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ORIGINAL ARTICLE

Splenectomy improves survival by increasing arterial blood supply in a rat model of reduced-size liver

Christian Eipel,[1]* Kerstin Abshagen,[1]* Jessica Ritter,[1] Daniel Cantré,[1] Michael D. Menger[2] and Brigitte Vollmar[1]

1 Institute for Experimental Surgery, University of Rostock, Rostock, Germany
2 Institute for Clinical & Experimental Surgery, University of Saarland, Homburg, Germany

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Correspondence
Christian Eipel PhD, Institute for Experimental Surgery, University of Rostock, D-18057 Rostock, Germany. Tel.: +49 381 494 2503; fax: +49 381 494 2502; e-mail: christian.eipel@uni-rostock.de

*Equal contribution of both authors.

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Summary

Prevention of acute portal hyperperfusion in small-for-size livers by inflow modulation results in beneficial postoperative outcome. The objective of this study was to unravel the underlying mechanism, emphasizing the intimate relationship between portal venous (PV) and hepatic arterial (HA) blood flow (BF). Rats underwent partial hepatectomy (pHx), splenectomy before pHx or splenectomy and ligation of the A. hepatica before pHx. Portal venous blood flow (PVBF), hepatic arterial blood flow (HABF), and tissue pO_2 were assessed during stepwise resection from 30% to 90%. Hepatic regeneration and hypoxia-responsive gene expression were analyzed in each group after nonlethal 85% pHx. 90% pHx caused a fourfold rise in PVBF, a slight decrease in HABF with a 50% reduction in pO_2, and high mortality. Splenectomy before pHx reduced the PVBF and caused a rise in HABF with doubling in tissue pO_2. An attenuation of hypoxia-responsive gene expression turned into enhanced hepatocellular regeneration and improved survival. A. hepatica ligation abolished the beneficial effect of splenectomy on tissue oxygenation, proliferation, and outcome. In conclusion, the beneficial effect of splenectomy in small-for-size livers can be attributed to a rise in HABF with sufficient oxygen supply rather than to a reduced portal venous hyperperfusion to the remnant liver.

Introduction

The liver has the unique capacity to regulate its mass after surgical removal or after cell loss caused by chemicals or viruses [1]. The increased blood flow to liver mass ratio immediately after hepatectomy (pHx), and the resulting increased intrahepatic shear stress have been proposed to stimulate and regulate liver regeneration [2–4]. On the other hand, the liver cannot directly control the portal venous blood flow (PVBF), and has to accept the entire outflow from the upstream splanchnic organs [5]. Portal hyperperfusion in reduced-size livers in case of living-donor or cadaveric split liver transplantation as well as after extended pHx has been shown to impair postoperative recovery seriously [6,7]. With the increasing practice of living-donor liver transplantation and the enlargement of the resectable liver mass, the small-for-size syndrome has emerged as an important clinical problem [8]. The ongoing debate on the causes of the small-for-size syndrome mainly focuses on portal hyperperfusion with high intravascular shear stress [9–11]. To avoid liver failure by portal hyperperfusion, techniques for reduction of portal inflow, such as portal-caval and mesocaval shunts, splenic artery ligation, and splenectomy have been established [12–14]. As the splenic fraction accounts for up to 52% of the total portal venous inflow [15], its contribution to portal hypertension is evident. As a consequence, the extent of spleen-derived blood perfusion is an important factor in determining portal inflow pressure. Glanemann *et al.* could demonstrate that simultaneous splenectomy

with extended partial hepatectomy in rats reduces the PVBF by approximately 44% compared with nonsplenectomized animals [11].

Under physiological conditions, alterations in PVBF are counteracted by concomitant changes in the hepatic arterial blood flow (HABF), reflecting the so called arterial hepatic buffer response (HABR) [16,17]. As a consequence of portal venous hyperperfusion in reduced-size-livers, HABR may lead to hepatic arterial hypoperfusion [18,19]. For the reason that the regenerating liver requires an enormous amount of oxygen for its increased metabolic load and for DNA synthesis, suboptimal arterial inflow may be poorly tolerated in the reduced-size-liver and may increase the risk for organ dysfunction [20]. However, the specific contribution of arterial hypoperfusion upon portal venous hyperperfusion in reduced-size-livers on liver oxygenation, liver regeneration, and final outcome has not yet been defined.

We hypothesize that beside reduced intrahepatic shear stress, lowered portal hyperperfusion mediates protection in reduced-size-livers mainly because of an improved oxygen supply via the HABR. To clarify this, we used an experimental setting of extended pHx with and without splenectomy. Additional ligation of the hepatic artery in splenectomized animals was performed to exclude the hepatic artery flow response.

