Transpl Int (2005) 17: 862–871
DOI 10.1007/s00147-005-0809-9

ORIGINAL ARTICLE

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Detrimental effect of sinusoidal overperfusion after liver resection and partial liver transplantation

Received: 28 May 2003
Revised: 14 November 2003
Accepted: 5 March 2004
Published online: 23 April 2005

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Abstract Liver resection exposes the remaining sinusoids to an over-proportional blood flow. This mechanism may aggravate ischaemia/reperfusion damage and rejection in partial liver transplants. We studied the potential relevance of this mechanism for the pathogenesis of partial liver transplant dysfunction. Eighty-four isogeneic Lewis rats were divided into four groups: (I) sham operation; (II) partial liver resection (30% residual liver volume); (III) orthotopic transplantation of a full-size liver; (IV) transplantation of a reduced-size liver (30% transplant volume). Microcirculation was determined by intravital microscopy 90 min after surgery. Survival rates, liver function and morphology were monitored over a period of 14 days. Lowest survival rates and impaired liver function were observed after partial liver transplantation (group IV). These transplants displayed the lowest perfusion rate and an increased rate of leukocyte–endothelium interactions in the presence of a significantly increased sinusoidal blood flow velocity compared with those in groups I and III. Sinusoidal overperfusion in groups II and IV resulted in widespread endothelium lesions. Sinusoidal overperfusion seems to be a significant factor impairing liver function after liver resection. In addition to other adverse factors, such as ischaemia/reperfusion injury, it can contribute to the pathogenesis of postoperative dysfunction of partial liver transplants.

Keywords Partial liver transplantation · Liver resection · Overperfusion · Microcirculation · Sinusoidal endothelium

Introduction

Partial liver transplantation aims at transplanting small-for-size liver grafts, which are expected to regenerate during the postoperative course and ultimately result in a transplant volume appropriate to the body size of the recipient [1, 2, 3]. The outcome of partial liver transplantation essentially depends on an adequate graft volume size, on the one hand, and a volume sufficient for survival of the living-related transplant donor, on the other. Division of the liver into two grafts of equal size is impossible because of the liver segment anatomy [2]. Accordingly, the use of a larger liver graft provides an adequate liver volume but imposes an increased risk of morbidity to the living donor, whereas the smaller liver graft is often functionally inadequate [4, 5, 6, 7, 8].

The exact mechanism of the damage observed in small-for-size liver grafts is not entirely understood. Haemodynamic disturbances seem to be a major cause of postoperative transplant dysfunction, in addition to ischaemia, reperfusion damage, and rejection. It has been suggested that the partial liver graft is exposed to excessive portal perfusion, as observed after massive hepatectomy, and that portal decompression could improve transplant survival [7, 8, 9, 10].

We studied the influence of increased blood flow velocity on graft function in a rat model of partial liver transplantation. The study focused on potential morphological and functional correlates, such as direct damage to endothelial cells, microcirculatory disorders, and increased blood flow velocity. In order to distinguish between the resection-related lesions and the transplantation-associated lesions, we used partial and full-liver models as well as transplantation and non-transplantation models.

Materials and methods

Animals and study design

All operations and handling procedures were performed after approval by the veterinary district administration of Münster and were in accordance with the *Animal Protection Law* of Germany.

Eighty-four isogeneic male Lewis rats (250 g–300 g; Charles River, Sulzfeld, Germany) were randomly allocated to four experimental groups for either short-term experiments ($n=7$ per group), in which hepatic microcirculation was assessed by intravital microscopy, or for long-term experiments ($n=7$ per group), in which the rats were kept under observation up to the 14th postoperative day (Table 1).

In group I (sham operation, $n=14$) the liver was mobilised by division of the hepatic ligaments (Fig. 1a). In group II (partial liver resection, $n=14$) two-thirds of the liver were resected so that only the left lobe, which makes up approximately 30% of total liver volume, remained in situ (Fig. 1b). In group III (full-size-liver transplantation, $n=14$ per donor and recipient rat) we performed a full-size-liver transplantation with portal venous reperfusion and transplant arterialisation (model of Engemann, Fig. 1a) [11]. In group IV (partial liver transplantation, $n=14$ per donor and recipient rat) we performed a partial liver transplantation. For this, the donor operation comprised a size reduction of the transplant by a two-thirds hepatectomy, just as in group II. The partial liver graft (30% of the original liver volume) was then orthotopically transplanted with portal venous reperfusion and transplant arterialisation, just as in group III (Fig. 1b).

