OPTIMIZING THE LIVING DONOR LIVER TRANSPLANTATION - EFFECTS OF VARIOUS DONOR PRETREATMENTS AFTER PARTIAL HEPATECTOMY IN THE RAT

PhD Thesis

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Introduction

Between 1955 and the end of 1967, the framework of clinical organ transplantation that exists today was established in few centers in continental Europe, Great Britain and North America. The kidney was at first the forerunner organ, but liver transplantation soon became the driving force in discoveries and advances that were applicable for other kinds of organs. These accomplishments included the development of better methods of organ preservation, the evolution of present-day immunosuppression, and the elucidation of several mechanisms of alloengraftment and acquired tolerance. In addition, research in liver transplantation is provided insight into the metabolic interrelations of the intraabdominal viscera in normal and pathological conditions, progress in the understanding and treatment of liver based inborn error of metabolism, and identification of growth factors that influence hepatic growth control and regeneration.

With the advances in immunosuppression, postoperative care and surgical technique, liver transplantation has become the golden standard in the treatment of many chronic liver diseases. Since then, the number of patients on the waiting list has increased and organ shortage appeared to be one of the major problems in clinical transplantation. In the past a very high death rate on the waiting list was common among children needing a liver transplant. Both split and living related liver transplants (LDLT) have contributed to a considerable reduction of their mortality while waiting for a liver transplant.

Major advantages of LDLT include the good viability of the liver harvested from a healthy individual, the careful selection of the timing of the transplantation, and the potential good tissue matching. The reduced waiting period for a living donor organ may decrease the risks of decompensation or death before transplantation, thereby improving the overall chances for success. Disadvantages are the risk to healthy donors and also the fact that, this modality has a potential psychological burden on the donor. A wide range of complication rates are reported in the literature in donors after LDLT. Donor safety has a major importance in LDLT.

In contrast to graft procurements from cadaver donors, graft procurements from living donors are usually elective procedures, offering the possibility for careful donor preparation including the application of substances that are beneficial for the graft and not harmful to the donor – or, even preferable, that are advantageous for the donor himself, protecting his residual liver during the harvesting of the graft. Potential candidates for these pre-treatments are α-tocopherol (vitamin E), the flavonoid silibinin and the amino acid L-glycine.

α -Tocopherol

α -Tocopherol is a potent, lipophilic antioxidant that has been shown to offer protection against ischemia/reperfusion injury to the liver and against hypothermic injury/cold-induced apoptosis.

Silibinin

The flavonolignane silymarin, isolated from the fruit of the milk-thistle, has well-known hepatoprotective properties. The whole extract, silymarin, composed of the three isomers silibinin, silidanin and silichristin, has been shown to provide protection in different models of experimental liver intoxication. Silibinin inhibits hypothermic injury/cold-induced apoptosis as well as reperfusion injury and oxidative components of inflammatory injuries.

L-Glycine

L-Glycine is the most simple, non-essential amino acid. A lot of evidence has accumulated that glycine is an effective anti-inflammatory, immunomodulatory and cytoprotective agent, that provides strong protection against hypoxic injury of hepatocytes as well as of other cell types. In addition, glycine has been shown to be a strong inhibitor of Kupffer cell activation and, by this property, to inhibit the manipulation-induced injury during liver harvesting.

Based on the increase in understanding the mechanisms underlying hepatic regeneration, a variety of strategies have been developed to bolster liver regenerating, including injection of several growth factors, preconditioning methods, etc. Exogenous application of tri-iodothyronine (T3) stimulated a liver regeneration response that resembled in timing and in magnitude of deoxyribonucleic acid (DNA) synthesis.

To guarantee sufficient liver function, in particular in small remnants, the preservation of vascular and biliary structures of the entire remnant liver is of paramount importance. While the need of an optimal arterial and portal venous blood supply is well documented, there is little information regarding the impact of hepatic outflow obstruction on both liver regeneration and function after major hepatectomy. From the experience gained in the field of living-related liver transplantation, it is known that an inadequate venous outflow may also result in reduction of arterial and portal inflow with subsequent impairment of liver function and liver regeneration.

Liver function parameters and regeneration are significantly better in patients undergoing a small resection than in patients undergoing a liver resection of more than 60%. This effect exceeds a linear size correlation, which led to the conclusion that graft or remnant

liver size influences regeneration. The underlying molecular mechanisms, however, are not well understood. In particular, the role of proregenerative cytokines and the role of transcription factors are ambiguous.

Research objectives

1. Determination of the effect of pre-treatment with α-Tocopherol, Silibinin and L-Glycine on the liver injury, after partial hepatectomy in the rat.

2. Determination of the effect of exogenous administration of T3. Whether it leads to a stimulated liver regeneration after 70% partial hepatectomy (PH) and whether treatment of T3 also confers a survival advantage after 90% partial hepatectomy.