Material and methods

Animals

Experiments were performed in accordance with German law on the protection of animals and the NIH guidelines for the Care and Use of Laboratory Animals ('Principles of Laboratory animal care', NIH publication Vol 25, No. 28 revised 1996). Male Wistar rats (body weight 230–350 g; Charles River Laboratories, Sulzfeld, Germany) were housed in standard animal laboratories with a 12-h light–dark cycle and held in single cages with access to tap water and standard laboratory chow *ad libitum*.

Experimental setting and groups

Experiments were performed in three separate sets of animals. For a condensed survey of the experimental schedule and the number of animals used in each set, see Table 1. Rats were divided into the following three groups: 30–90% pHx alone (group: pHx), splenectomy prior to 30–90% pHx (group: S + pHx) and splenectomy combined with ligation of the A. hepatica prior to 30–90% pHx (group: S + L + pHx). The latter group is designed to inhibit HABR and thus to decipher the impact of increased HABF upon splenectomy.

Survival study (set 1)

For analysis of 10-day survival, rats of each group (15 animals per group) were subjected to a lethal 90% pHx. For this purpose, rats were anesthetized by breathing isoflurane (1.8 vol%) and placed in supine position on a heating pad for maintenance of body temperature at 37 °C. After midline laparotomy and exposure of the upper abdominal organs, the liver was freed from its ligaments. The relevant lobes of the liver were then resected by placing 4–0 suture ties most proximally to their origin (left lateral and median lobes) or with slight distance to its origin (right superior lobe), guaranteeing adequate blood flow to the neighboring residual lobe.

In addition, animals underwent splenectomy only (S + pHx) or in combination with ligation of the A. hepatica (S + L + pHx) before 90% pHx. For this purpose, livers were disconnected from the main hepatic artery by ligating it, using a double suture just above the level of the bifurcation of the gastroduodenal and celiac

Table 1. Experimental setting and number of animals per group.

Experimental subsets	Experimental procedure	Animals (*n*)	
Set 1	pHx 90% alone	15	
Survival study	pHx 90% + splenectomy	15	
	pHx 90% + splenectomy and ligation of A. hepatica	15	
Set 2	pHx 30, 70, 85, 90% alone	5	
Stepwise hepatectomy	pHx 30, 70, 85, 90% + splenectomy	5	
Analysis of macrocirculation	pHx 30, 70, 85, 90% + splenectomy and ligation of A. hepatica	5	
Set 3		24 h	4 days
Regeneration model	pHx 85% alone	5	5
Analysis of liver tissue and plasma 24 h and 4 days post surgery	pHx 85% + splenectomy	5	6
	pHx 85% + splenectomy and ligation of A. hepatica	5	6

pHx, partial hepatectomy.

artery. After irrigation with warm physiological saline and leaving a 2 ml saline depot for volume replacement, the abdomen was closed with running 5–0 sutures. Postoperatively, animals were allowed to recover from anesthesia and surgery under a red warming lamp. Rats that lived longer than 10 days were considered survivors.

Analysis of hepatic macrocirculation (set 2)

For analysis of PVBF, HABF and hepatic tissue oxygenation, stepwise liver resection (removal of 30%, 70%, 85%, and 90% of the liver in the same animal) was performed in each group. For an overview of the experimental schedule and the number of animals analyzed at each timepoint, see Fig. 1. The animals of all groups (n = 15; pHx, S + pHx, S + L + pHx) served for assessing baseline values (B) before pHx as well as before splenectomy and ligation of the A. hepatica. Then, rats received splenectomy (n = 10; S + pHx, S + L + pHx) and served for evaluation of initial values after splenectomy only (S). Furthermore, splenectomized rats which additionally underwent ligation of the A. hepatica (n = 5; S + L + pHx) served for data of splenectomy combined with ligation of the A. hepatica before pHx (L). Afterwards, macrocirculatory parameters of the individual groups were measured after each step of resection (pHx30, pHx70, pHx85, pHx90). Briefly, spontaneously breathing pentobarbital-anesthetized rats were catheterized and laparotomized. The portal vein and the hepatic artery were then prepared and ultrasonic perivascular flowprobes (Transonic Systems, Ithaca, NY, USA) connected to a flowmeter (T402 Animal Research Flowmeter, Transonic Systems) were placed to record basal hepatic inflows. Liver tissue oxygenation was assessed by means of a flexible polyethylene microcatheter Clark type pO_2 probe (diameter 0.5 mm, length 200 mm; Licox Systems, GMS, Kiel-Mielkendorf, Germany), which was positioned inside the left lateral lobe penetrating the liver surface. The catheter was allowed to equilibrate for approximately 10 min. During this period, perivascular flow probes were re-placed around the hepatic artery and portal vein for subsequent blood flow measurements. Online temperature compensation was performed by a temperature probe (thermocouple microprobe; Licox Systems), which was positioned between liver lobes to protect hepatic parenchyma from further penetration. After performing stepwise liver resection (removal of 30%, 70%, 85%, and 90% of the liver), the ultrasonic perivascular flow probes were again positioned and hepatic inflows were recorded for 30 min. Tissue oxygenation was again measured as described above, using the remnant liver lobe for probe positioning. Data from ultrasonic flowmetry were digitalized online by an analog-to-digital converter (LabJack U12; LabJack Corp., Lakewood, CO, USA), transferred to a computer and recorded by scientific data acquisition software (DAQFactoryExpress; AzeoTech, Ashland, OR, USA). The animals were killed at the end of the experiment by an overdose of pentobarbital.

