Transplant International ISSN 0934-0874

ORIGINAL ARTICLE

Auxiliary liver transplantation with flow-regulated portal vein arterialization offers a successful therapeutic option in acute hepatic failure – investigations in heterotopic auxiliary rat liver transplantation

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Keywords
acute hepatic failure, animal models, experimental transplantation, heterotopic auxiliary liver transplantation, orthogonal polarization spectral imaging, portal vein arterialization, rats, regulation of the portal blood flow.

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[1]Principles of Laboratory animal care were followed, as well as the current version of the German Law on the Protection of Animals. The experimental protocol was reviewed and approved by the Animal Care Committee of the District President of Cologne, FRG (K 40, 17/99).

Received: 17 October 2005
Revision requested: 10 November 2005
Accepted: 23 January 2006

doi:10.1111/j.1432-2277.2006.00291.x

Summary

Heterotopic auxiliary liver transplantation (HALT) with portal vein arterialization (PVA) was proposed in acute hepatic failure (AHF). However, clinical results of PVA are controversial because of lacking standardized flow-regulation. In rats, we examined HALT with flow-regulated PVA in AHF. Group A: HALT with flow-regulated PVA and 85% resection of the native liver to induce AHF [acute experiments ($n = 8$), killing after 7 days ($n = 8$) and after 6 weeks ($n = 11$)]. Group B: 85% liver-resection ($n = 10$). The average blood-flow in the arterialized portal vein in HALT achieved normal values (1.7 ± 0.4 ml/min/g liver-weight). After reperfusion, the diameters of the sinusoids (6.4 ± 0.6 μm), the postsinusoidal venules (31.1 ± 3.3 μm) and the intersinusoidal distance (17.9±0.7 μm) also achieved normal values. The functional sinusoidal density amounted to 335 ± 48/cm. The 6-week survival was nine of 11 with excellent liver function (Quick's value: 110% ± 7.8%). The hepatobiliary radioisotope scanning with (^{99m}Tc) ethyl hepatic iminodiacetic acid (EHIDA) showed no significant differences between the native livers and grafts. The hepatocellular morphology was regular, apart from low-grade necroses in two grafts. The grafts' sinusoidal endothelial cells did not show any morphological changes. In group B, however, all rats died from AHF within 6 days. HALT with flow-regulated PVA achieved good results regarding microcirculation, morphology and function and can reliably bridge AHF.

Introduction

Acute hepatic failure (AHF) is defined as a syndrome of severe hepatic dysfunction and hepatic encephalopathy in individuals with no evidence of preexisting liver disease; its mortality without transplantation ranges between 60% and 80% [1]. Orthotopic liver transplantation has markedly improved the prognosis of patients with AHF and is recommended, when spontaneous recovery appears unlikely. A US multicentre study showed a 1-year-survival rate of 76% after liver transplantation for AHF [2]. This result is obtained at the price of lifelong immunosuppression and the associated long-term risks (infection and tumor growth) and considerable costs.

On the other hand, the enormous regeneration potential of the liver is well known: most patients surviving AHF

without liver transplantation experienced a complete morphological and functional recovery of their native liver [3]. Therefore, auxiliary liver transplantation is an interesting alternative approach in AHF. After regeneration of the native liver, the immunosuppression can be discontinued and the liver graft can be explanted or left to atrophy [4].

The European Auxiliary Liver Transplant (EURALT) study group compared auxiliary versus orthotopic liver transplantation for AHF [5]. One-year-patient survival did not differ between standard orthotopic (61%) and auxiliary liver transplantation (62%) for AHF. In that study, 65% of the patients surviving auxiliary liver transplantation were free of immunosuppression within 1 year. However, portal vein thrombosis was more frequent after both heterotopic auxiliary liver transplantation (HALT; 42%) and auxiliary partial orthotopic liver transplantation (APOLT; 14%) than after standard orthotopic liver transplantation (0.5%).

