Transpl Int (2005) 17: 822–833
DOI 10.1007/s00147-004-0751-2

ORIGINAL ARTICLE

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Arterialisation of the portal vein as a model for the induction of hepatic fibrosis: description of microsurgical models in the rat

Received: 10 September 2003
Revised: 12 February 2004
Accepted: 5 March 2004
Published online: 13 April 2005

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Abstract Within the framework of liver transplantation, arterialisation of the portal vein in the case of non-recanalisable thrombosis has been reactivated. However, one of the consequences of this vascular reconstruction is the development of hepatic fibrosis. Clinical experience has shown that the development of fibrosis can be avoided by reducing portal inflow. We present, as a model for the induction of hepatic fibrosis, techniques of PVA, including transplantation. For PVA, several different techniques were used: the first with reduction of the portal inflow over a stent inserted in the right renal artery (PVA-B), the second with unrestricted flow using an aortic-portal segment (PVA-APS). The third technique was orthotopic liver transplantation with unrestricted portal arterialisation (OLTx-APS). Portal blood flow was measured with an ultrasonic flow probe. To determine the degree of hepatic fibrosis the amount of hydroxyproline was measured. Quantification of relative transcript levels of procollagen I was effected with real-time PCR using the Taq-Man technology on a lightcycler instrument. The extracellular matrix was visualised with picro-sirius staining. Measurements with the ultrasonic probe showed a significant increase in flow rates, both with reduced (PVA-B) and unrestricted inflow (PVA-APS; OLTx-APS). The lowest survival rate (58%) was found in the group with unrestricted portal inflow. The reason for this was a high rate of thrombosis in the in the portal vascular tree (4 out of 12). In the OLTx-APS group four animals died within the first 3 post-operative days (69%), as a result of protracted postoperative shock. The overall survival rate was the highest (85%) in the group undergoing PVA with reduction of the portal inflow. PVA with unrestricted inflow was followed by a significant increase in extracellular collagen, which showed a clear correlation with the increase in the amount of hydroxyproline, the level of the mRNA for procollagen I and picro-sirius staining. With the operative PVA techniques presented herein, different arterial flow rates in the portal vein can be investigated. In our opinion these techniques represent an excellent animal model for studying the genesis of fibrosis and antifibrotic substances. By regulating the blood flow in the arterialised portal vein hepatic fibrosis can be reduced or even avoided. After a brief period of learning the microsurgical techniques, the surgeon can limit clamping times and achieve good results with these techniques.

Keywords Hepatic fibrosis · Portal vein arterialisation · Unrestricted and reduced portal flow · Liver transplantation

Abbreviations AS: Aortic segment · APS: Aortic-portal segment · ECM: Extracellular matrix · HYP: Hydroxyproline · IHVC: Infrahepatic vena cava · OLTx-APS: Orthotopic liver transplantation with unrestricted portal arterialisation · PBF: Portal blood flow · PV: Portal vein · PVA: Portal vein arterialisation · PVA-APS: Portal vein arterialisation with unrestricted flow via an aortic-portal segment · PVA-B: Portal vein arterialisation with reduction of flow ("banding") · SHVC: Suprahepatic vena cava

Introduction

A therapeutic option for dealing with a non-recanalisable thrombosis of the portal vein during the course of liver transplantation is the arterialisation of the portal vein [1, 2, 3, 4, 5]. The effects of this unphysiological vascular reconstruction on the function and morphology of the graft are not yet known in adequate detail [6]. Reported consequences of PVA are the development of vascular ectasis with thrombosis in the PV, right heart decompensation and hepatic fibrosis. More recent clinical experience shows that a reduction in portal inflow can avoid these undesired effects [7, 8]. To investigate the mechanisms involved, we recommend having a microsurgical animal model available. Various procedures for inducing hepatic fibrosis have been reported. Via ligation or division of the common bile duct, secondary biliary cirrhosis can be induced [9]. Occlusion of the bile duct system by a retrograde injection of sodium amidotrizoate (Ethibloc; Ethicon, Germany), induces the same effect of progressive portal fibrosis [10, 11]. A further procedure that has been reported is the administration of tetrachlorides or dimethylnitrosamines [12]. In recent years, progress has been made in clarifying the molecular and cellular processes leading to fibrosis [13, 14]. Currently, no rational treatment of the imbalance between fibrinolysis and fibrogenesis is available. The in vivo model could be used for evaluating substances with antifibrotic potential, such as silymarin, or for investigating the genesis of hepatic fibrosis [15, 16]. The above-mentioned procedures are of only limited value in this respect, since they induce fibrosis through the action of free radicals or necrosis. In our opinion, PVA is a model for hepatic fibrosis that could replace or supplement previous methods. Numerous microsurgical techniques with which to accomplish arterialisation of the rat portal vein have been proposed [17, 18, 19, 20]. All stem from the era of shunt surgery, and all are associated with a high rate of thrombosis [21, 22]. This prompted modifications to the techniques in an attempt to simplify and standardise them. For PVA with reduced portal flow, a Teflon catheter was used. PVA with undiminished inflow was carried out with the aid of an interposed aortic-portal segment. The technique of orthotopic liver transplantation using microsurgical suturing is a widely accepted technique [23, 24, 25]. Here, too, modification may enable PVA to investigate the changes to the graft [26, 27]. The aim of our study is to describe in detail various operative techniques of portal vein arterialisation after creating a portacaval shunt, and during orthotopic liver transplantation.

Material and methods

A total of 38 operations using various techniques of portal vein arterialisation were carried out. The animals used were male Lewis inbred rats (LEW/CrlBR; MHC Haplotype RT1). At the start of the procedure, the rats weighed 260–280 g. Postoperatively, the animals were kept in individual Macrolon cages and had unrestricted access to water and pelleted feed (Standard Diet, Rats and Mice 1324, Altromin, Lage). Ambient temperature was kept constant at 21°C, and humidity at 60%, with a12-h light/dark cycle. As an optical aid, we used a fine-focussing operating microscope with a magnification of ×8 to ×32 (Operating Microscope 7, Zeiss, Munich). All operations were performed using microsurgical instruments (Aesculap, Tuttlingen) under sterile conditions. The animals received pre-medication consisting of 0.1 mg/kg buprenorphine (Temgesic; Essex Pharma) via s.c. injection. All operations were done under general anaesthesia with 1.0–1.5% isoflurane (Forene: Abbot) applied with the aid of a closed-circuit anaesthesia vaporiser (Völker; Kaltenkirchen, Germany). For maintaining anaesthesia, an isoflorane/oxygen mixture was applied via a head chamber at a dose of 1.0–1.5%. For postoperative treatment of pain, the animals routinely received 0.1 mg/kg buprenorphine Temgesic; (Abbot) s.c. at 12-h intervals for 3 days. This study was approved by the animal ethics committee of the government of Bavaria, Munich.