All procedures were carried out under nitrous oxide/isoflurane anaesthesia ($N_2O:O_2=2:1+1.5\%$ isoflurane). Preoperatively, the rats were starved for 24 h but had

Table 1 Experimental groups. *SHAM* sham operation (group I), *PLR* partial liver resection (group II), *FSLT*full-size-liver transplantation (group III), *PLT* partial liver transplantation (group IV)

Group	Number of animals (short-term experiments)	Number of animals (long-term experiments)	Total
I (SHAM)	7	7	14
II (PLR)	7	7	14
III (FSLT)	7 (donor) 7 (recipient)	7 (donor) 7 (recipient)	28
IV (PLT)	7 (donor) 7 (recipient)	7 (donor) 7 (recipient)	28
Total			**84**

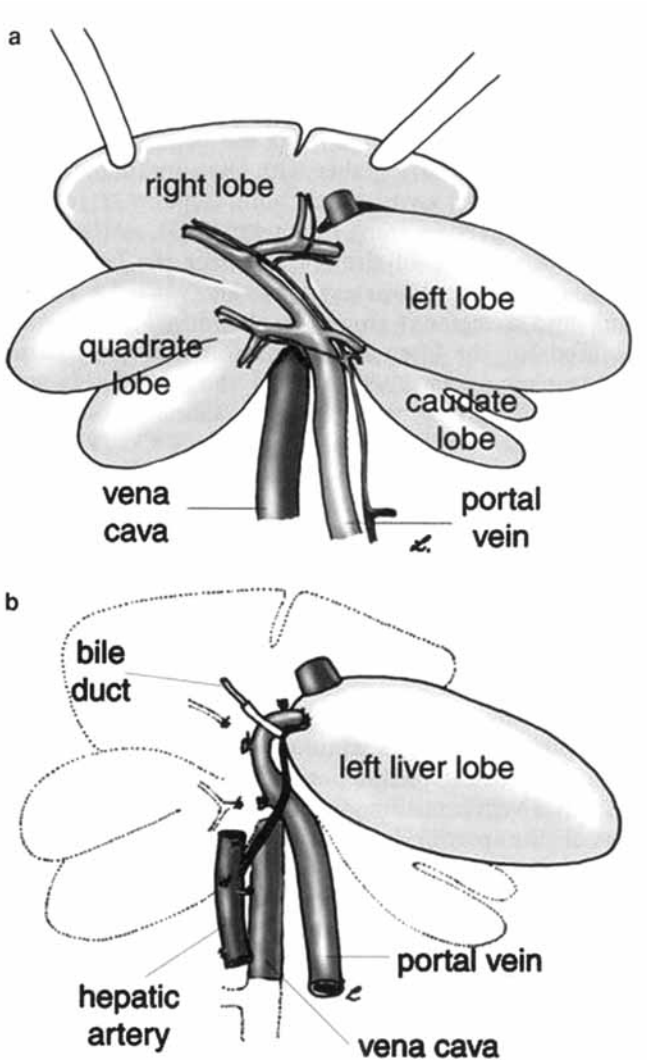

Fig. 1a,b Liver volumes in the full-liver groups (I and III) and partial liver groups (II and IV). Full-size liver groups (**a**) consisted of group I (sham operation) and group III (full-size liver transplantation) in which a full-size liver was orthotopically transplanted with portal venous and hepatic artery reperfusion. In the partial liver groups (**b**) two-thirds of the liver were resected so that only the left lobe, which makes up approximately 30% of total liver volume, remained in situ (group II) or served as partial liver transplant (group IV)

free access to water. Postoperatively, they received water and feed ad libitum.

Surgical technique

In all sham-operated animals (group I) after a median laparotomy the liver was mobilised by division of the hepatic ligaments.

Partial liver resection (group II) comprised a size-reduction of the liver by resection of the right, caudate and quadrate lobes, corresponding to a two-thirds hepatectomy. Only the left liver lobe (equivalent to 30% liver volume) remained.

Partial liver transplantation experiments (in group IV) were started with a size-reduction of the donor liver by a two-thirds hepatectomy (Fig. 1b). The partial liver transplant (30% of the original liver volume) was explanted together with the suprahepatic and infrahepatic vena cava, portal vein, hepatic artery and bile duct. A Teflon stent (diameter 26G) was inserted into the distal end of the bile duct for the later anastomosis. The hepatic artery, together with the coeliac trunk and a segment from the abdominal aorta, were prepared for the later artery anastomosis. The partial liver transplant was perfused via the portal vein and the hepatic artery with cold histidine–tryptophan–ketoglutarate (HTK) solution at 4°C (Koehler, Germany) for 5 min at a pressure of 10 mmHg and stored in HTK-solution at 4°C for 3 h until required for implantation.

The recipient's operation began with the hepatectomy of the recipient's liver. The partial liver transplant was then orthotopically placed in the recipient site. First, the suprahepatic vena cava was sutured end-to-end to the suprahepatic vena cava with 8-0 Monofil (Serag-Wiessner, Germany) with a running suture. Then, the transplant portal vein was sutured end-to-end with 10-0 Monofil to the recipient's portal vein. Immediately after the portal vein anastomosis had been finished, reperfusion of the partial liver transplant followed. Subsequently, the aortic segment was sutured end-to-side to the infrarenal abdominal aorta of the recipient with 10-0 Monofil with a running suture, and the partial liver transplant was reperfused arterially. The transplant bile duct was implanted into the recipient's bile duct with the splint technique [11].

The surgical technique of full-size-liver transplantation (group III) was similar except for the size reduction of the transplant in the donor operation.