Heterotopic auxiliary liver transplantation with portal vein arterialization (PVA), which avoids portal vein thrombosis of the auxiliary graft, was first described by Erhard *et al.* [6]. The main advantage of this approach is that it leaves the hilum and the portal vein of the native liver untouched, which may reduce the incidence of possible complications such as portal vein thrombosis and disturbance of native liver recovery because of portal flow steal phenomenon. Furthermore, this technique avoids the surgical trauma of more extensive liver dissection of the native liver and the graft, leading to reduced postoperative bleeding and more remaining liver mass for bridging (graft) and regeneration (native liver). The clinical results demonstrated, that a sufficient liver function can be achieved using this technique [7,8]. In the last years, artificial liver support like molecular adsorbents recirculating system (MARS) seemed to have potential for bridging an acute liver failure and auxiliary liver transplantation was neglected. However, new clinical studies have demonstrated, that MARS treatment had no significant survival benefit on patients with liver failure when compared with standard medical therapy [9]. As MARS has not lived up to expectations, auxiliary liver transplantation should be reconsidered as a therapeutic concept for AHF.

The clinical results of orthotopic liver transplantation with PVA have been controversial [7,8,10–13]. The lack of flow regulation in the arterialized portal vein of the graft might be responsible for the problems observed. Therefore, investigations of flow-regulated PVA in liver transplantation are needed.

In former experiments, we performed HALT with PVA in AHF, but we did not examine the portal vein flow, the microcirculation, or the histology of the grafts [14]. In these experiments, we carried out more detailed investigations:

1 Examination of the microcirculation, morphology and function of an auxiliary graft with flow-regulated PVA,
2 Therapy of acute liver failure by HALT with flow-regulated PVA.

For this purpose we modified our technique of HALT with standardized PVA in the rat [15], using stents with different diameters to achieve adequate blood flow in the arterialized portal vein. Those pilot experiments with different stent diameters had shown, that – using a stent of 0.3 mm inner diameter – a physiological average blood flow in the arterialized portal vein was achieved.

Materials and methods

Animals and experimental groups

Syngeneic male Lewis rats (Charles River Wiga GmbH, [1]Sulzfeld, Germany) weighing 375 ± 24 g were operated upon. The animals were given water as well as standard laboratory rat chow *ad libitum*[1].

Donor and recipient operations were performed under ether inhalation anesthesia. At the end of these experiments, the animals were killed by an ether overdose.

The operation procedure has been described before [15]. In summary, the right lobe and the caudate lobes of the graft (approximately 30% of a whole liver mass) were perfused with histidine-tryptophan-ketoglutarate (HTK)-solution (Custodiol, Fa. Köhler, Germany) and were implanted into the right renal fossa of the recipient. Using an operating microscope (Zeiss, Jena, Germany, 16- to 25-fold magnification), the graft's infrahepatic caval vein was anastomosed to the recipient's caval vein end-to-side (8–0 Ethilon, running suture). The graft's portal vein was anastomosed to the recipient's right renal artery in stent technique (polyurethane catheter: 0.3 mm inner diameter, 8 mm length) end-to-end. Using stents with a standard inner diameter, this technique allows an arterial-venous anastomosis with standardized flow rates (Fig. 1). The celiac trunk of the graft was anastomosed to the recipient's infrarenal aorta end-to-side. In the long-term experiments, the stented bile duct (polyurethane tube, Abbocath, Wiesbaden, Germany, 20-G) was inserted into the duodenum. Approximately 85% of the liver mass of the native liver was resected to induce an AHF [16]. Cold ischemia was 64 ± 7 min; warm ischemia was always <25 min.