Liver regeneration model (set 3)

To evaluate liver regeneration of reduced-size livers, individual groups of rats were subjected to a nonlethal 85% pHx as described above. At 24 h after 85% pHx, i.e. dur-

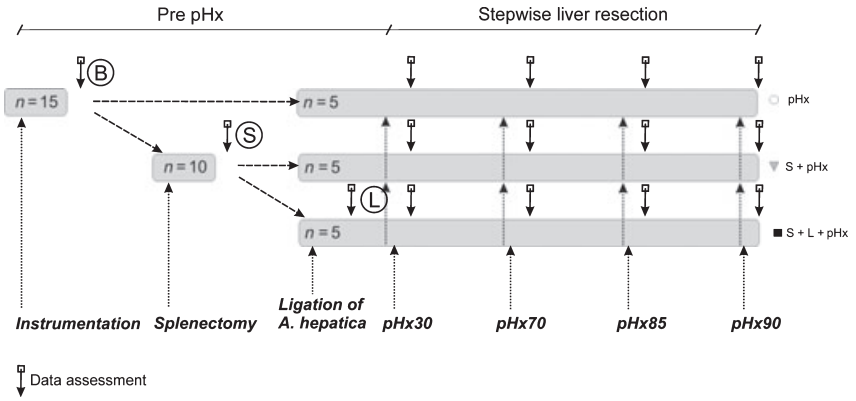

Figure 1 Experimental set-up of set 2. Animals were divided into three experimental groups: pHx, S + pHx, S + L + pHx. Stepwise liver resection (removal of 30%, 70%, 85%, and 90% of the liver) was performed in each group. Animals of all groups (n = 15; pHx, S + pHx, S + L + pHx) served for assessing baseline values (B) before pHx as well as before splenectomy and ligation of the A. hepatica. Then, rats received splenectomy (n = 10; S + pHx, S + L + pHx) and served for evaluation of initial values after splenectomy only (S). Furthermore, splenectomized rats, which additionally underwent ligation of the A. hepatica (n = 5; S + L + pHx), served for data of splenectomy combined with ligation of the A. hepatica before pHx (L). Afterwards, macrocirculatory parameters of the individual groups (n = 5) were measured after each step of resection (pHx30, pHx70, pHx85, pHx90). pHx, partial hepatectomy; S, splenectomy; L, ligation.

ing maximum of DNA synthesis [21] as well as after 4 days, rats were sacrificed with an overdose of pentobarbital to collect blood and liver tissue samples (5–6 animals per group and timepoint). Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were measured spectrophotometrically as an indicator of hepatocellular disintegration. The residual livers were recovered, weighed, and processed for subsequent analysis. The weight of the regenerated liver was used to calculate the growth of residual liver lobes according to weight of regenerated liver/preoperative liver weight × 100 (%). Preoperative liver weight was assumed as 3.5% of body weight.

Western blot analysis of liver tissue

For Western blot analysis of the hypoxia responsive erythropoietin receptor (EpoR), liver tissue was homogenized in lysis buffer (1 M Tris pH 7.5, 5 M NaCl, 250 mM EDTA, 10% Triton-X 100, 4% NaN_3, and 100 mM phenylmethyl sulfonyl fluoride), incubated for 30 min on ice, and centrifuged for 15 min at 10 000 *g*. Prior to use, all buffers received a protease inhibitor cocktail (1:100 vol/vol; Sigma Aldrich, St. Louis, MO, USA). Protein concentrations were determined using the bicinchoninic acid protein assay (Pierce, Rockford, IL, USA) with bovine serum albumin as standard. Equal amounts of whole protein extracts (40 μg) were separated discontinuously on sodium dodecyl sulfate polyacrylamide gels (12%) and transferred to a polyvinyldifluoride membrane (Immobilon-P transfer membrane; Millipore, Billerica, MA, USA). After blockade of nonspecific binding sites, membranes were incubated for 2 h at room temperature with polyclonal rabbit anti-EpoR antibody (1:200; Santa Cruz Biotechnology, Santa Cruz, CA, USA), followed by secondary peroxidase-conjugated goat anti-rabbit antibody (1:20 000; Cell Signalling Technology, Frankfurt, Germany). Protein expressions were visualized by means of luminol-enhanced chemiluminescence (ECL plus; Amersham Pharmacia Biotech, Freiburg, Germany), densitometrically assessed (Quantity One, Gel Doc XR; Bio-Rad Laboratories GmbH, Munich, Germany) and normalized to the β-actin signals (monoclonal mouse anti-β-actin antibody, 1:20 000; Sigma).