Biochemistry

Preoperatively, and again 90 min after reperfusion and 1, 2, 7 and 14 days after operation, blood samples (200 µl; micro-method; Ektachem-Kodak) were drawn via a jugular vein catheter for assessment of the detoxication function of the liver by serum ammonium (NH_3) and bilirubin and, additionally, for the assessment of the synthetic liver function by protein. The degree of liver damage was assessed by measurement of the aspartate aminotransferase (AST) levels. NH_3 was determined at 4°C and bilirubin, AST, bilirubin and protein were determined at 37°C by standard enzymatic techniques.

Intravital microscopy

Intravital microscopy was performed in all rats under constant conditions of anaesthesia and temperature. Body temperature was kept between 36°C and 37°C by an adjustable heating system integrated into the table. A polyethylene catheter was inserted into the carotid artery for measurement of arterial blood pressure. Another polyethylene catheter, for injection of contrast medium, was inserted into the superior vena cava via the external jugular vein.

The hepatic microcirculation was studied on the lower surface of the left liver lobe, 60 min after the onset of reperfusion, by intravital fluorescence microscopy as previously described [12]. Sodium fluorescein (2 µmol/kg b.w. intravenously; Sigma, Deisenhofen, Germany) was used to enhance contrast for the assessment of sinusoidal perfusion, while rhodamine 6 G (0.2 µmol/kg b.w. intravenously; Sigma) was used for vital staining of leukocytes. Microcirculatory parameters were quantified off-line by frame-to-frame analysis of video-recorded microscopic images via the computer-assisted image-analysis system AnalySIS (SIS, Münster, Germany). A final magnification of 750 was applied for measurement of the microcirculation. Ten to 12 acini and 10–12 post-sinusoidal venules were randomly selected and observed for 30 s in each, per experiment.

The following five parameters were determined: sinusoidal perfusion rate [perfused sinusoids/total number of sinusoids observed (as a percentage)], sinusoidal diameters [measured in 100 sinusoids per liver both in the periportal and pericentral zone (in micrometres)] and diameters of post-sinusoidal venules (in micrometres); number of sticking leukocytes in sinusoids [stagnant leukocytes not moving during a period of 20 s (number per square millimetre)] and sticking leukocytes in post-sinusoidal venules [stagnant leukocytes not moving during a period of 20 s (number per square millimetre)]; number of rolling leukocytes in sinusoids [temporarily stagnant leukocytes with adhesion times between 0.2 s and 20 s (number per square millimetre)] and number of rolling leukocytes in post-sinusoidal venules [leukocytes rolling along the endothelial lining of post-sinusoidal venules (number per square millimetre)], as well as the velocity of free-flowing leukocytes in

sinusoids and post-sinusoidal venules (in micrometres per second) [12, 13, 14, 15].

Immunohistochemistry, light microscopy and electron microscopy

Ninety minutes after reperfusion (short-term experiments) or 14 days after operation (long-term experiments) the rats were killed. Successively, their liver volumes were determined after in toto explantation by water displacement, and liver specimens were taken.

For histological examination all liver specimens were fixed by immersion for at least 2 days in 4% formaldehyde solution and were subsequently dehydrated, embedded in paraffin wax and cut into 5-μm-thick sections. The sections were stained with Ehrlich's haematoxylin/eosin to display nuclei and cytoplasm. From all sections photomicrographs were taken of the periportal and pericentral zones of the liver lobule and from the corresponding region of the control livers in group I (sham operation). The sections were examined by light microscopy for proliferation, atrophy and necrosis of the hepatic parenchyma.

Immunohistochemistry, for labelling of Kupffer cells and sinusoidal endothelium, was determined from cryo-frozen tissue by the immuno-alkaline phosphatase (APAAP) method with fuchsin as chromogen and the antibodies ED1 (specific for Kupffer cells, dilution 1:400) and HIS-52 (rat endothelial cell antigen, RECA, dilution 1:40; Biozol Diagnostica, Eching, Germany). The slides were counted and assessed independently for the presence of APAAP-positive cells. For this purpose, the entire section was scanned, and approximately 150 square fields were examined with an eyepiece integration grid.

For transmission electron microscopy, liver specimens were cut into 1 mm×1 mm×1 mm blocks and immersed in 2.5% glutardialdehyde for 8 h. After being washed out in PBS buffer, the tissue specimens were fixed with 1% OsO_4 for 2 h, dehydrated in graded series of ethanol and embedded in epoxy. Ultra-thin sections were stained with uranyl acetate and lead citrate and viewed with a transmission electron microscope (CM-10, Philips, Germany).

For scanning electron microscopy, the liver specimens were cut into 2 mm×2 mm×5 mm blocks, dehydrated in ethanol, freeze-fractured in liquid nitrogen, prepared by critical-point drying, coated with ion-sputtered platinum, and viewed with a high-resolution scanning electron microscope (1530 VP, LEO, Germany). Photographs were taken at a magnification of ×20,000.

