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Analysis of the microcirculation during xenogeneic liver perfusion in the guinea pig – rat model. The contribution of leukocytes to the rejection process

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Abstract Since the main feature of hyperacute rejection is a disturbance of the xenograft's microcirculation, we analyzed microhemodynamic parameters during xenogeneic hemoperfusion of the guinea pig (GP) liver and investigated the contribution of leukocytes to the rejection process using intravital fluorescence microscopy. Isolated GP livers were hemoperfused via the portal vein in a recirculating system with a constant flow of 1 ml/min per g liver. In contrast to isogeneic perfusion with heparinized GP blood, a disturbance in the microcirculation was observed during xenogeneic perfusion using heparinized rat blood, with significantly higher values of perfusion pressure, reduced sinusoidal perfusion rates, and a larger number of stagnant leukocytes. A complete breakdown of the microcirculation, with the highest values of perfusion pressure and the smallest perfusion index, was associated with 100% accumulated leukocytes when rat blood was anticoagulated with sodium citrate. Almost isogeneic perfusion values were obtained when fucoidin, which inhibits L-selectin-dependent cell interaction, was added to heparinized rat blood. These data indicate that leukocyte-endothelial cell interaction contributes to xenogeneic rejection.

Key words Xenogeneic liver hemoperfusion · Intravital microscopy · Fucoidin

Introduction

The combination of guinea pig (GP) and rat is often used as a discordant model to investigate xenogeneic rejection [1–3]. Although the disturbance of the microcirculation caused by endothelial cell activation via the complement system and xenogeneic natural antibodies is the main rejection mechanism, there are no quantitative measurements of microhemodynamics in this model. Histological findings of hyperacute rejected organs [4] and in vitro studies suggest an involvement of leukocytes, especially natural killer cells, in the early rejection phase [5]. The contribution of early cell interaction to the rejection process in vivo is not clear. In the present study, intravital fluorescence microscopy (IVM) was used to analyze microhemodynamic parameters during xenogeneic hemoperfusion of GP livers and to investigate the role of leukocyte-endothelial cell interaction in the early rejection process. For this purpose we used the selectin-binding polysaccharide, fucoidin, which is known to interfere with the ability of leukocytes to interact with sulfate-containing proteoglycans on the surface of vascular endothelium by inhibiting the selectin-dependent rolling of leukocytes [6, 7].

Material and methods

Livers of GPs (180–220 g) were hemoperfused via the portal vein and drained via the inferior thoracic vena cava using a closed perfusion circuit, as described elsewhere [8], with a constant flow of 1 ml/min per g. Perfusate consisted of blood diluted with hydroxyethyl starch to a hematocrit of 20%. IVM was performed using a fluorescence microscope with epi-illumination (Leica, Germany) as described in detail [9]. Briefly, the plasma of the perfusate was

stained with fluorescein sodium [4.5 μg/ml; Sigma, Germany) to enhance the contrast of plasma and parenchymal cells. Rhodamine 6G (0.6 μg/ml; Sigma) was used to label vital leukocytes and platelets. A water-immersion objective and different filter blocks were employed to visualize the distinct fluorescent dyes. The microscopic pictures were transferred via CCD camera (Pieper Fk 6990, Switzerland) to a monitor-video unit. A video timer allowed the time related interpretation of the video sequences. The quantification of microhemodynamic parameters in sinusoids was performed off-line by frame-to-frame analysis of the videotaped images [10]. Ten to twelve microscopic fields were randomly selected and recorded at 10 min time intervals for a total observation period of 45 min. The following parameters were measured: (1) perfused sinusoids (given as a percentage of the total number of sinusoids observed), (2) sinusoidal leukocyte stagnation (defined as white blood cells stagnant within sinusoids and not moving during an observation period of 20 s per mm^2), and (3) number of moving leukocytes in sinusoids of recorded microscopic fields. Portal perfusion pressure was monitored continuously, and bile flow was measured via the cannulated bile duct. The following groups ($n = 6$) were compared: (1) isogeneic perfusion with heparinized GP blood (ISO), (2) xenogeneic perfusion with heparinized rat blood (XENO), (3) xenogeneic perfusion with citrated rat blood (CIT), and (4) xenogeneic perfusion with heparinized rat blood and fucoidin (0.36 mg/ml) (FUC). Results are expressed as means (SEM), and differences were considered significant at $P < 0.05$ when tested with the unpaired Students t-test.

Results

Microhemodynamic parameters

Isogeneic hemoperfusion with heparinized GP blood resulted in a homogeneous sinusoidal perfusion rate of 100% in the first 5 min and a slow decrease to 81% after 45 min of perfusion. In the XENO group the number of perfused sinusoids dropped rapidly from 90% in the first 5 min to 35% at 45 min. In the CIT group only 4% of the sinusoids were perfused by the end of the experiment. Treatment with Fucoidin improved the perfusion to a level of 80%, thus comparable to isogeneic values (Fig. 1).

Poor perfusion rates in xenoperfused groups were accompanied by a significantly higher number of stagnant leukocytes in observed, perfused sinusoids. Ninety-eight percent of leukocytes in the XENO group and 100% in the CIT group adhered firmly after 25 min of perfusion. In the FUC group the total number of observed leukocytes was increased, whereas the proportion of stagnant cells was comparable to the ISO group with 54% at the end of perfusion (Fig. 2). With reference to the low perfusion index, the perfusion pressure of the XENO group (27.5 mmHg) and CIT group (31.3 mmHg) was significantly higher as compared to pressure values of the ISO group (13.6 mmHg) and FUC group (21.5 mmHg) after 5 min of perfusion (Fig. 3).