Three experimental groups were formed:

Group A: HALT with flow-regulated PVA and 85% resection of the native liver to induce AHF. In group A evaluation was performed at three different points of time:

Acute experiments: 30 min after reperfusion of the portal vein, the blood flow in the portal vein was measured and orthogonal polarization spectral (OPS) imaging was performed on the right lobe of the graft. Ninety minutes

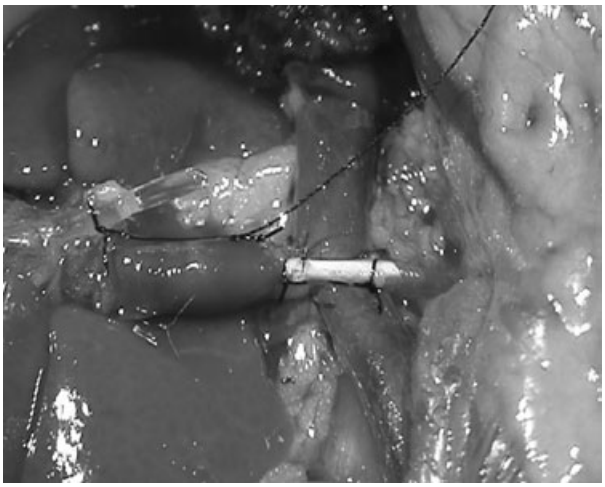

Figure 1 Macroscopic aspect 30 min after reperfusion of the portal vein during HALT with PVA.

after portal reperfusion, the bile flow was measured during an observation period of 60 min. After this, blood samples were taken for biochemistry [aspartate aminotransferase activity (ASAT) and alanine aminotransferase activity ALAT)], and at the end of these acute experiments, the grafts were explanted for histological and immunhistological examination ($n = 8$ transplantations).

Medium-term experiments: after 7 days, OPS imaging was performed on the right lobe of the graft. Blood samples were taken for biochemistry (ASAT, ALAT, Quick's value and AT III), histological and immunhistological examinations were performed ($n = 8$ transplantations).

Long-term experiments: the body weight was measured weekly. After 6 weeks, we performed hepatobiliary radioisotope scanning with (^{99m}Tc)-ethyl hepatic iminodiacetic acid (EHIDA) in order to differentiate the function of the native liver and the graft. Quick's value and AT III were determined as indicators of synthetic hepatic function. At the end of these long-term experiments, the native liver and graft were explanted and weighed. Histological and immunhistological examinations were performed ($n = 11$ transplantations).

Group B: resection of 85% of liver mass to induce AHF ($n = 10$).

Group C: laparotomy (sham group) ($n = 8$). After laparotomy, OPS imaging was performed to obtain normal values.

Blood flow measurement in the portal vein

The blood flow in the portal vein was measured using a Transonic^R Animal Research Flowmeter T 206 (Transonic Systems Inc., Ithaca, NY, USA), which was equipped with a perivascular ultrasonic volume flow-sensor (1.5 RB perivascular probe). The flow was detected by means of the Doppler effect.

Orthogonal polarization spectral imaging

Hepatic microcirculation was evaluated by OPS imaging, a reflection spectroscopy technique, allowing real-time observation of hepatic microcirculation *in vivo* without the use of a fluorescent dye. A detailed description of this process has been published recently [17,18]. The Cytoscan device (Cytoscan^{TM}, Cytometrics, Inc., Philadelphia, PA, USA), now available from Rheologics (Exton, PA, USA), was used. The probe was gently positioned on the lower surface of the right liver lobe under physiologic saline immersion without compression of the liver parenchyma. This has been described previously [19]. During each examination, 10 different liver acini and postsinusoidal venules of the right liver lobe were visualized, scanning the lower surface of the lateral right liver lobe from caudal to cranial. Because of extensive adhesions between the graft's surface and the small bowel, OPS imaging could not be performed after 6 weeks. A final magnification of 465-fold was achieved on the video screen. The analysis of the acquired data was carried out utilizing a computer-assisted microcirculation analysis system (Cap-Image, Dr Zeintl, Heidelberg, Germany). Cap-Image is the current state-of-the-art program, which is used to quantify data from images obtained using intravital microscopy. The analysis of microvascular parameters by Cap-Image has been described in detail previously [20]. The following parameters were analyzed:

1 Sinusoidal diameter (D) of the midzonal segment of each acinus (μm): 10 sinusoids were examined per acinus,
2 Intersinusoidal distance (ISD; μm),
3 Red blood cell velocity within the sinusoids (RBCV; μm/s),
4 Diameter of the postsinusoidal venules (μm) and
5 Functional sinusoidal density (FSD): it is defined as the length of red blood cell perfused sinusoids per observation area (1/cm).