Statistics

All data are presented as means ± SD. Statistical analysis was performed by the Kruskal–Wallis test using SPSS software. A level of significance of $P < .05$ was considered as sufficient in all experimental groups.

Results

Survival rate and liver regeneration

In groups I and II all the rats survived up to the 14th day. In group III one rat died of cholestatic complications on the seventh postoperative day. In group IV five rats survived to the 14th day. Two rats died on the first postoperative day, all with pallor and discoloration of the liver in the presence of intact vascular anastomoses.

After 14 days all rats in the full-liver groups (groups I and III) showed a macroscopically homogeneous liver with normal histological architecture and a liver volume of 7.4 ± 0.4 ml (group I) and 8.0 ± 1.2 ml (group III). In the partial-liver groups (groups II and IV), after 14 days, all livers had regenerated and regained their original size, their volumes being 7.5 ± 1.2 ml (group II) and 9 ± 1.3 ml (group IV). In both transplantation groups (groups III and IV) the liver transplants showed a homogeneously perfused appearance with intact anastomoses, except for one animal in group IV in which the regenerated liver transplant had led to kinking of the bile duct.

Biochemistry

Preoperatively, all biochemical values lay within the normal range, and there were no significant differences between the groups.

Immediately after operation, liver damage in the transplantation groups III and IV was significantly more severe than in the non-transplanted rats in groups I and II. In all rats with partial livers (groups II and IV) the most severe liver damage was evident on the first postoperative day and was significantly more severe than in the full-size-liver groups (groups I and III). In the later postoperative course liver damage in the transplantation groups (groups III and IV) was still significantly great compared with that in the non-transplanted rats, returning to normal at day 14 (Table 2).

During the postoperative course, the detoxication function of the liver, measured in terms of NH_3 levels, was significantly more impaired in the rats with partial livers (groups II and IV) than in the full-liver groups (groups I and III). Immediately after the operation the NH_3 level after partial liver transplantation was significantly higher than in groups II and III, whose NH_3 levels were in turn significantly higher than those of the animals in group I. Whereas, after full-size-liver transplantation, the NH_3 level had already returned to normal after the second day, in the partial liver groups (groups II and IV) it remained significantly raised up to

Table 2 Biochemistry results. *SHAM* sham operation (group I), *PLR* partial liver resection (group II), *FSLT* full-size-liver transplantation (group III), *PLT* partial liver transplantation (group IV)

Parameter	Group	Pre-operatively	Ninety minutes after reperfusion	First post-operative day	Second post-operative day	Seventh post-operative day	Fourteenth post-operative day
ALT (U/l)	SHAM	21.9 ± 1.9	28.3 ± 4.3	44.7 ± 8.9	33.0 ± 5.8	29.9 ± 3.6	27.3 ± 3.9
	PLR	17.6 ± 3.2	50.3 ± 16.0[d]	838.0 ± 350.6	338.0 ± 165.9[d]	46.3 ± 11.8	56.8 ± 15.2
	FLT	28.0 ± 8.4	357.7 ± 83.7[e]	491.7 ± 281.4[e]	400.9 ± 505.3[e]	120.3 ± 122.9[e]	79.4 ± 97.2
	PLT	34.3 ± 16.2	289.7 ± 108.3[a,c]	1144.1 ± 435.2[c]	668.6 ± 333.4[c]	294.6 ± 369.1[a,c]	59.5 ± 20.2
NH_3 (μg/dl)	SHAM	68.7 ± 12.7	86.6 ± 22.5	39.7 ± 21.0	40.6 ± 24.2	58.7 ± 13.9	51.7 ± 18.5
	PLR	79.6 ± 25.9	166.7 ± 26.6[d]	172.3 ± 67.5	124.0 ± 41.0[d]	175.6 ± 57.5[d]	180.2 ± 68.7
	FSLT	63.5 ± 19.6	210.7 ± 11.0[e]	103.7 ± 38.9	54.4 ± 38.7	67.4 ± 22.8	102.5 ± 83.7
	PLT	69.7 ± 17.2	399.8 ± 141.3[a,b,c]	195.3 ± 16.9[a,c]	103.6 ± 58.2[a]	136.3 ± 79.1[a]	118.4 ± 38.3
Albumin (mg/dl)	SHAM	2714.3 ± 90	2328.6 ± 95.1	2371.4 ± 95.1	2428.6 ± 95.1	2500.0 ± 129.1	2885.7 ± 90.0
	PLR	2780.6 ± 24	2871.4 ± 298.4	2285.7 ± 211.6	2200.0 ± 216.0	2185.7 ± 134.5	2600.0 ± 260.8
	FSLT	2725 ± 198.2	1983.3 ± 421.5[e]	1814.3 ± 296.8[e]	2128.6 ± 597.0[e]	2675.0 ± 319.6	2650.0 ± 119.5
	PLT	2750 ± 0.0	2106.7 ± 90.2	1828.6 ± 111.3[c]	1957.1 ± 171.8[c]	2466.7 ± 196.6	2683.3 ± 160.2
Bilirubin(mg/dl)	SHAM	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
	PLR	0.2 ± 0.1	0.1 ± 0.1	0.4 ± 0.3	1.0 ± 1.3	1.6 ± 2.0	0.3 ± 1.5
	FSLT	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.3	0.1 ± 0.7	1.6 ± 4.0	0.2 ± 2.9
	PLT	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.4 ± 0.4	2.0 ± 2.1[a,c]	0.8 ± 3.2

[a] $P < 0.05$, PLT vs FSLT
[b] $P < 0.05$, PLT vs PLR
[c] $P < 0.05$, PLT vs SHAM
[d] $P < 0.05$, PLR vs SHAM
[e] $P < 0.05$, FSLT vs SHAM

the seventh postoperative day. On the 14th day the NH_3 levels of all rats had returned to normal range without significant differences between the groups (Table 2).

