitney U-test)

control groups (perfusion of an anhepatic circuit with human blood or rat blood, $n = 5$). Blood samples were drawn at given time points for blood counts, biochemical analysis (GOT, LDH) and determination of titres of preformed natural antibodies by haemagglutination. As an indicator of liver function, bile was collected in intervals of 15 min.

Intravital microscopy

For IVM the right liver lobe was exteriorised on a specially designed mechanical stage and covered with a saran wrap to prevent drying of the tissue. The perfusate was stained with fluorescein sodium and rhodamine 6G. The microvessels of the liver were investigated directly using a microscope with epi-illumination and different filter blocks, a CCD video camera and a SVHS video recording unit. During perfusion eight to ten liver fields, postsinusoidal venules and portal venules were observed at a × 800 magnification (on the monitor) and videod. At the end of the perfusion, 30–50 acini were videod at a lower magnification (× 400). Quantification of the hepatic microcirculation was performed off line by frame-to-frame analysis of the videotapes as described elsewhere [5].

Results

During isogeneic in situ perfusion of rat livers, portal pressure remained in the physiological range, whereas perfusion with human blood resulted in a significantly increased portal pressure within the first minutes. The maximum pressure was reached in this group after 15 min, thereafter portal pressure decreased slowly to values comparable to the isogeneic group. Bile flow as a gross indicator of liver function was severley depressed in the xenogeneic group but not in the isogeneic one. Biochemical analysis of the perfusate revealed no significant changes or unphysiological values in the isogeneic perfusion group, except for a marked rise in LDH due to haemolysis that was also present in the isogeneic control group. GOT and LDH showed no major changes in the xenogeneic control group, indicating a less expressed haemolysis of human blood compared to rat blood. In contrast, GOT and LDH increased significantly in the xenogeneic perfusion group, indicating severe parenchymal cell injury in the liver. A reduction in preformed natural antibodies (PNAB) to almost zero was found in the xenogeneic perfusion group, but not in the xenogeneic control group.

Isogeneic perfusion resulted in a slight reduction in white blood cell (WBC) counts after 45 min and in no reduction in platelet (PLT) counts compared to the control group. In contrast, most of the human WBC and PLT accumulated in the liver within the first 5 min – predominantly portal and periportal as observed by IVM – resulting in a rapid reduction in WBC and PLT counts in the perfusate. In situ perfusion of rat livers with isogeneic blood showed a homogenous perfusion of the liver and an almost physiological WBC behaviour, whereas in the xenogeneic group, 24 % of acini were not perfused and most of the remaining ones were irregularly perfused as reflected by a low index of acinar perfusion. The observed microcirculatory parameters can be seen in Table 1.

Discussion

In situ rat liver perfusion with isogeneic rat blood showed an almost intact microcirculation with a sinusoidal and acinar perfusion rate of over 90 %. The small reduction in the perfusion rate – which in normal livers in vivo is nearly 100 % – is considered to be due to the lack of hepatic arterial perfusion [6] or the formation of microemboli in extracorporeal perfusion systems [7], a fact that was confirmed by IVM. In postsinusoidal venules, WBC flow behaviour and WBC adherence were similar to the values of in vivo observations, indicating – together with normal bile flow and enzyme levels – no relevant ischaemic damage [5] of the liver. In contrast, xenogeneic perfusion resulted in a breakdown of the microcirculation within minutes. Comparable values for perfusion deficits are reached in allograft rejection in the rat only after 4–6 days [8]. The observed dilatation of sinusoids, which was expressed less in the first 15 min after reperfusion but was markedly so after 30 min, was in agreement with histological findings [Pascher et al., submitted for publication] of hyperaemic areas in xenografts.

The predominantly portal and periportal accumulation of WBC during HXR and its speed and extent was probably mediated by early complement activation [9, 10] enhancing thrombocyte aggregation [11], together with subsequent adhesion of WBC. This is in agreement with the binding of PNAB and the activation of complement predominantly in afferent vessels [2, 3; Pascher et al., submitted for publication]. Because no gold standard exists for evaluation of hepatic function [12], direct observation of the microcirculation and, in particular, determination of the sinusoidal and acinar perfusion rate is a step forward towards sensitive and early characterisation of the severity of rejection in xenogeneic models.

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