Immunohistochemistry of liver tissue

Liver tissue was fixed in 4% phosphate buffered formalin for 2–3 days and embedded in paraffin. From the paraffin-embedded tissue blocks, 4 μm sections were cut and for immunohistochemical demonstration of hepatocellular proliferation, sections collected on poly-L-lysine-coated glass slides were treated by microwave for antigen unmasking and were incubated with monoclonal mouse anti-Ki-67 antibody (1:25; Dako Cytomation, Hamburg, Germany) overnight at 4 °C, followed by horseradish peroxidase (HRP)-conjugated goat anti-rat immunoglobulin (LSAB kit plus; Dako). The sites of peroxidase binding were detected using 3,3′-diaminobenzidine. The sections were then counterstained with hemalaun and examined by light microscopy (Axioskop 40; Zeiss, Göttingen, Germany). Ki-67-positive hepatocellular nuclei were counted within 30 consecutive high power fields (×40 objective, numerical aperture 0.65) and are given as cells/mm^2. Immunohistochemical analysis of Ki-67 was performed in an investigator-blinded fashion.

Statistics

Data are presented as mean ± SE. After testing for normality and equal variance across groups, we assessed differences between the groups of 85% pHx using one-way ANOVA followed by the *post hoc* Holm-Sidak test for pairwise comparisons. When criteria for parametric tests were not met, Kruskal–Wallis ANOVA on ranks, followed by Dunn's test, was employed. Statistical differences of macrohemodynamic parameters among the three groups and extent of liver resection were assessed by two-way ANOVA for repeated measurements followed by the appropriate *post hoc* comparison. For reasons of clarity and comprehensiveness, only statistically significant differences between groups are indicated in the figures, with the exception of changes within groups prior to stepwise resection (B, S, L). Differences within these groups were assessed by one-way ANOVA for repeated measures followed by the *post hoc* Holm-Sidak test for pairwise comparisons. Furthermore, for analysis of survival data from the three groups, we performed a Log-Rank test followed by the *post hoc* Holm-Sidak test for pairwise comparisons. Data were considered significant when $P < 0.05$. Statistical analysis was performed using the SIGMA STAT and SIGMA PLOT software package (Jandel Scientific, San Rafael, CA, USA).

Results

Survival (set 1)

Only four of 15 animals survived 90% liver resection. Reduction in portal venous inflow by means of splenectomy before 90% pHx significantly increased the 10-day survival as compared with extended liver resection alone ($P = 0.013$) (Fig. 2). Additional ligation of the A. hepatica before splenectomy and extended pHx resulted in an almost comparable survival as observed in animals subjected to 90% pHx alone (Fig. 2). All animals subjected to 85% pHx with or without additional splenectomy as

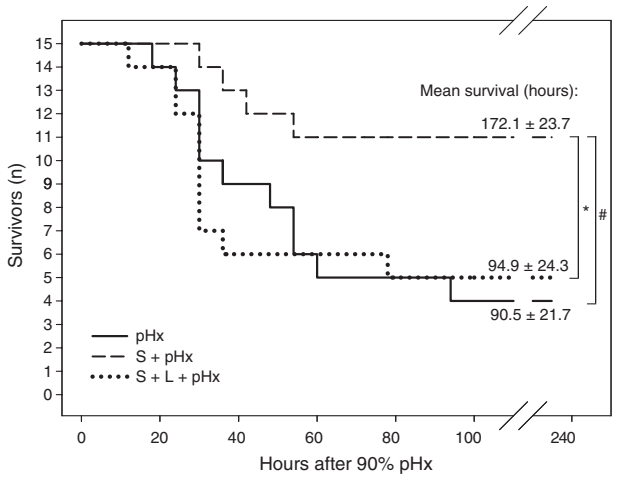

Figure 2 Survival curves of a 10-day survival study of animals, which underwent 90% pHx (pHx, $n = 15$), splenectomy before 90% pHx (S + pHx, $n = 15$), and splenectomy, ligation of the A. hepatica and 90% pHx (S + L + pHx, $n = 15$). Survival Log-Rank test and *post hoc* comparison; $^{\#}P < 0.05$ vs. pHx; $^{*}P < 0.05$ vs. S + L + pHx. pHx, partial hepatectomy; S, splenectomy; L, ligation.

well ligation of the A. hepatica survived the observation period.