Biochemical liver functions, measured in terms of albumin levels, were more impaired in all rats in groups II, III and IV up to the second postoperative day than they were in the sham-operation group, but from the seventh day onwards they returned to normal without any significant differences between the groups (Table 2).

After liver transplantation, one rat from group IV showed significantly increased bilirubin level on the seventh day (Table 2).

Intravital microscopy

Measurement of arterial blood pressure (mean 90 ± 10 mmHg) and heart rate (mean 422 ± 12/min) and temperature (37.1 ± 0.5°C) revealed no detectable differences between any of the groups.

In all transplanted rats (groups III and IV) the perfusion rate was below 90% and was, hence, significantly lower than in groups I and II (Fig. 2a). Within the transplant groups (groups III and IV) and the non-transplant groups (groups I and II) no significant difference in perfusion rate was detected (Fig. 2a).

Leukocyte velocity (Fig. 2b, c) was significantly lower in rats with a full-size liver (groups I and III) than in rats with a partial liver (groups II and IV, Fig. 2b, c).

The periportal and pericentral sinusoidal diameters in the two transplantation groups, following full-size and partial liver transplantation, were significantly decreased in comparison with the non-transplanted rats (groups I and II, Fig. 2d). The diameter of the post-sinusoidal veins in group IV (partial liver transplantation) at 30.2 ± 4.3 μm was significantly lower than in the other groups (Fig. 2d).

A significant difference was found between the non-transplanted rats (groups I and II) and the transplanted rats (groups III and IV) with regard to the numbers of rolling and sticking leukocytes in the acinus. In groups III and IV there were significantly larger numbers of rolling leukocytes and sticking leukocytes than in the non-transplanted rats (Fig. 2e). With regard to leukocyte activation in the postsinusoidal veins, the number of rolling and sticking leukocytes after partial liver transplantation was significantly higher than in the non-transplanted rats (groups I and II). On the other hand, a significant difference between the full-size-liver groups (groups I and III) could not be detected (Fig. 2f).

Histology

Ninety minutes after reperfusion, light microscopy observation of sections from group I revealed normal liver architecture in all sections, without any conspicuous features of cellular damage or destruction in the