Bile production measurement

During the donor operation, a 20-G polyurethane stent was inserted into the bile duct. A plastic tube (1.1 mm inner diameter) was placed onto the stent 90 min after portal reperfusion. Bile production was calculated from the bile outflow into the plastic tube during a time period of 60 min.

For biochemical analysis, blood samples were collected at the end of the experiments by puncture of the aorta.

ALAT, ASAT and parameters of liver-synthesis (Quick's value and AT III) were determined.

Hepatobiliary radioisotope scanning was performed using (^{99m}Tc)-EHIDA, a gamma-emitter. After intravenous injection, (^{99m}Tc)-EHIDA is bound to plasma-proteins. It is taken up by the hepatocytes by active transportation (like bilirubin) and it is then secreted into the bile ductuli. (^{99m}Tc)-EHIDA with an activity of 2 MBq was injected intravenously. Tissue samples of the native liver and the graft were taken after 4 min. Blood and tissue samples of both livers and of the duodenum were taken after 7 min. The rat was then killed. The activity per gram of tissue was determined in a scintillation counter; this activity was calculated as fraction of the applied activity.

Liver weight

In the acute experiments, the graft was weighed and the weight of the remaining native liver was calculated by the following formula: complete liver weight [2.66% of body weight (this value with a standard deviation of ±0.49% was determined in previous experiments with $n = 80$ animals of the same strain and age)] – weight of the resected liver lobes. In the medium-term and long-term experiments, the graft and the remaining native liver were weighed after the killing by an ether overdose.

Histology

The graft's right liver lobe was placed in 4% buffered formalin. The tissue was embedded in paraffin and processed further using standard histological procedures. The slides were stained with hematoxylin and eosin.

Immunhistology

Immunohistochemical staining was performed using anti-CD 31 monoclonal antibody specific for the endothelial cell marker CD 31 (mouse anti rat CD 31, Serotec, Düsseldorf, Germany).

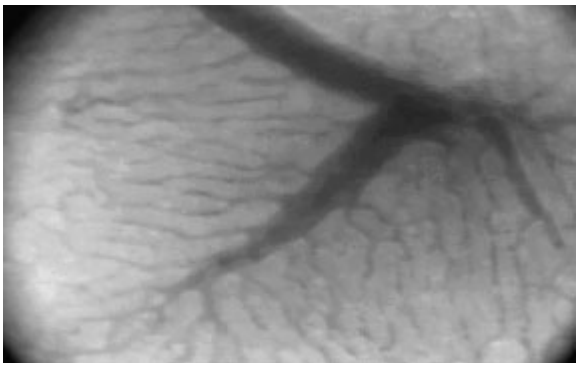

Figure 2 OPS imaging after reperfusion of the portal vein and the hepatic artery during HALT with PVA, showing a hepatic acinus with a physiological configuration.

Statistics

All calculations were performed using SPSS for Windows version 12.0.1 (SPSS Inc. Chicago, IL, USA). Data are presented as mean ± SD. Continuous parameters were compared by a two-sided *t*-test for unpaired data. *P*-values ≤0.05 were considered statistically significant.