Four operative procedures were carried out and described below: (1) Control group ($n = 10$); (2) arterialisation of the portal vein with reduced inflow ("banding") ($n = 13$) PVA-B; (3) arterialisation of the portal vein with unrestricted inflow ($n = 12$), PVA-APS; (4) orthotopic liver transplantation with unrestricted portal arterialisation via an aortic-portal segment ($n = 12$), OLTx-APS.

Operations

Control group: Via a laparotomy, the liver was freed from its supporting ligaments and mobilised. After 160 min, the abdomen was closed in layers.

Creation of the portacaval shunt: As the necessary first step in the arterialisation of the portal vein, portal flow was first shunted to the vena cava. This end-to-side anastomosis was done using the technique described [21, 28]. The abdomen was opened by a longitudinal caudo-cranial incision carried to the xiphoid. After exposing the infrahepatic vena cava, the portal vein was mobilised to the entry of the splenic vein. To accomplish this, the gastroduodenal vein has to be divided. The vena cava was then partially clamped at the level of the renal vein using a Satinsky microclamp. When doing this, an adequate venous return to the right heart must be ensured. The vessel was then opened by a longitudinal 4-mm incision, and two holding sutures were placed (8/0; Nylon DR6, Serag-Wiessner, Naila). After clamping the portal vein at the level of the splenic vein, the superior mesenteric artery was clamped to minimise venous congestion in the splanchnic bed. After dividing the portal vein at the takeoff point of the gastroduodenal vein, the holding sutures were used to create an anastomosis with continuous suture. The anastomosis was cleared of air by opening the Biemer clamp on the portal vein and the superior mesenteric artery, and the venous blood flow into the vena cava released, whereupon the livid blue discoloration of the bowel spontaneously changed and pulsation of the mesenteric vessels was seen (Fig. 1).

Remarks

For the construction of the portacaval shunt it is important to expose as long a segment of the portal vein as possible. After clamping the portal vein, it must be divided at the level of the takeoff point of the gastroduodenal vein to obtain an adequately long vascular stump, both for portacaval anastomosis and to ensure a sufficiently long stump in the hilus. The clamping time of the portal vein should not exceed 15 min.

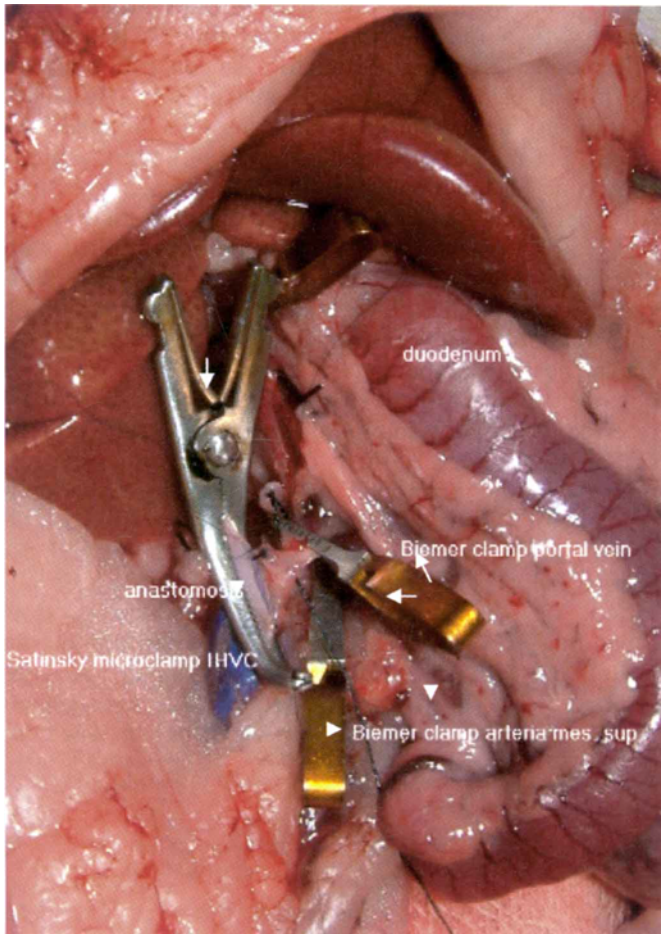

Fig. 1 Creation the portacaval shunt

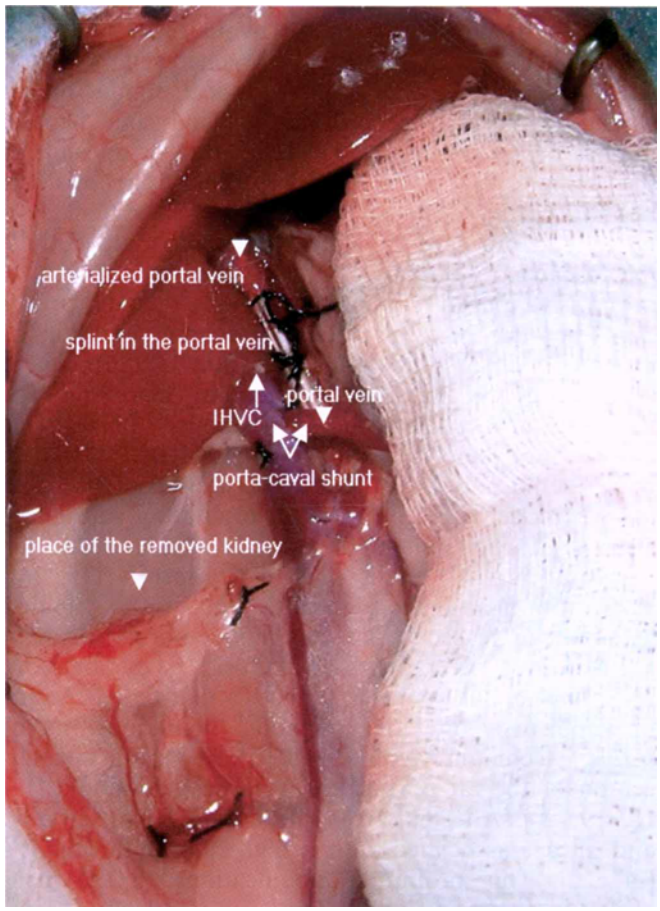

Fig. 2 Portal vein arterialisation with reduction of flow ("banding"); (PVA-B)