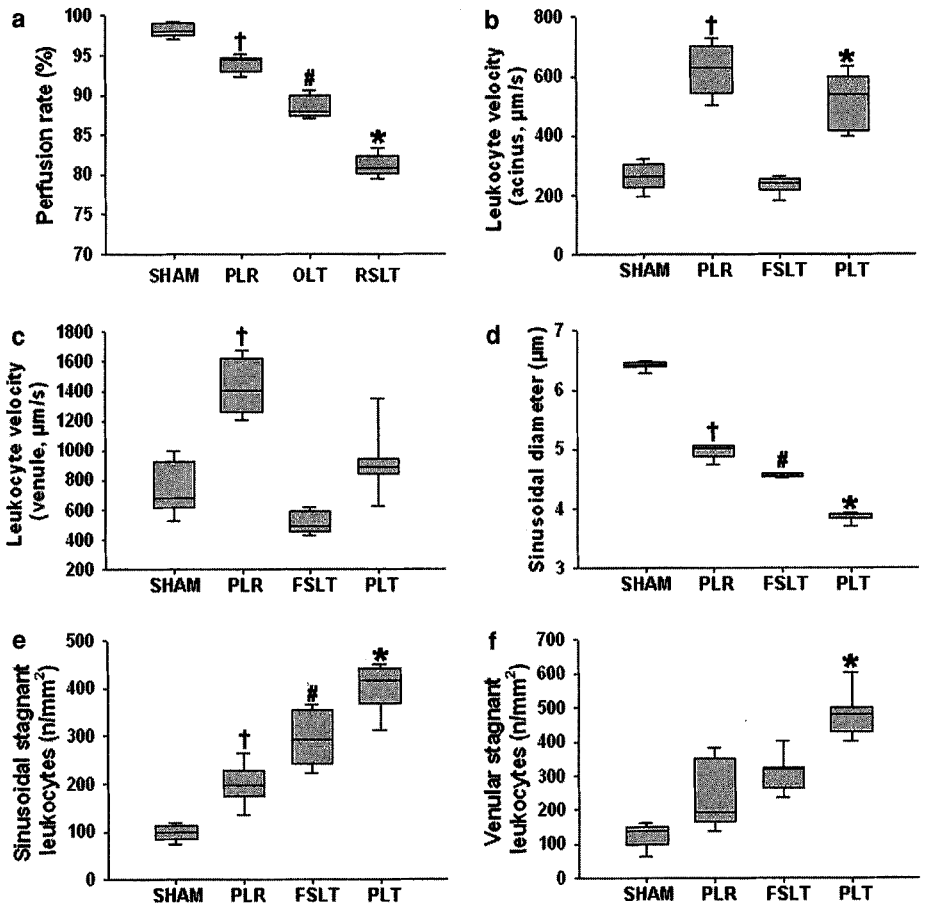

Fig. 2a–f Results of intravital microscopy. *SHAM* sham operation (group I), *PLR* partial liver resection (group II), *FSLT* full-size-liver transplantation (group III), *PLT* partial liver transplantation (group - IV). $*P < 0.05$, PLT (group IV) vs FSLT (group III); $\# P < 0.05$, FSLT (group III) vs SHAM (group I); † $P < 0.05$, PLR (group II) vs SHAM (group I)

hepatocytes and non-parenchymal cells. In group III a noticeable swelling of hepatocytes, with consequent narrowing of the sinusoids as evidence of an ischaemic cellular lesion, was visible. All rats that had undergone liver resection (groups II and IV) showed a characteristic pattern of damage in the hepatocytes by cytoplasmic vacuolisation (Fig. 3).

Immunohistochemical labelling of endothelial cell damage in the sinusoids showed moderate endothelial damage in group III and definitely more increased endothelial cell reactivity in the partial-liver groups (groups II and IV). Immunostaining with antibodies against Kupffer cells revealed moderate detection in group II and group III. In group IV there was the strongest expression of the marker in the sinusoids, with a distribution gradient increasing from the periportal tracts towards the central vein (Fig. 3).

In group I transmission and scanning electron microscopy revealed an intact liver ultrastructure with a regular appearance of sinusoidal endothelium with normal endothelial fenestrations and Disse's space. Ninety minutes after full-size-liver transplantation (group III), the sinusoidal endothelium was still intact, though the density and the diameter of the endothelial fenestrations was clearly smaller than in group I. All rats that had undergone liver resection (groups II and IV) presented a characteristic ultrastructural alteration with absence of fenestrations of openings and cytoplasmatic broadening of sinusoidal endothelium. In addition, after partial liver transplantation (group IV) we observed patchy endothelial cell desquamation, resulting in a complete loss of endothelium in certain areas and leading to exposure of underlying hepatocytes (Fig. 4).

Discussion

The continuous expansion of indications for liver transplantation has led to increased demand for donor organs that cannot be adequately met. For this reason, the technique of partial liver transplantation has been developed, either by dividing a liver graft or by the use of livers from living donors [16]. In the light of the favourable results from the use of size-compatible partial liver transplants for children, partial liver transplants have recently been used for adults as well [17].

The clinical outcome of partial liver transplants is, however, significantly poorer than that after full-size-