Results

Acute experiments

In group A1, the macroscopic aspect showed a homogeneous reperfusion of the graft (Fig. 1). The average blood flow in the arterialized portal vein in HALT was within the normal range (1.7 ± 0.4 ml/min/g liver weight). OPS imaging also demonstrated homogeneous perfusion-patterns (Fig. 2). After portal and arterial reperfusion, the FSD amounted to 335 ± 48/cm. It was significantly lower than the normal value (439 ± 22 /cm), which we had determined directly after laparotomy. The diameters of the sinusoids (6.4 ± 0.6 μm), the postsinusoidal venules (31,1 ± 3,3 μm) and the ISD (17.9 ± 0.7) were in accordance with the normal values (Table 1). Ninety minutes after reperfusion of the portal vein, the bile-production amounted to 27 ± 8 μl/h/g liver weight and the values of

	HALT with flow-regulated PVA, acute experiments	HALT with flow-regulated PVA, after 7 days	Normal values
Functional sinusoidal density (1/cm)	335 ± 48	398 ± 20	439 ± 22
Sinusoidal diameter (μm)	6.4 ± 0.6	7.05 ± 0.4	6.5 ± 0.4
Intersinusoidal distance (μm)	17.9 ± 0.7	19.8 ± 0.7	19.3 ± 1
Red blood cell velocity in the sinusoids (μm/s)	223 ± 51	164 ± 16	157 ± 10
Diameter of the postsinusoidal venules (μm)	31.1 ± 3.3	35.4 ± 3.8	30.9 ± 3.5

Table 1. Parameters of microcirculation, measured 5 min after reperfusion of the portal vein and hepatic artery after HALT with flow regulated PVA ($n = 8$), 7 days after HALT with flow regulated PVA ($n = 8$) and directly after laparotomy in native livers to obtain normal values ($n = 8$).

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the transaminases reached 698 ± 386 U/l for ASAT and 643 ± 296 U/l for ALAT.

Histology

In group A1, one animal showed slight intraparenchymatous tissue damage. In another animal, a slight vacuolization of hepatocytes was seen in zone I. Beyond these findings, no further tissue damage and no edema formation was visible. The sinusoidal endothelial cells of the grafts, stained by anti-CD 31 monoclonal antibody, did not show any morphological changes, in particular there was no disruption of the sinusoidal lining.

Medium-term experiments

After 7 days, the ASAT and ALAT values decreased to 301 ± 166 U/l and 153 ± 61 U/l, respectively. The parameters of liver-synthesis were within the normal range (Quick's value: 128% ± 3%, AT III: 125% ± 20%). The results of OPS imaging are shown in Table 1. There were no significant differences to the normal values regarding diameters of sinusoids ($P = 0.09$), ISD ($P = 0.4$), RBCV in the sinusoids ($P = 0.5$) and the diameters of the post-sinusoidal venules ($P = 0.14$). The FSD had increased within the first postoperative week, but again it was significantly lower than the normal value ($P = 0.01$). The sinusoidal endothelial cells of the grafts did not show any pathological morphology.

Long-term experiments

In group A III, the 6-week survival rate was nine of 11 animals. Two rats died from peritonitis in the first postoperative week, caused by leakage of the choledochoduodenostomy. On average, the transplanted animals lost 28 g of body weight in the two first postoperative weeks (from 389 ± 16 to 361 ± 25 g). Thereafter, the body weight increased steadily. After 6 weeks, the weight amounted to 440 ± 9 g and was significantly above the initial weight (389 ± 16 g, $P \leq 0.001$). The animals' general health condition was excellent. The parameters of liver-synthesis were within the normal range (Quick's value: 110% ± 7.8%, AT III: 104 ± 6%).

In the hepatobiliary radioisotope scanning, there were no significant differences between native liver and graft, 4 and 7 min after injection of (^{99m}Tc)-EHIDA, with low activity in the blood and high activity in the duodenum (Fig. 3).