PVA with reduction of the flow ("banding") (PVA-B) ($n = 13$)

In a first step, the right renal artery was dissected free. To accomplish this, the takeoff point of the suprarenal artery had to be divided. Thereafter, the right renal vein and right urethra were divided between ligatures, and the kidney excised. The long mobilised right renal artery was put under tension and a Biemer clamp was placed at the point of exit of the artery from the abdominal aorta. After making a V-shaped incision in the vessel, an approximately 8-mm long Teflon-coated splint (Vasofix Braunüle, 1.0 mm/20 G, OD Braun, Melsungen) was inserted into the lumen of the vessel and secured with a ligature. With the aid of two microforceps, the stump of the portal vein in the hilus was then drawn over the splint and secured with a ligature. The arterial flow to the portal vein was then released by removing the Biemer clamp. Prior to closing the abdominal wall in layers, the portal blood flow (PBF) was measured. The average overall duration of the procedure was 160 ± 21 min (Fig. 2).

Arterialisation of the portal vein with unrestricted flow (PVA-APS) ($n = 13$)

PVA with undiminished blood flow was effected by interposing an aortic-portal segment (APS) between the portal vein and the infra-renal abdominal aorta. The first step towards accomplishing this was, after first ligating all the tributaries, removing from a donor animal the segment of aorta extending from the iliac bifurcation to the point of its passage through the diaphragm. After performing a longitudinal laparotomy, a portacaval shunt was created as described above. The caudal end of the aortic segment was then anastomosed, end-to-end, with the portal vein stump in the hilus. For this purposes, two holding sutures were placed. With the upper holding suture, the posterior and ventral walls were then anastomosed using a continuous suture (8/0; Nylon; DR6; Seralon). On completion of this anastomosis, the infrarenal abdominal aorta was then freed by blunt dissection and clamped with two Biemer clips. After washing out the clamped aorta, an approximately 4 mm-long incision was made in the aortic wall, and a Biemer vascular clip placed in about the middle of the APS and put under tension. Suturing was begun at the caudal end of the incision with the placement of a holding suture. The posterior wall was then sutured from the inside, followed by the anterior wall from the outside using an everting continuous suture (8/0; Nylon; DR6; Seralon). Beginning caudally, the clips were removed to permit arterial portal perfusion of the liver. Finally, the portal blood flow (PBF) was measured with the aid of an ultrasonic probe. The average time for the operative procedure was 160 ± 25 min (Fig. 3).

Remarks

Most problems seen with this PVA variant are experienced with the construction of the anastomoses. Here, in particular the posterior wall of the vessel must be fully exposed. This is necessary to ensure that suturing does not result in constriction or leakage. The end-to-side APS anastomosis should be primarily leak-free, since any further clamping and the use of a new suture are associated with the risk of suture-related constriction and secondary thrombosis of the aortic segment.

Orthotopic liver transplantation with unrestricted portal arterialisation via an aortic-portal segment (APS) ($n = 12$)

Orthotopic liver transplantation with PVA was developed on the basis of a previously described microsurgi-

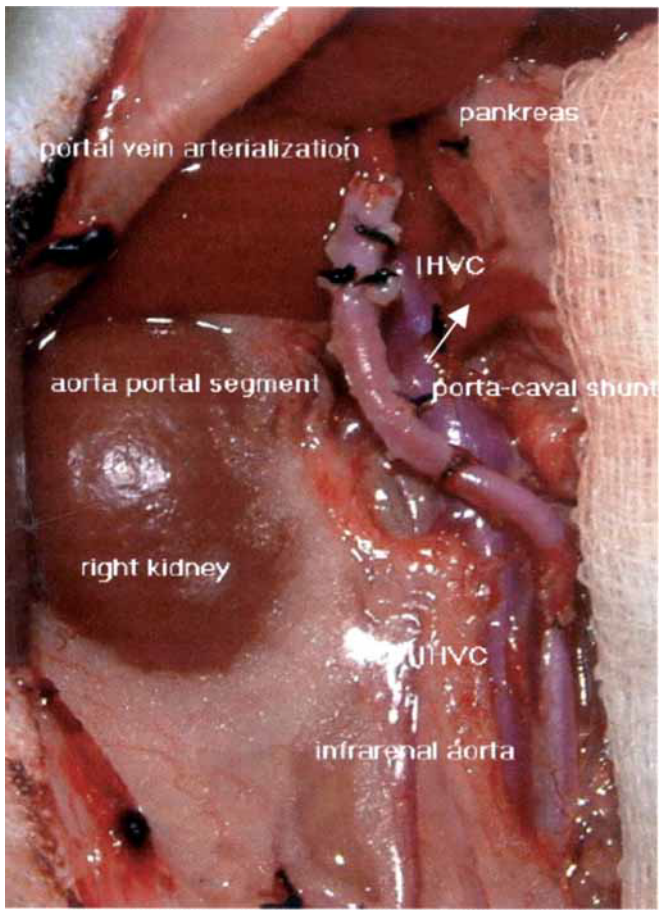

Fig. 3 Portal vein arterialisation with unrestricted flow via an aortic-portal segment (PVA-APS)

cal technique [23]. This technique was modified by re-arterialisation of the graft using a hepatico-aortic segment [29]. Portal arterialisation of the liver graft was effected using this hepatico-aortic segment [27]. With this operative technique, all anastomoses are carried out using microsurgical suturing methods. The bile duct was reconstructed over a stent [30].