Bile flow as a parameter for liver function dropped to 2% of the basal value in intact animals in the CIT group. The ISO group reached 41% of basal bile flow and this was significantly higher than in the XENO group (21%) and FUC group (27%). There was no significant difference between the XENO and FUC groups (Fig. 4).

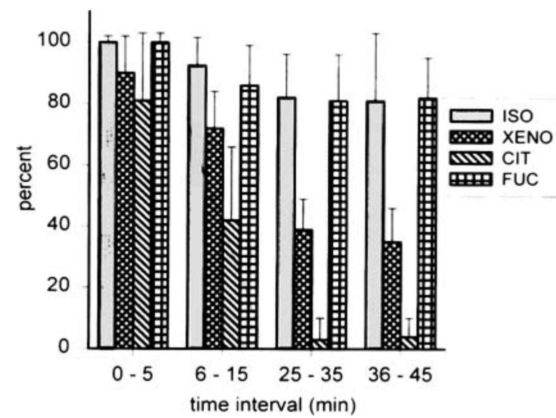

Fig. 1 Number of perfused sinusoids as a percentage of total number of observed sinusoids (*ISO* Isogeneic perfusion with heparinized guinea pig blood, *XENO* xenogeneic perfusion with heparinized rat blood, *CIT* xenogeneic perfusion with citrated rat blood, *FUC* xenogeneic perfusion with heparinized rat blood and fucoidin)

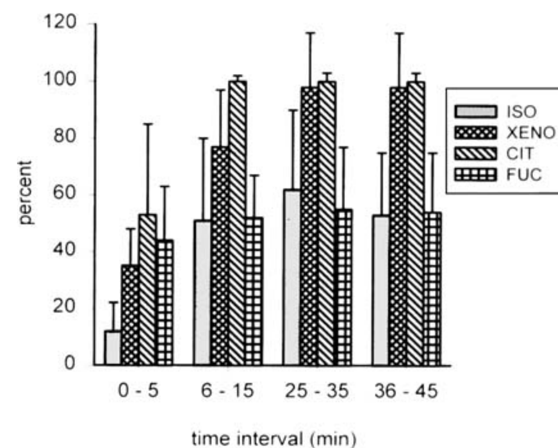

Fig. 2 Proportion of stagnant leukocytes in sinusoids as a percentage of observed leukocytes

Discussion

IVM has been used recently to investigate different organs, including skeletal muscle, lung, small bowel, and liver [11]. Small animals, such as rats and hamsters, are commonly used for this technique and microhemodynamic parameters during allograft rejection [12] and ischemia-reperfusion injury [13] are well documented.

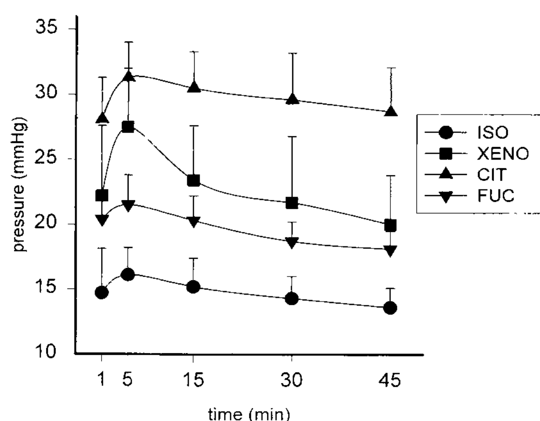

Fig. 3 Portal perfusion pressure in mm Hg

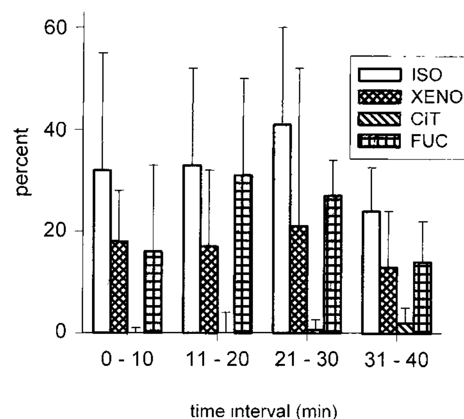

Fig. 4 Bile flow as a percentage of bile production in intact animal (baseline)

Seehofer et al. [9] described IVM in a liver xenoperfusion model of the rat as a sensitive method to detect alterations of microcirculation during xenogeneic rejection. The results of this study indicated that IVM is also suitable for quantifying microhemodynamic parameters during xenoperfusion of the GP liver with rat blood. We observed a deterioration of perfusion rate within the first 20 min during xenogeneic perfusion, which was accompanied by a significantly larger number of stagnant leukocytes in perfused sinusoids. In contrast, isogeneic perfusion had a constant high perfusion rate during the whole observation period and a lower and stable number of stagnant leukocytes. Fucoidin was able to improve the microhemodynamic parameters with higher perfusion rates and a simultaneous reduction in the proportion of stagnant leukocytes to almost isogeneic perfusion conditions. It is well documented that fucoidin is a substance which is known to reduce L-selectin-dependent rolling of leukocytes, and thus interferes with inflammatory events in different animal models [14, 15]. The improvement of the perfusion by fucoidin in our model indicates the contribution of early leukocyte-endothelial cell interactions to xenogeneic rejection in vivo. Anticoagulation of blood with sodium citrate resulted in a complete breakdown of the microcirculation with the lowest perfusion values. Beside the anticoagulant potency, it is known that heparin oligosaccharides bind L- and P-selectin and inhibit acute inflammation [16]. This could be the explanation for the better results in the heparinized XENO group versus the CIT group. In summary, IVM is suitable for investigating early changes of microhemodynamic parameters during the xenoperfusion of GP livers. Our results indicate the contribution of early leukocyte-endothelial cell interaction in the perfusion failure during xenogeneic liver perfusion.

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