Hepatic macrohemodynamics (set 2)

Animals of all groups exhibited values of mean arterial blood pressure (80–120 mmHg) and heart rate (400–450 beats/min) throughout the experimental period without differences among the three groups (data not shown). During baseline conditions (B), PVBF (Fig. 3a) and HABF (Fig. 3b) were about 1.6 ± 0.1 ml/min × g and 0.21 ± 0.01 ml/min × g, respectively. Splenectomy (S) resulted in a significant reduction in PVBF to 1.2 ± 0.1 ml/min × g (Fig. 3a), which induced a HABR with a significant increase in HABF averaging 0.31 ± 0.02 ml/min × g (Fig. 3b). As expected, ligation of the A. hepatica after splenectomy (L) decreased HABF to zero, while PVBF remained unchanged.

With increasing extent of liver resection, PVBF in relation to the mass of the liver remnant was found dramatically increased up to fourfold of baseline (Fig. 3a). This increase in PVBF did not affect the HABF up to 85% pHx, but induced a slight decrease after 90% pHx (Fig. 3 b). After splenectomy, pHx also induced a fourfold increase in PVBF (Fig. 3a). However, this increase in PVBF was associated with a steep increase in HABF (Fig. 3b). Calculation of the total hepatic blood flow upon 90% pHx resulted in values of 6.0 ± 1.0, 5.3 ± 0.8, and 4.6 ± 0.7 ml/min × g in animals with hepatectomy alone, splenectomy before hepatectomy, and splenectomy

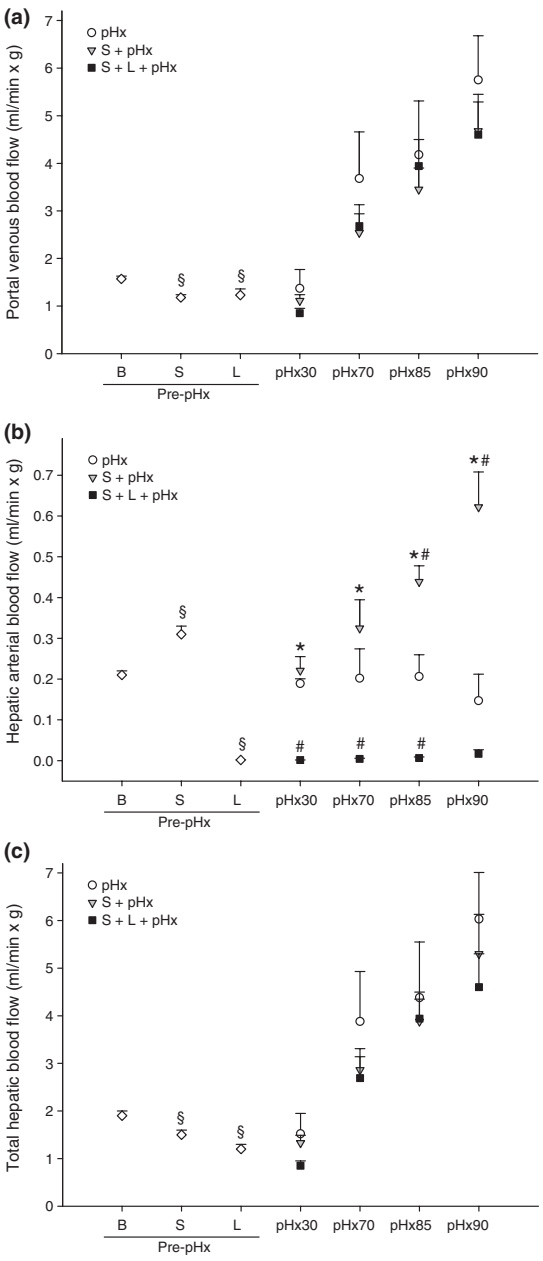

Figure 3 Ultrasonic flowmetry of PVBF (a) and HABF (b) and calculation of the total hepatic blood flow (c). Prior to the surgical procedure baseline values (B; $n = 15$) were measured. Initial values of splenectomy only (S; $n = 10$) and ligation of the A. hepatica after splenectomy (L; $n = 5$) before pHx as well as data after each resection step of the individual groups were assessed: pHx alone (pHx), splenectomy before pHx (S + pHx) and splenectomy, ligation of the A. hepatica and pHx (S + L + pHx). Values are given as mean ± SE. Two-way ANOVA and *post hoc* comparison; $^{\#}P < 0.05$ vs. pHx; $^{*}P < 0.05$ vs. S + L + pHx. One-way ANOVA for repeated measures and *post hoc* comparison; $^{\S}P < 0.05$ vs. B. PVBF, portal venous blood flow; HABF, hepatic arterial blood flow; pHx, partial hepatectomy; S, splenectomy; L, ligation; SE, standard error; ANOVA, analysis of variance.