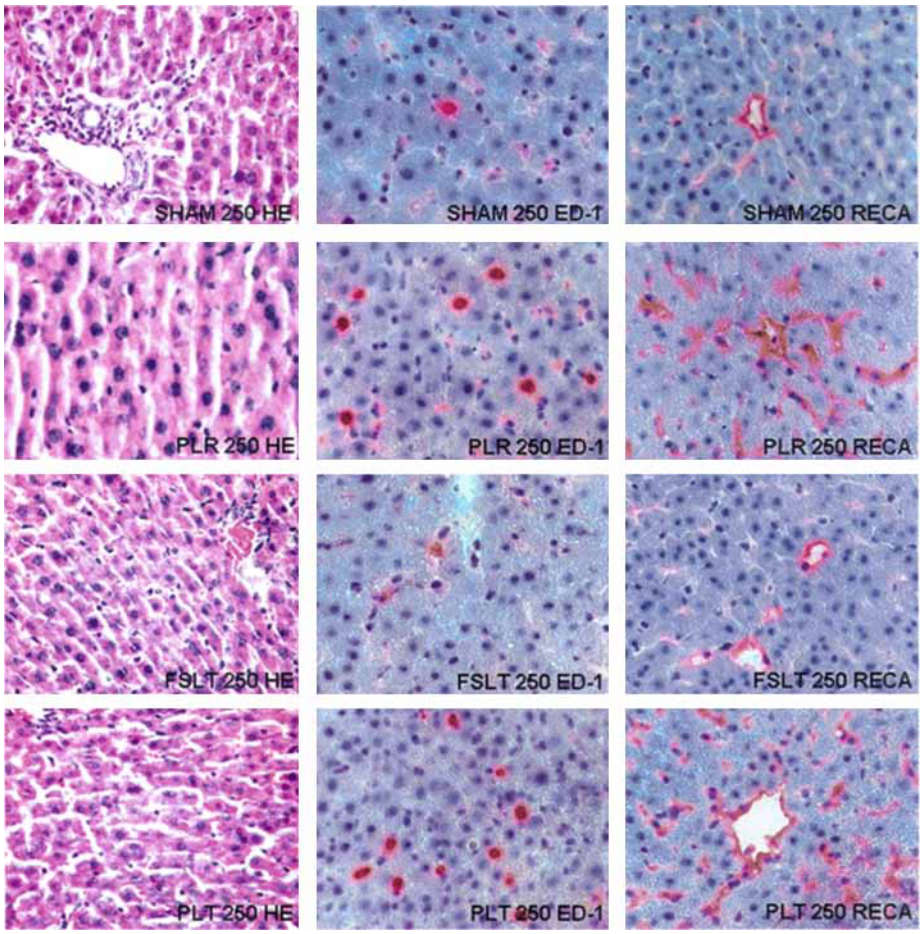

Fig. 3 Light microscopy and immunohistology results. *Left column* haematoxylin/eosin (*HE*) staining, *middle column* Kupffer cell labelling antibodies (*ED-1*), *right column* endothelial cell antigen (*RECA*); magnification ×250

liver transplantation. Although liver resections with substantial reduction in parenchymal volume of up to 80% are usually well tolerated by patients, comparable reductions in liver transplants are frequently complicated by a clinically manifest disorder of liver function, known as "small-for-size syndrome" [4, 5, 18]. The details of the pathomechanism of the small-for-size syndrome of partial liver grafts are still largely unknown. It had been postulated that extensive hepatectomy may cause a harmful increase in blood flow velocity and haemodynamic disturbance in the liver, and that these adverse consequences could, presumably, be minimised by portal decompression, e. g. by portocaval shunting [1, 7, 8, 9, 10].

The present study addresses the consequences of an increased flow rate observed after liver resection on postoperative liver function. We performed all experiments in isogeneic donor–recipient combinations, in order to exclude the confounding factor of potential rejection. By comparing partial liver transplantation with full-size-liver transplantation and with partial liver resection without transplantation, we were able to observe the effects of resection-related increased flow rate separately from those arising from the transplantation-associated damage due to ischaemia/reperfusion injury.

In support of our hypothesis, we show that an increased flow rate indeed has a distinct potential for damage after liver resection, which, in addition to other damaging mechanisms, contributes to clinically manifest postoperative dysfunction in partial liver grafts. By means of electron and scanning electron microscopy supplied by immunohistochemistry we have demonstrated that the increased blood flow velocity following liver resection (groups II and IV) leads to damage to the endothelium with the morphological correlates of swelling of endothelial cells, endothelial desquamation, loss of Disse's space and loss of endothelial cell fenestration. These lesions are associated with a reduction in the exchange surface between liver parenchyma and metabolites in the blood and could, therefore, be regarded as being responsible for the biochemically proven deterioration in detoxication capability of the partial liver groups (groups II and IV) compared with the full-size-liver groups (groups I and III).

Furthermore, intravital microscopy demonstrated a typical pattern of damage to the microcirculation after