After 6 weeks, regeneration of the native liver had occurred with an increase of the liver-weight (from 2.3 ± 0.8 to 9.8 ± 1 g) and regular histology. By this time, the graft was atrophic with a decrease of liver

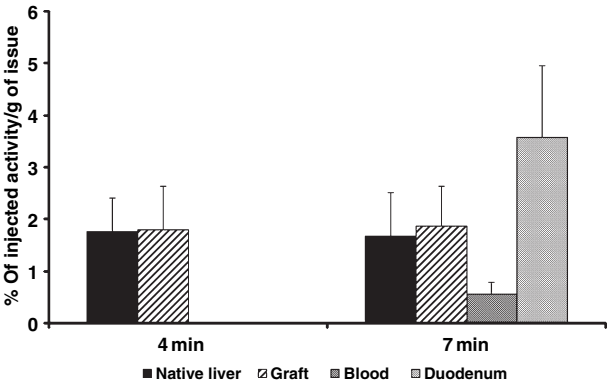

Figure 3 Hepatobiliary radioisotope scanning, 4 and 7 min after injection of (^{99m}Tc)-EHIDA, $n = 9$ rats (blood and duodenal activity was only measured at 7 min).

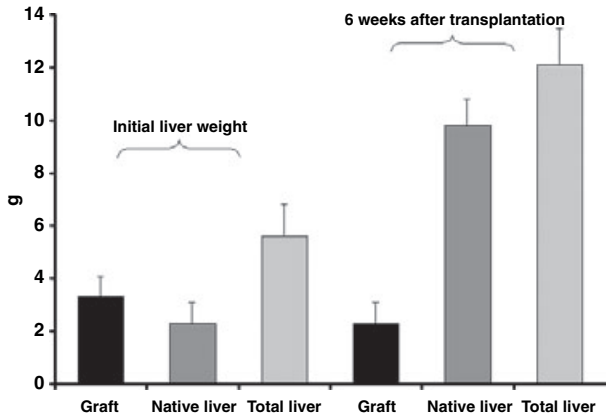

Figure 4 Weight of graft, native liver and total liver weight at the time of operation and after 6 weeks, $n = 9$ rats.

weight from 3.3 ± 0.8 to 2.3 ± 0.8 g. The total weight of both livers was within the normal range of liver weight/body weight (Fig. 4).

Histology

The hepatocytes were regular, without edema formation or fatty degeneration at 6 weeks after transplantation. Only in two grafts, low-grade intraparenchymatous hepatocellular necroses were recognized. Proliferation of bile ductuli and cholangitis because of sludge formation could be seen in all grafts (Fig. 5). A continuous layer of endothelial cells stained by anti-CD 31 monoclonal antibody could be detected without any morphological change or disruption (Fig. 6).

In group B (AHF without transplantation), the ASAT values reached 1964 ± 413 U/l (ALAT: 1984 ± 525 U/l) and albumin decreased to 25.3 ± 0.4 g/l on the first

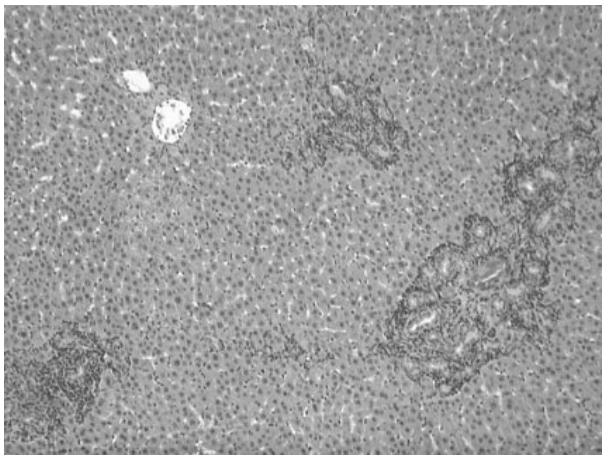

Figure 5 Microscopic aspect (hematoxylin and eosin, histology) 6 weeks after HALT with flow-regulated PVA, showing good preservation of hepatic architecture and slight proliferation of bile ductuli because of sludge formation in the bile duct.