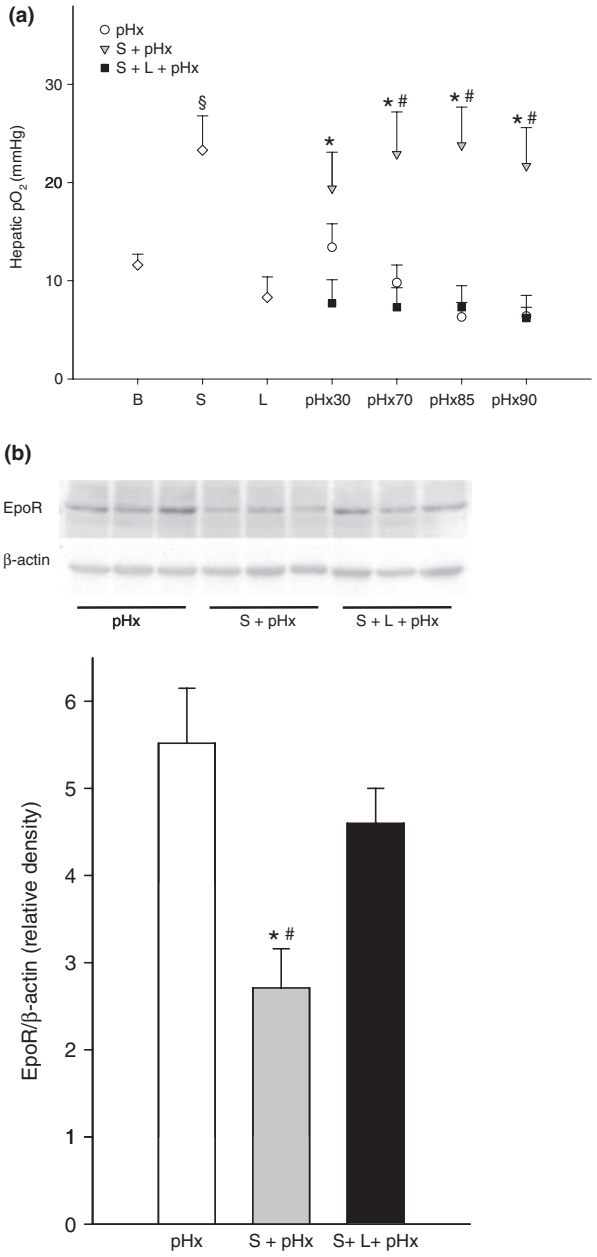

Figure 4 Hepatic tissue oxygenation (a) of pHx alone (pHx), splenectomy before pHx (S + pHx), and splenectomy, ligation of the A. hepatica and pHx (S + L + pHx). Values are given as mean ± SE of five independent experiments per group. Two-way ANOVA and *post hoc* comparison; $^{\#}P < 0.05$ vs. pHx; $^{*}P < 0.05$ vs. S + L + pHx. One-way ANOVA for repeated measures and *post hoc* comparison; $^{\S}P < 0.05$ vs. B. Representative Western blot analysis and densitometric analysis of erythropoietin receptor protein expression 24 h after 85% liver resection in the three groups (b). Signals were corrected with that of β-actin serving as internal control. Values are given as mean ± SE of 5–6 independent experiments per group. One-way ANOVA and *post hoc* comparison; $^{\#}P < 0.05$ vs. pHx; $^{*}P < 0.05$ vs. S + L + pHx. pHx, partial hepatectomy; S, splenectomy; L, ligation; SE, standard error; ANOVA, analysis of variance.

and ligation of the hepatic artery before hepatectomy (Fig. 3c).

Hepatic tissue oxygenation (set 2)

Polarographically assessed tissue pO_2 was found significantly increased upon splenectomy (23.3 ± 3.5 mmHg) when compared with baseline values of 11.6 ± 1.1 mmHg (Fig. 4a), most supposedly as a consequence of the elevated HABF (Fig. 3a). As expected, ligation of the A. hepatica after splenectomy abolished the effect of splenectomy on hepatic pO_2, resulting in pO_2 values even below baseline conditions (Fig. 4a).