Removal of the donor liver

After removing the liver from its ligamentous structures and dissecting free the coeliac trunk and abdominal aorta, it was perfused with Ringer's lactate solution via the aorta. Thereafter, under cold perfusion, the cranial end of the prepared aortic segment was anastomosed end-to-end with the portal vein stump (8/0; Nylon; DR6; Seralon). The graft was then finally completely removed from the donor and stored at 4°C until used.

Transplantation

In contrast to the donor procedure, the abdomen of the recipient was opened with a transverse laparotomy in order to optimally expose the upper abdomen to facilitate suturing of the suprahepatic vena cava (SHVC). After creating the portacaval shunt, hepatectomy was then performed. This was carried out after placing a mosquito clamp on the infrahepatic vena cava (IHVC), and a modified Satinsky clamp on the suprahepatic vena cava (SHVC). The placement of these two clamps marks the start of vena cava clamp time. The success of the transplantation was decisively dependent on the duration of clamping, which should not exceed 25 min. During this clamping phase, general anaesthesia was stopped, and the animal was ventilated with oxygen alone. The venous return to the heart then had to be rapidly re-established by anastomosing the SHVC and IHVC, since portal connection to the transplant had not jet been established. The APS was therefore rapidly anastomosed, end-to-side, to the infrarenal aorta (8/0; Nylon; DR6; Seralon) to secure reperfusion of the graft. After opening the Biemer clamps on the aorta, arterial portal and arterial reperfusion of the graft occurred via the APS. On reperfusion, the graft showed a rapid homogeneous change in colour to bright red. As the final step of the transplantation, the recipient bile duct was pulled over the splint already in position in the donor bile duct, and adapted using a ligature. Thereafter, the abdomen was irrigated twice with warm saline solution and closed in layers (3/0 Dexon, Braun, Melsungen). The average duration of the operation was 159 ± 12 min. No postoperative antibiotic cover was applied (Fig. 4).

Remarks

Rapid suturing of the suprahepatic vena cava is a prerequisite for keeping the vena cava clamp time as short as possible. Good exposure of the upper abdomen via a transverse laparotomy with maximal cranial retraction of the abdominal wall is helpful here. During hepatectomy, a portion of the diaphragm is included in the bite of the Satinsky clamp. In this manner, a maximum length of vessel stump is obtained for the SHVC suture. Vena cava clamp time must not exceed 25 min.

Measurement of portal venous blood inflow (PBF)

After completion of the anastomoses measurement of portal venous blood inflow was carried out with an ultrasonic probe. Saline solution was used as the immersion solution. The probe (1.5RB129, Transonic Systems, Ithaca, N.Y., USA) comprises two ultrasonic

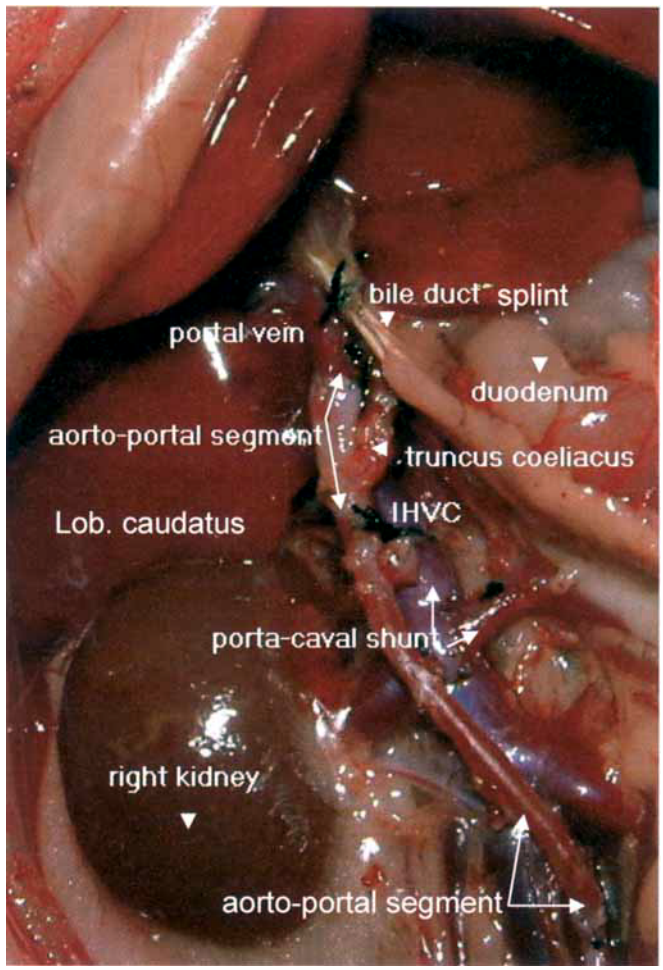

Fig. 4 Overview of the completed vascular anastomoses after OLTx with PVA, showing the aortic-portal segment (OLTx-APS)

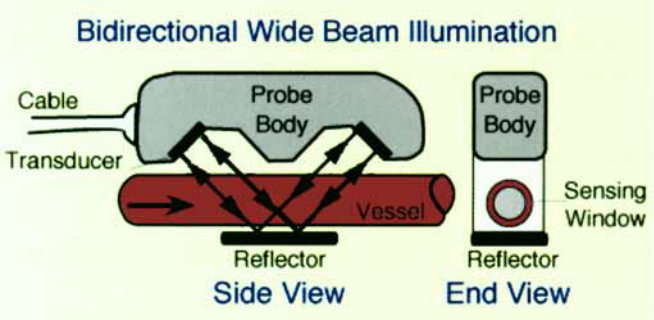

Fig. 5 Schematic representation of the ultrasonic probe and measuring method

transducers and an acoustic reflector. For the measurement, the two transducers are placed on one side of the vessel being investigated, and the reflector on the opposite side between the two transducers. Ultrasonic signals are transmitted and received alternately by the transducers after crossing the vessel upstream and downstream of the blood flow. The flowmeter (T209 dual channel flowmeter, Transonic Systems) computes the exact time needed for an ultrasonic signal to pass from one transducer via the reflector to the other transducer. On the basis of the difference between the upstream and downstream signal run times, the flowmeter calculates the blood flow in the vessel in ml/min (Fig. 5).