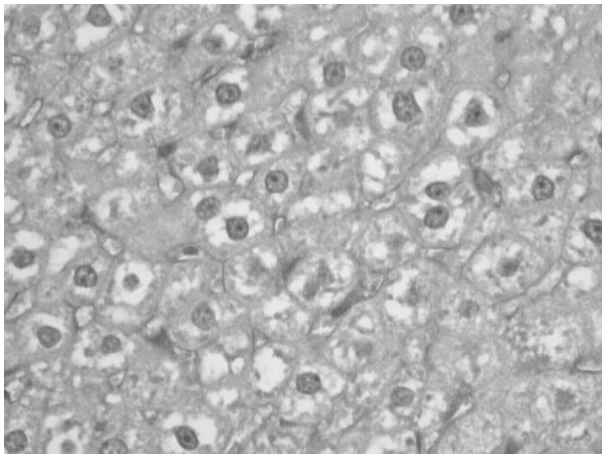

Figure 6 Immunhistochemistry: endothelial cells stained by anti-CD 31 monoclonal antibody, 6 weeks after HALT with flow-regulated PVA.

postoperative day. The rats became comatose or suffered from intra-abdominal bleeding. All animals died from AHF within the first six postoperative days.

Discussion

Blood flow regulation in the arterialized portal vein

Permanent PVA has been proposed for auxiliary liver transplantation in AHF, in order to avoid portal blood steal phenomenon, portal vein thrombosis and primary nonfunction. Erhard *et al.* [7] and Margerit *et al.* [8] reported promising results in a total of five patients. In experimental auxiliary liver transplantation with PVA, the results were controversial. In heterotopic rat liver transplantation with PVA, Aguirrezabalaga achieved good results with 100% survival in a short-term experiment of 7 days [21]. Hong, however, reported spontaneous intra-operative rupture of the graft capsule, severe bleeding, and portal and sinusoidal congestion [22]. In those two experiments in rats, no intra-operative blood flow quantification and regulation in the arterialized portal vein was performed.

In former experiments we performed HALT with hyperperfusion of the arterialized portal vein. In those hyperperfusion experiments we found massive disturbances of the microcirculation. The 6 week survival rate was 82%. However, six of nine grafts had shown massive hepatocellular necroses after 6 weeks [23].

In order to avoid the negative effects of portal hyperperfusion on the graft's microcirculation and morphology, we regulated the portal blood flow in HALT with PVA. Using a stent with 0.3 mm inner diameter and 8 mm length, the average portal blood flow was in the upper range of the values reported by other investigators under physiological conditions (0.85 ± 0.17 ml/min/g liver weight [24]; up to 20.6 ± 2.6 ml/min in 337 g rats; this corresponds to 2.1 ml/min/g liver weight [25].

Microcirculation after HALT with flow-regulated PVA

Functional sinusoidal density represents an established parameter for quantification of nutritional tissue perfusion [26]. The FSD is defined as the length of red blood cell perfused sinusoids per observation area. Directly after reperfusion, the FSD (in group A I) was 335 ± 48/cm, equivalent to 76% of normal value, which we had determined directly after laparotomy (439 ± 22/cm, sham group). This result is in accordance with results of human hepatic microcirculation following full-size orthotopic liver transplantation [27]: 5 min after reperfusion a FSD of 310 ± 63/cm was measured, equivalent to 73% of the normal value (424 ± 27/cm). In our experiments, the FSD had increased to 91% of the normal value, 7 days after HALT with PVA. The diameters of the sinusoids and the postsinusoidal venules were within the normal range, both directly after transplantation and after 7 days. This is in accordance with the normal values for rats of similar size, as reported by Vollmar *et al.* [28] (sinusoidal diameter: 6.7 ± 0.2 μm, diameter of postsinusoidal venules: 30 ± 2 μm). In addition, the ISD was measured. This parameter is particularly important because an increase of ISD in response to ischemia-reperfusion or hyperperfusion may indicate edema formation caused by swelling of both endothelial and parenchymal cells. Alternatively, the ISD could also increase because of partial sinusoidal no-reflow with perfusion of only every second

or third sinusoid. In group A, the ISD was within the normal range, both after reperfusion and after 7 days. This result is in agreement with the histological examinations, in which no edema formation could be seen in endothelial or parenchymal cells. The RBCV, measured 5 min after reperfusion after HALT with flow-regulated PVA was significantly elevated; after 7 days it returned to values within the normal range.