Of note, extended liver resection of 85% and 90% resulted in an impaired tissue oxygenation, demonstrating pO_2 values below 10 mmHg (Fig. 4a). In parallel with the increased hepatic arterial inflow after stepwise resection (Fig. 3b), splenectomy before pHx prevented the decrease in hepatic tissue pO_2, guaranteeing maintenance of pO_2 values between 19 mmHg and 23 mmHg even after extended hepatectomy (Fig. 4a). Ligation of the A. hepatica in splenectomized and hepatectomized animals resulted in hepatic pO_2 values as low as those found in animals with pHx alone (Fig. 4a).

Consistent with this, splenectomy before 85% pHx attenuated the hypoxic response as indicated by a significantly reduced protein expression of EpoR. This confirms a sufficient hepatic oxygenation as a result of the increased hepatic arterial inflow (Fig. 4b).

Liver regeneration (set 3)

Accordingly, indicators of hepatocellular regeneration, i.e. the number of Ki-67 positive hepatocytes, revealed significantly higher values 24 h after 85% pHx in liver tissue of animals that underwent splenectomy when compared with animals, which underwent pHx alone or ligation of the A. hepatica after splenectomy (Fig. 5a and b). Furthermore, the weight of the regenerated livers 24 h and 4 days after splenectomy and 85% pHx was noticeably increased compared with the two other groups (Fig. 5c and Table 2), reflecting the beneficial effects of splenectomy on the regenerative capacity of the liver. Plasma analysis revealed reduced activities of transaminases in group S + pHx in comparison with pHx alone, whereas additional ligation of the hepatic artery showed a tendency to revert this positive effect of splenectomy (Table 2).

Discussion

The herein presented study communicates the following major findings: Firstly, extended pHx resulted in portal

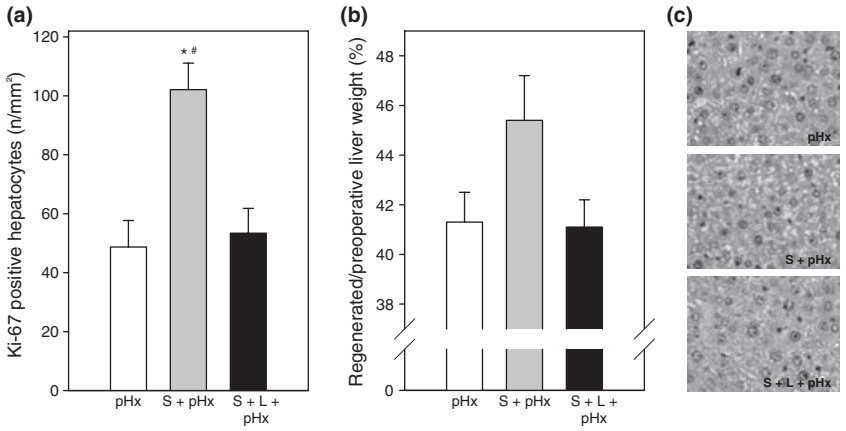

Figure 5 Quantitative analysis of Ki-67-stained parenchymal cells in liver tissue sections (a, original magnification ×400) as well as representative immunohistochemical images (b), and liver weight (c) at 24 h upon 85% pHx alone (pHx), splenectomy before 85% pHx (S + pHx) and splenectomy, ligation of the A. hepatica and 85% pHx (S + L + pHx). Values are given as mean ± SE of 5–6 independent experiments per group. One-way ANOVA and *post hoc* comparison; $^{\#}P < 0.05$ vs. pHx; $^{*}P < 0.05$ vs. S + L + pHx. pHx, partial hepatectomy; S, splenectomy; L, ligation; SE, standard error; ANOVA, analysis of variance.

hyperperfusion of the remaining liver lobes, which only affected arterial perfusion little, but induced low hepatic tissue oxygenation. Secondly, splenectomy induced an increase in HABF according to a HABR, which was capable of increasing hepatic tissue oxygenation by a factor of 2. Thirdly, pHx after splenectomy resulted also in a marked portal hyperperfusion, but additionally increased HABF, thereby maintaining the high tissue oxygenation level. This resulted in enhanced regeneration with a significant improvement of survival. Fourthly, ligation of the A. hepatica abolished the beneficial effect of splenectomy on tissue oxygenation and outcome after extended hepatectomy, proving the pivotal role of the enhanced tissue oxygenation for survival of the small-for-size syndrome.