Ultrasonic Doppler examination visualisation of the arterialised portal vein

Before killing the animals on the 84th postoperative day, a Doppler ultrasonographic examination of the arterialised portal vein was performed. This study was done using a device manufactured by GE Vingmed Ultrasound A/S/Norway and a 5 MHz paediatric transducer. For this purpose, the transducer was directed towards the liver hilus. In the so-called M-mode, the arterial flow profile was then determined. The evaluation was strictly qualitative.

Hydroxyproline determination in liver tissue

To determine the collagen content, the concentration of hydroxyproline was quantified by calorimetric methods using the analysis methods as described [31, 32]. The concentration of hydroxyproline was recorded in µg/mg.

RNA preparation, reverse transcription and real-time polymerase chain reaction

Snap-frozen rat liver tissue was pulverised in liquid nitrogen and homogenised in RNA Pure (PeqLab, Erlangen, Germany), and total RNA was isolated in accordance with the manufacturer's recommendations. Reverse transcription of 1 µg of each total RNA to complementary DNA (cDNA) was performed in 20 µl reactions with superscript RTII reverse transcriptase (Gibco BRL). Quantification of relative transcript levels of procollagen 1 and the housekeeping gene β-2 microglobulin in the cDNA samples was performed by real-time quantitative PCR using the TaqMan technology (PE-Biosystems, Foster City, Calif., USA) on a LightCycler instrument (Roche, Mannheim, Germany). Primer and probes were synthesised at MWG Biotech AG (Ebersberg, Germany). Real-time PCR was performed using the LightCycler FastStart DNA Master Hybridization Probe Kit (Roche Molecular Biochemicals, Mannheim, Germany). Generally, PCR samples were amplified with an initial Taq DNA polymerase activation step at 95°C for 10 min, followed by 45 cycles of denaturation at 95°C for 20 s, annealing at 60°C for 15 s and extension at 72°C for 6 s. For analysis, the proportional second derivative maximum option of the LightCycler software (version 3.5) and a standard curve generated by parallel amplification of 1:2 to 1:32 serial dilutions of pooled cDNA from the control group were used. To normalise variations in reverse transcription efficacy, transcript levels of the housekeeping gene β2-microglobulin were determined, and procollagen I transcript levels were expressed as the ratio of procollagen I to β2-microglobulin mRNA (Table 1).

Histology: picro-sirius staining

On the 28th and 84th days, biopsies were obtained from the liver, fixed in 4% formalin and embedded in paraffin. Staining with picro-sirius red stain was applied to represent the collagen content of the tissue [33]. For this purpose, the sections were deparaffinised and hydrated in a decreasing series of alcohols ending in distilled water. They were then placed in Weigert's haematoxylin for 10 min and washed three times in distilled water. Thereafter, they were placed in 10 ml aqueous Sirius

Table 1 Real-time RT-PCR systems

	5′-Primer	5′-FAM and 3′-TAMRA labelled probe	3′-Primer
Procoll. α1(I)	TCCGGCTCCTG CTCCTCTTA	TTCTTGGCCATGCGTCAGGAGGG	GTATGCAGCTGACT TCAGGGATGT
β2-Microglob.	CCGATGTATATGCTTGCA GAGTTAA	AACCGTCACCTGGGACCG AGACATGTA	CAGATGATTCAGAGCTCCATAGA

Red solution (Waldeck Division Chroma, Münster, Germany) and in 90 ml saturated aqueous picric acid solution. All sections were examine with bright light optics using a Zeiss photomicroscope.

Statistical analysis

The statistical analysis was done with the SPSS Programme (Version 11). With approximately normal distribution, the results were presented as means ± standard error of the mean (SEM). In the case of presumed non-normal distribution, or in the event of small numbers of cases, the medians and interquartile ranges (IQR) have been given. Inferential statistics between factors were assessed using ANOVA models or, in the case of non-normal variables, the Kruskal-Wallis test. Post hoc t-test using adjustment for multiple testing was performed for bivariate comparison. Statistical significance was assumed at a P value of less than 0.05.

Results

Survival rates/premature deaths

For the analysis of survival rates and premature deaths, all operated animals were considered, including those in which fatal intra-operative complications (circulatory collapse, haemorrhage, air embolism or thrombosis) occurred. The observation period was 84 days.

Control group ($n=10$)

All animals of the control group survived the observation period.

PVA with reduction of the inflow ("banding") (PVA-B) (n = 13)

Here, one animal that had experienced an anaesthetic incident died on the 28th postoperative day, and another animal died on the 56th postoperative day, the autopsy showed an extensive abscess in the abdominal cavity. In this group the survival rate was 85%.

PVA with unrestricted inflow (n = 13) (PVA-APS)

In this group, PVA via an interposed aortic segment was performed in 13 cases. Of these, one animal each died prematurely on the 5th, 6th, 8th and 16th postoperative day. In each of these animals, the autopsy showed thrombosis of the APS and the portal vascular tree. Another animal died on the 66th postoperative day, but the cause of death could not be established with certainty. Overall, 4 of the 12 animals developed a thrombosis of the portal venous bed; in this group, the survival rate was 58%.

Orthotopic liver transplantation with unrestricted PVA via an APS ($n=12$)

Overall, a total of four animals died of cardiovascular failure during the observation period, two of them within 12 h after the operation. Here the vena-caval clamp time was more than 25 min for the first operations in the group. Two more animals died on the 2nd and 3rd postoperative days. The reason for these premature deaths was progressive liver failure consequently from insufficient perfusion of the graft. At the end of the surgical phase no more fatal complication were observed, so that in this group a learning curve effect can be surmised. As 4 out of 12 animals died, the survival rate was 69% (Table 2; Fig. 6).