HALT with flow-regulated PVA in AHF

We used the method of 85% liver resection, first described by Emond, to induce AHF [16]. Without HALT, the transaminases rose to nearly 2000 U/l on day one, liver-synthesis decreased, and the animals became comatose or suffered from edema formation and intraabdominal bleeding. All the animals of this group died within the first six postoperative days. HALT with flow-regulated PVA resulted in a 6-week survival of nine of 11 animals. After an initial weight loss, the rats grew continuously. After 6 weeks, the body weight was significantly higher than the initial weight. The surviving rats' general health condition and the liver synthesis were excellent. The grafts' function was equivalent to that of the native livers – investigated by hepatobiliary radioisotope scanning. The total liver weights were within the normal range. Thus, AHF was successfully treated by HALT with flow-regulated PVA.

The method of 85% liver resection for induction of AHF has the following advantages: In contrast to the application of hepatotoxic agents, this method is standardized, reproducible and no side-effects on other organ-systems or the graft are expected. However, it must be taken into consideration, that after 85% resection of healthy liver tissue a good regeneration can be expected. The regeneration of a whole, but intoxicated liver may be different. However, our experiments show, that the model of 85% liver resection is appropriate to induce AHF. This AHF can be treated successfully by HALT with flow-regulated PVA.

The inter-liver competition between native liver and the graft is still under discussion. Hess demonstrated that impairment of the native liver (by means of bile duct ligation, resection, or absence of portal venous blood) resulted in atrophy of the native liver and hypertrophy of the graft [29]. In our experiments by contrast, the remaining liver lobes of the native liver had hypertrophied during 6 weeks and the graft had atrophied. Two factors must be taken into account when trying to explain this phenomenon:

1 The influence of the hepatotrophic factors of the portal vein on the liver's growth should be considered [30]. It is very likely that the small remnant of the native liver cannot bind the total amount of the hepatotrophic factors in the first pass so that they can reach the systemic circulation and the graft. When the native liver regenerates, a larger amount of the hepatotrophic factors is bound in the native liver and the amount that reaches the systemic circulation and the graft decreases. Thus, the native liver is favored and regenerates, whereas the graft atrophies with time.

2 After 6 weeks, proliferating bile-ductuli were seen in the grafts, as signs of an incipient biliary cirrhosis. This biliary cirrhosis is most likely caused by sludge formation in the stent that we used for construction of the choledochoduodenostomy. In earlier experiments, we found the same phenomenon after orthotopic rat liver transplantation, when the choledocho-choledochostomy was performed in stent-technique with a 22-G stent [31]. In these experiments, we used the widest stent that can be placed in the rat's bile duct (20-G), but nevertheless, sludge formation and beginning biliary cirrhosis could not be avoided completely. This is a second possible explanation for a long-term advantage of the native liver compared with the graft.

Conclusion

These experiments show, that HALT with flow-regulated PVA can reliably bridge an AHF until the native liver regenerates. The feasibility of flow regulation in PVA, leading to good results regarding microcirculation, morphology and graft function is demonstrated. These results should be taken into consideration for possible clinical applications. However, in human transplantation plastic stents are avoided, because prostheses require long-term anticoagulation therapy. Therefore, in human transplantation blood flow regulation may be performed by banding of the portal vein (7).

Acknowledgment

The authors thank Dr Langer for his support in OPS analyzing.

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