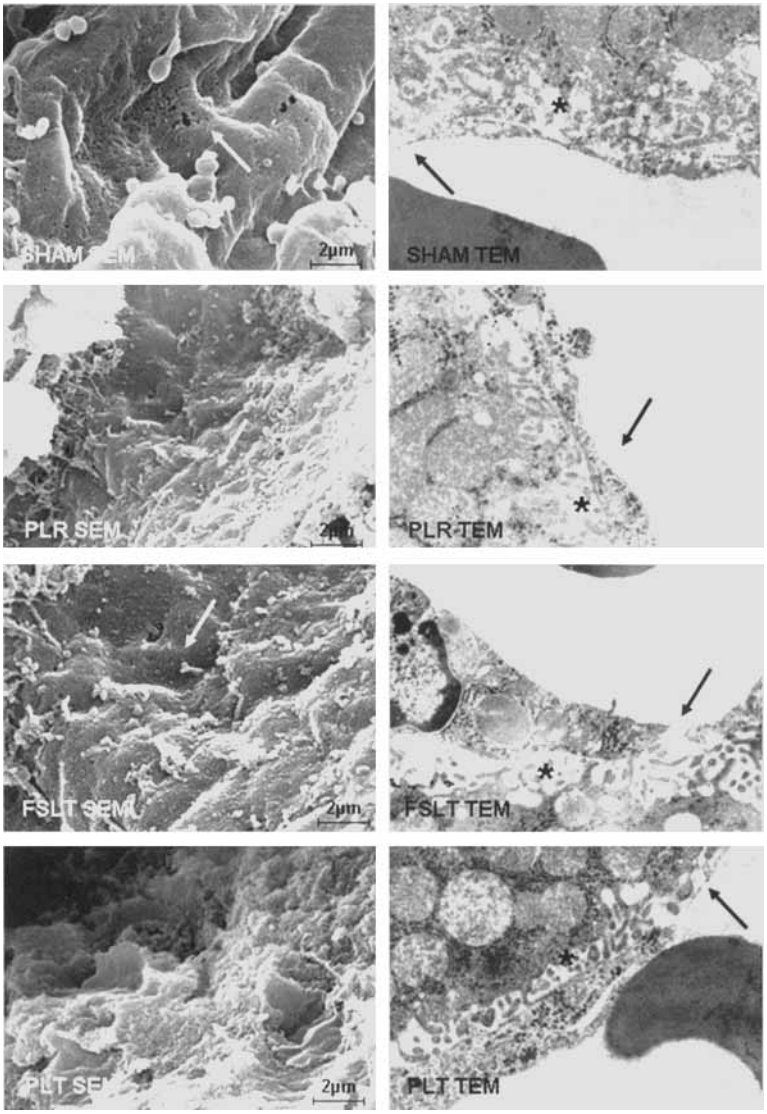

Fig. 4 Ultrastructure results. *Right column* transmission electron microscopy (*TEM*), *left column* scanning electron microscopy (*SEM*). *Arrows* sinusoidal endothelium, * Disse's space. *SHAM* sham operation (group I), *PLR* partial liver resection (group II), *FSLT* full-size-liver transplantation (group III), *PLT* partial liver transplantation (group IV). Magnification ×20,000

liver resection (groups II and IV), with a significant increase in flow rate, sinusoidal vasoconstriction, leukocyte adhesion and diminished perfusion rate. Owing to the significant reduction of perfusion rate, the functional liver mass following liver resection was still further reduced. It can also be postulated that in those areas that are still perfused, metabolic exchange will be further restricted by the increased flow rate, which shortens the contact time between the blood constituents and the liver parenchyma. As possible causes for the vasoconstriction and increased leukocyte adhesion observed after liver resection, we might suggest the demonstrable endothelial cell damage or a diminished oxygen input caused by the shortened contact time between blood and liver parenchyma due to the increased flow rate, leading to increased release of vasoconstrictors, e.g. endothelin-1, and increased expression of adhesion molecules [19, 20, 21, 22].

Both the parenchymal lesions shown at ultrastructural level and the microcirculatory disorders revealed by intravital microscopy were responsible for the liver damage demonstrated in the present study by biochemical parameters and the diminished synthetic and detoxification capabilities that follow liver resection.

In partial liver transplants, the unavoidable ischaemia/reperfusion damage resulting from transplantation can be subdivided into a vascular component, with sinusoidal vasoconstriction, an inflammatory reaction, with increased leukocyte–endothelium interaction, and damage by reactive oxygen radicals [19, 22]. All these factors will be superimposed and will act in concert [19, 21, 22, 23, 24]. In the present study the ischaemia/reperfusion lesions were revealed morphologically by swelling of the hepatocytes and subsequent narrowing of sinusoids, by increased Kupffer cell activation and damage to the sinusoidal endothelium, and by a reduced

number and diameter of endothelial fenestrations [23]. At microcirculatory level, comparison with the non-transplantation groups (groups I and II) showed a significantly lower perfusion rate, sinusoidal vasoconstriction and increased leukocyte activation. Biochemically, in agreement with the haemodynamic and histological changes on the first day, we found significantly more severe liver damage than in the non-transplantation groups (groups I and II).

It is conceivable that the morphological and microcirculatory lesions observed after partial liver transplantation may be triggered by the increased blood flow velocity rate and the ischaemia/reperfusion lesions, thus generating a vicious circle. This phenomenon may be regarded as the cause of the poorer survival in group IV. In clinical practice other factors, such as previous liver damage, e.g. fatty change, or immunological reactions, such as acute rejection, may further impair transplant function. Reliable prognosis of liver function after partial liver transplantation is, therefore, crucial for the success of such partial liver transplantation, especially when marginal donor organs or living-donor organs have been employed.

In conclusion, the status of the microcirculation after partial liver transplantation is closely correlated with postoperative liver function, and there is a specific pattern of microcirculatory damage, together with the resection-induced increase in flow rate and the ischaemia/reperfusion lesions [24]. In the light of these results it might be conceivable that, in future, a technique for rapid and reliable assessment of the microcirculation in partial liver transplants, performed immediately postoperatively, would be able to offer prognostic guidance regarding graft function and the probability of regeneration. However, owing to the elaborate set-up required, intravital microscopy is of limited value in clinical practice. Thus, simpler methods for the examination of the microcirculation, such as the recently introduced technique of orthogonal reflex spectrophotometry, which has already been applied to humans in full-size liver transplantation, should be investigated [25].

Furthermore, an improvement of the microcirculation in partial liver transplants, in particular in reducing sinusoidal overperfusion, e. g. by pharmacological regulation of sinusoidal diameter and perfusion or even by portal decompression via a mesocaval shunt, might offer new and interesting therapeutic approaches of clinical relevance in the near future [26].

Acknowledgements Dr. D. Palmes was supported by a grant from the Else Kröner-Fresenius-Stiftung. We thank Mrs. K. Cebulla and Mrs. G. Krull for their technical assistance, Dr. M. Langer for the excellent drawing and M. Bühner, Department of Physics, for his technical assistance in performing scanning electron microscopy.

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