Measurement of portal venous blood inflow (PBF)

The flow was measured before closing the abdominal wall and after intravenous injection of 2–3 ml saline solution. In the intact portal vein, a flow of 16 ml/min ± 6.1 (mean ± SD) was measured. Measurements done after PVA with reduced and unrestricted blood

Table 2 Survival rate, time and cause of death

Group	Survival rate (%)	Dead animals (n)	Time of death (days post-op)	Cause of death/autopsy findings
Control	100	0/10/		
PVA–B	85	2/13	28, 56	1x Anaesthetic event 1x Thrombosis of PV
PVA–APS	58	5/13	5, 6, 8, 16, 66	4x Thrombosis of PV and AS 1x n.d.
OLTx–APS	69	4/12	1, 1, 2, 3	2x Cardiovascular failure post-op 2x Primary non-functional graft with necroses in the graft

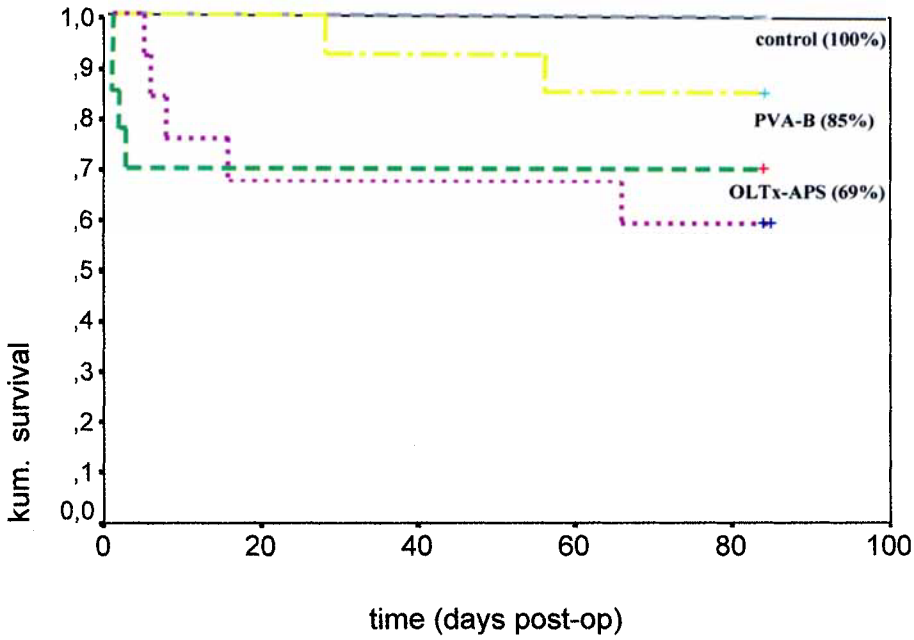

Fig. 6 Cumulative survival rate as percentage (Kaplan–Meier)

flow showed significantly increased values. Statistical significance was also found between the PVA-B and PVA-APS group (Fig. 7).

Ultrasonic Doppler examination at the end of the observation period

In the groups in which experimental arterialisation was effected via the aortic segment (PVA-APS and LTx-APS), all surviving animals had a positive Doppler demonstration of arterial blood flow before being killed. In the group with reduced blood flow, no arterial signal was detectable in the portal vein of 2 out of 13 (18%) animals, and these were excluded from further analysis.

Hydroxyproline

Hydroxyproline is a product of the hydroxylation of the amino acid proline. The collagen content in the ECM can be measured by determining hydroxyproline. At the start of the experiment, the average hydroxyproline

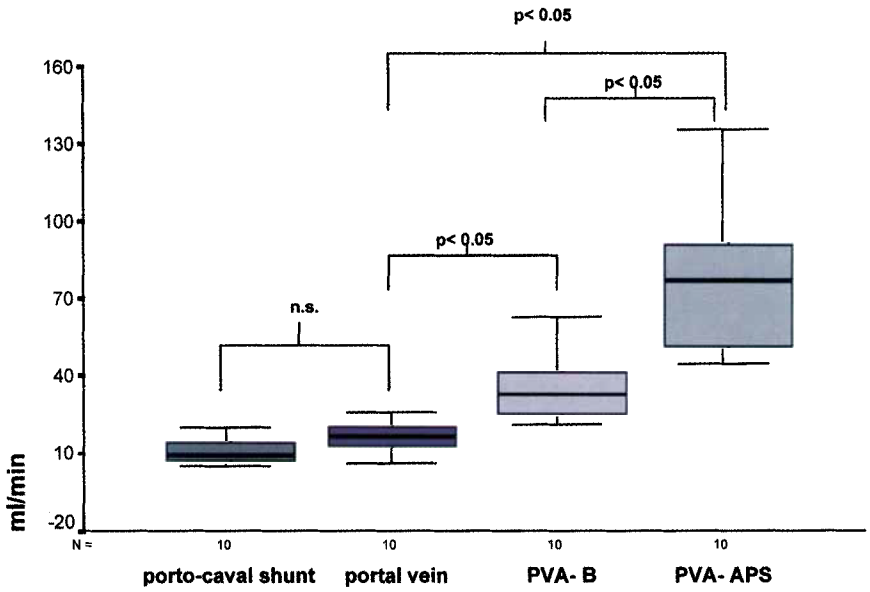

Fig. 7 Portal venous blood flow (PBF) graph: box plot; Mann–Whitney U-test; portal vein (control) vs PVA-B; $P = 0.001$; portal vein vs PVA-APS ; $P = 0.001$; PVA-B vs PVA-APS; $P = 0.001$. Statistical significance was assumed at $P < 0.05$