Excessive portal blood flow is thought to contribute to the dysfunction and failure after extended liver resection, segmental liver transplantation as well as transplantation in patients with uncompensated severe portal hypertension [8,22–24]. Whereas under normal conditions most cells have an adequate oxygen concentration, under critical conditions, hepatocytes may have an oxygen concentration at which mitochondrial respiration and ATP synthesis are partially oxygen limited [25]. Reduced-size livers may suffer from an unbalance between liver regeneration and liver function, which leads to a severe graft dysfunction. Its pathogenesis is still debated, although it is assumed that the syndrome is primarily linked to portal hyperperfusion.

Table 2. Regenerative/preoperative liver weight and transaminase activities at 4 days upon 85% pHx alone (pHx), splenectomy before 85% pHx (S + pHx) and splenectomy, ligation of the A. hepatica and 85% pHx (S + L + pHx).

	pHx	S + pHx	S + L + pHx
Regenerative/preoperative liver weight (%)	93.9 ± 4.6	102.7 ± 4.0	94.9 ± 2.7
AST (U/l)	193 ± 29	132 ± 11	158 ± 32
ALT (U/l)	94 ± 12	66 ± 5	76 ± 12

Values are given as mean ± SE of 5–6 independent experiments per group.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; pHx, partial hepatectomy; S, splenectomy; L, ligation.

In humans, 60% pHx results in a doubling of the portal flow in the remnant liver tissue [24]. This extent of pHx is followed by a transient small-for-size syndrome of minor degree, which usually resolves spontaneously within a few days. In contrast, major liver resection >75% is followed by a more pronounced and long-lasting small-for-size syndrome with much higher morbidity and mortality [24]. However, occurrence and pathogenesis of small-for-size syndrome remain highly controversially discussed [26]. In our study, 70% liver resection led to approximately 2.3-fold increase in PVBF with a further elevation up to fourfold in case of 90% pHx.

Modulation of liver inflow after segmental liver transplantation or massive liver resection was advocated as a way to overcome organ hyperperfusion, including splenic artery ligation and splenectomy or diversion of portal vein blood by means of portocaval and mesocaval shunts [24,27–29]. However, these procedures have been used despite the lack of hemodynamic data [30,31]. In the present study, we observed a significantly increased survival of simultaneously splenectomized and hepatectomized animals compared with animals with 90% pHx alone. It has been discussed that this effect is mainly

caused by associated suppression of the intrahepatic flow and thus less sinusoidal shear stress [7,19,32]. However, reduction in total hepatic inflow in simultaneously splenectomized and hepatectomized animals was marginal and not that pronounced what it would take to improve survival by reduced shear stress. Instead, splenectomy before pHx caused a doubling of hepatic tissue pO_2 due to a HABR and, paradoxically, was associated with a further rise in HABF during extended, i.e. 85% and 90% pHx, which led to high tissue pO_2 values and reduced hypoxic stress. Supposedly, the paradoxical increase in arterial inflow covered the oxygen demand and thereby improved organ regeneration and animal survival.

The spleen is involved in immune defenses as a component of the reticuloendothelial system and splenectomy has been associated with decreased antibody formation, decreased IgM production, and decreased clearance of particulate antigens [33]. However, the immunological effect of splenectomy and the incidence of septic complications in liver transplantation and major liver resection are controversial [29,34–38].

It has been reported that platelet-derived serotonin is involved in the initiation of liver regeneration [39] and that platelets promote liver regeneration [40]. As splenectomy caused an increment in peripheral platelet counts [41,42], these findings suggest that the spleen may compromise the regenerative capacity of the liver and *vice versa* splenectomy may promote liver regeneration. However, the herein observed benefit in small-for-size livers can be clearly attributed to a rise in HABF with sufficient oxygen supply rather than to splenectomy itself because the beneficial effect of splenectomy was abolished by simultaneous ligation of the hepatic artery.

Extended liver resection causes a complex competitive scenario of hepatocellular proliferation. Moreover, at the same time, hepatocytes perform all essential functions needed for homeostasis [43]. To maintain the balance between regeneration and liver function, the regenerating liver consumes large amounts of oxygen to restore the hepatic energy charge in response to a greatly increased demand for energy [20,44]. The present data indicate that improved tissue oxygenation correlates with increased regeneration capacity of the liver.

In summary, our data provide evidence that improved arterial inflow rather than reduction in portal venous hyperperfusion is of high significance for the beneficial effect after surgical decompression of portal inflow in small-for-size livers.

Authorship

CE: contributed to conception as well as design of the study and drafted the manuscript. KA: performed the Western blots, evaluated the statistics, and drafted the manuscript, JR: carried out the animal experiments, evaluated the immunohistological sections, DC: carried out animal experiments, MDM: contributed to conception of the study and revised the manuscript critically for important intellectual content, BV: contributed to conception as well as design of the study and revised the manuscript critically for important intellectual content.

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