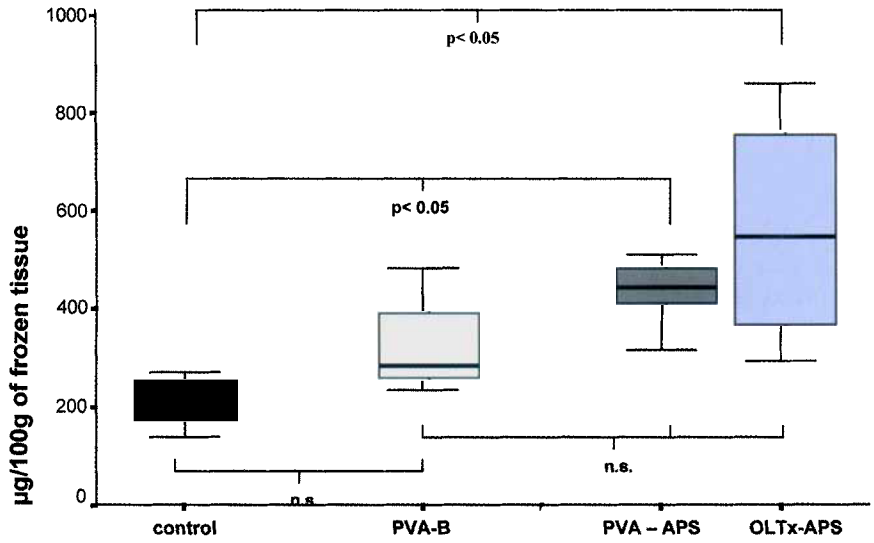

Fig. 8 Concentration of hydroxyproline in liver tissue on day 84. Kruskal Wallis test; Dunn's method: control vs PVB-APS: $P=0.001$; control vs LTx-APS: $P=0.001$; control vs PVA-B: n.s.; PVA vs PVA-APS /LTx-APS: n.s.

concentration was 229 ± 33.5 µg/100g liver dry weight (mean ± SD). Up until the 28th postoperative day after PVA, a clear increase was recorded. After 84 days, this development was even more pronounced. This increase was significant in the two groups with unrestricted portal flow (PVA-APS; OLTx-APS), but not in the groups with reduced flow (PVA-B) (Fig. 8).

Rat liver procollagen I mRNA transcript levels

The control group and the groups undergoing PVA and LTx with unrestricted portal inflow were analysed at the end of the observation period. In the former group a significant up-regulation of procollagen I transcript levels by fivefold to sixfold was seen. The increase in liver collagen was paralleled by a significant up-regulation of procollagen I mRNA, which encodes the major collagen type in fibrosis (Fig. 9).

Histology: picro-sirius red stain

In the tissue specimen obtained 84 days after PVA with no portal flow reduction, this stain showed marked periportal fibrosis with incipient septal formation and low-grade perivascular and interstitial fibrosis. Also diagnosed was a severe obliterative vasculopathy of the portal vein accompanied by intimal fibrosis and narrowing of the lumen (Figs. 10 and 11)

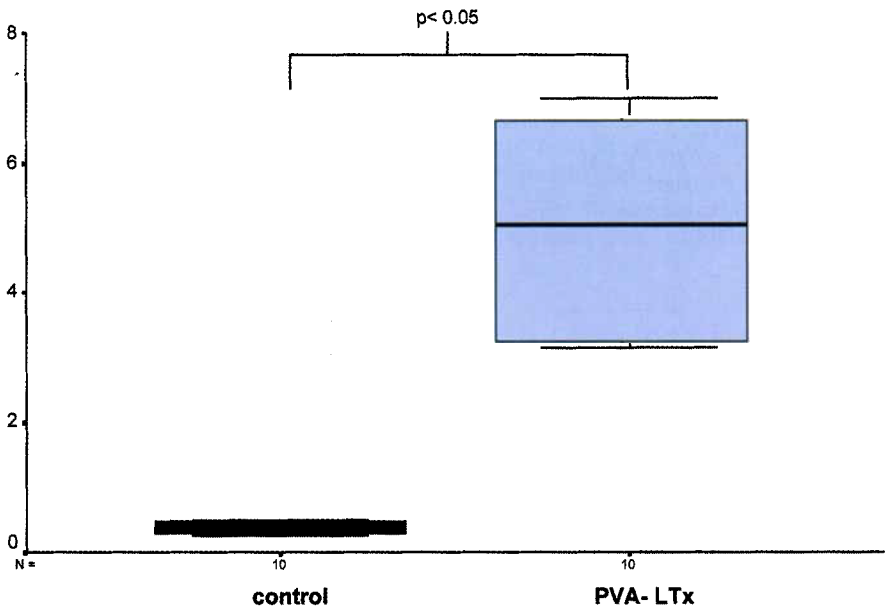

Fig. 9 Procollagen I mRNA transcript levels on day 84. Control vs OLTx-APS: $P=0.001$

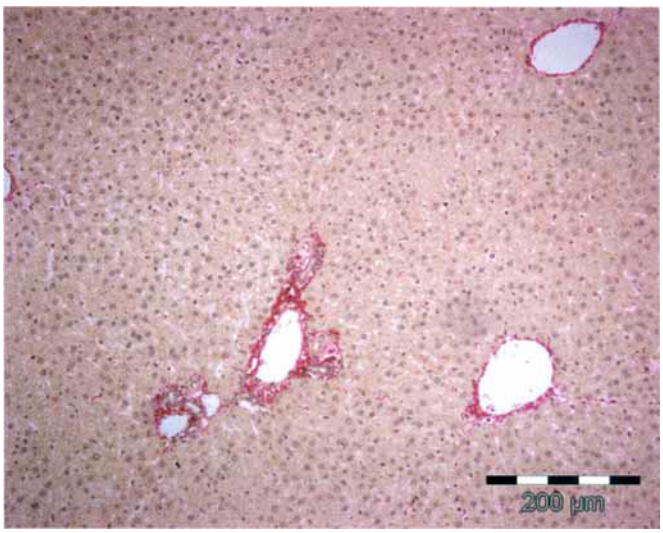

Fig. 10 Picro-sirius red staining; 84 days after PVA with reduction of blood flow (PVA-B). Mild periportal and perivascular fibrosis. The periportal vessels showed normal wall architecture

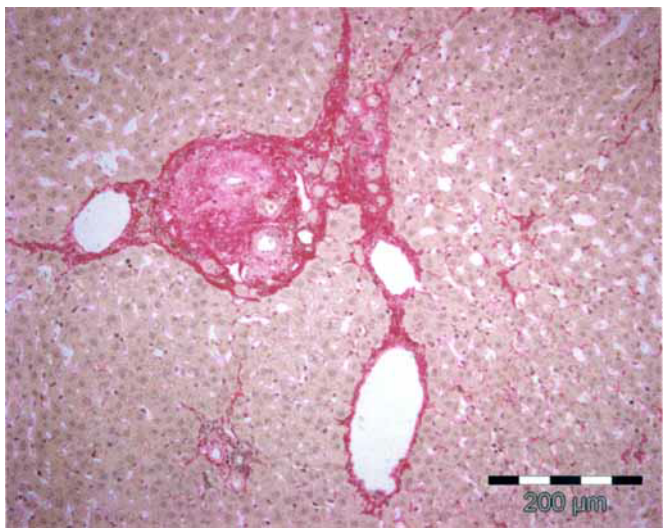

Fig. 11 Picro-sirius red staining; 84 days after PVA without reduction of blood flow (PVA-APS). Marked periportal fibrosis with incipient formation of septa. Obliterative vasculopathy of the portal vein with fibrosis of the intima and narrowing of the lumen

Discussion

The technique of PVA has been re-activated for liver transplantation [2, 4, 5, 34, 35]. The basis for the present study was our own experience with PVA during clinical liver transplantation and arterialisation of the liver in the pig and rat model [4, 6, 27]. Clinical experience and findings indicate that structural changes in the graft occur as a result of "over-arterialisation" of the graft. As one of the long-term sequelae of PVA with unrestricted inflow in the PV, the development of a fibrosis has been reported [8, 36, 37]. The purpose of reducing the portal inflow is to avoid the development of hepatic fibrosis [7]. To investigate these mechanisms, the microsurgical animal models described were developed. By varying portal inflow different situations can be simulated. Various microsurgical techniques have been proposed for arterialisation of the portal vein [17, 18, 19, 20, 28]. With the aim of standardising the procedure and achieving different portal inflow rates, operative techniques are now presented. To simplify the PVA with reduced inflow a Teflon stent was placed in the renal artery after excision of the right kidney. This technique involving the placement of a splint in the portal vein was successfully used for arterial revascularisation in an auxiliary liver transplantation model [38]. An additional advantage of this technique is that it enables standardisation of the portal inflow. The associated thrombosis rate was 12% after the observation period, and the reported thrombosis rate of the portal vein was about 20 to 30% [21, 22]. For the technique of PVA with unrestricted inflow, an aortic segment obtained from the donor animal was used in analogy to the technique described in liver transplantation. In this group, an appreciably reduced survival rate (58%) was observed. In 4 out of 5 animals autopsy showed thrombosis of the portal vein system. This phenomenon is known from the clinical setting, where ectasis with multiple thromboses has been found in the arterialised portal vein system [6]. The same problem was thus also found in our microsurgical rat model as a consequence of unrestricted portal venous inflow.

Orthotopic liver transplantation in the rat has already been reported [39, 40, 41, 42]. The technique with arterialisation of the PV via an AS is based on that described by Engemann [26]. Here the re-arterialisation of the graft is accomplished with the aid of an AS. The cranial end of this AS was anastomosed end-to-end with the PV. The PV was thus arterialised with unrestricted inflow. In comparison with conventional technique, this procedure has two peculiarities. With the conventional technique the clamp time of the SHVC, IHVC and the PV before resection of the liver up to the release of the SHVC and portal vein is referred to as the anhepatic phase. All authors consider the duration of this phase to be critical for the survival of the recipient animal. The reported duration for survival ranges widely between 11 and 30 min [26, 39, 43, 44, 45]. In the case of OLTx with PVA, hepatectomy is followed by the suturing first of the SHVC and then the IHVC. Thus the clamp time of the vena cava corresponds to the anhepatic phase. In our own experience this time was an average 21 ± 2 min. It became shorter during the course of the study, presumably as a result of the learning curve. The long-term

survival of the recipient animal was achieved with a clamp time of less than 25 min in all cases [46].

The second peculiarity of this technique is that reperfusion of the graft is first achieved when the end-to-side anastomosis between the aortic segment and the infrarenal abdominal aorta the graft is established. An ischaemia/reperfusion injury might be considered the cause of early graft failure. Overall, a survival rate of 69% was achieved, which was lower than the average value reported following the conventional technique in rats [30, 42, 43, 45]. The portal blood flow was determined by measurements with an acoustic reflector. In this way, a significantly increased flow in comparison with a normally perfused PV was seen both after PVA with unrestricted flow and PVA with flow reduction. This means that two techniques producing different flow rates are available. The objective of the described microsurgical models in the rat was to induce hepatic fibrosis. To prove induction of hepatic fibrosis, various components of the ECM were determined. Extracellular matrix components in the liver comprise types I–VII collagens and elastin, with the collagens forming the major portion of the matrix. Hydroxyproline is a post-translational product of the hydroxylation of the amino acid proline, and occurs in all the various types of collagen. This means that the collagen content of liver tissue can be estimated by determining the concentration of HYP [47, 48]. A significant increase in HYP was observed both after PVA with unrestricted inflow and in the group undergoing liver transplantation. In the group undergoing PVA with inflow reduction an increase was also observed, but was not significant.

One of the main extracellular matrix components of fibrotic liver is the expression level of procollagen I mRNA [49]. In hepatic fibrosis of the liver, the mRNA level of procollagen I is a powerful parameter [50]. Here, PVA with unrestricted inflow has been associated with a significant five to sixfold up-regulation of procollagen I mRNA, an effect that has also been observed in rat liver with biliary cirrhosis [50, 51]. The increase in liver collagen is paralleled by a significant up-regulation of procollagen I mRNA, which encodes the major collagen type in fibrosis. The development of periportal and perivascular fibrosis, have also been demonstrable by the picro-sirius stain. Therefore we conclude that: firstly, PVA with unrestricted portal inflow, a discernible change in the ECM, together with an increase in the collagens and up-regulation of procollagen I mRNA occurs; secondly, these effects were not observable to the same extent when portal blood flow was reduced; thirdly, the validity of the microsurgery models with various portal inflow is proven and can enable investigations of the genesis and treatment of hepatic fibrosis. Aided by the detailed description, and the analysis of possible sources of error provided, it should be possible for a microsurgeon to learn the surgical technique presented herein, rapidly and easily.

Acknowledgements This study was supported by the ELAN Foundation of the University of Erlangen-Nuremberg

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