3. Studying the role of the venous outflow after major hepatectomy. We established a rat model to investigate the impact of hepatic vein deprivation on both hepatic regeneration and function after major hepatectomy.

4. Determination of the activation of transcription factors and cytokines after hepatectomy. While previous studies have largely focused on the molecular events after partial hepatectomy, the aim of this work was to investigate liver regeneration after subtotal hepatectomy. We analyzed whether the extent of liver resection has an impact on the activation of transcription factors and the expression of pro- and anti-regenerative cytokines using a rat resection model and compared 70% (partial hepatectomy, PH) and 90% resection (subtotal hepatectomy, SH), respectively.

Material and methods

1. Pre-treatment with α-tocopherol, silibinin and L-glycine

Animals and methods

All animals received humane care in compliance with the German Law for the Protection of Animals and the institutional guidelines, and permission for the use of the animals for liver resection was obtained from the local authorities. Male 6-8 weeks-old Wistar rats were fed ad libitum and maintained under a 12-hour light/dark cycle. Animals were divided randomly into five groups:

- One group of rats received a daily intragastric administration of α-tocopherol (vitamin E; Uno-Vit-600, C.P.M. Contract-Pharma, Bruckmühl, Germany; 100 mg/kg body weight for the last 3 preoperative days).
- One group of rats received intraperitoneal injections of the flavonoid silibinin (silibinin-dihydrogen succinate; kindly provided by Madaus, Cologne, Germany, and dissolved in normal saline) in a daily dose of 100 mg/kg body weight for the last 5 days preoperatively.
- Another group of rats was fed a glycine-enriched chow (5% L-glycine; Fa. Ssniff, Soest, Germany) for the last 5 preoperative days; the standard rat chow contained 0.87% L-glycine.
- In the combination group, animals were treated with all three pre-treatments simultaneously.
- The control group did not get any pre-treatments.

Operative procedure

90% partial hepatectomy was performed under isoflurane anesthesia according to the method of Higgins and Anderson.

Sham animals were also operated under inhalation anesthesia, laparotomy was performed and the liver lobes were freed from their ligaments. "Anaesthetized rats" received anesthesia only, without laparotomy.

Samples

Animals were sacrificed after 0 hours, 6 hours, 12, 24, 48, 72 and 168 hours and 4 weeks after surgery. The residual livers were removed, weighed and fixed or rapidly frozen in liquid nitrogen. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and total bilirubin were assessed in serum samples using standard assays. Prothrombin time (PT) was assessed in plasma samples using a standard assay.

Liver histology

Liver samples fixed in buffered 4% formaldehyde were embedded in paraffin, cut in 5 µm sections and stained with hematoxylin-eosin. Additional sections were used for naphtol-AS-D-Chloracetate-esterase (ASDCL) staining.

Western blots

Tissue samples were homogenized in the extraction buffer provided with the Nuclear protein extraction kit (Pierce: NE-PER kit). Nuclear proteins were extracted according to the manufacturer's instructions. Protein concentrations were determined using the Bio-Rad kit. Nuclear extracts per lane were subjected to 7.5 % SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto a nitrocellulose membrane (Schleicher and Schuell). HIF-1α was detected using a rabbit polyclonal antibody against mouse HIF-1α (Novus Biologicals). α-tubulin (Sigma) served as a loading control.

TUNEL assay

TUNEL reaction mixture was prepared according to protocol (*In Situ* Cell Death Detection Kit). Hepatocyte apoptosis was quantitated by counting the number of TUNEL-positive cells in 10 random microscopic high-power fields (x100).

Real Time - Polymerase Chain Reaction (RT-PCR)

One–step RT-PCR was performed using the iCycler iQ Real Time PCR (Bio-Rad Laboratories) and the QuantiTect SYBR Green RT-PCR Kit (Qiagen) according to the manufacturers instructions. To account for possible concentration errors, the house keeping gene β-actin served as a reference control.

2. Pre-treatment with tri-iodothyronin

Animals and methods

Tri-iodothyronine (Sigma) was dissolved and administered at a dose of 4 mg/kg bodyweight in each rat in the early morning hours. In a dose-response curve, we demonstrated that the application of tri-iodothyronine 10 days before surgery results in the highest Ki-67 proliferation index as well as liver body weight ratio.

- To investigate the stimulatory effect of exogenous tri-iodothyronine 6 rats each received tri-iodothyronine or placebo in the above-mentioned concentration and were subjected to a 70% partial hepatectomy (PH) 10 days after the injection. 24 h after hepatectomy, rats were sacrificed.
- To investigate whether the exogenous administration of tri-iodothyronine confers a survival advantage 20 rats each received tri-iodothyronine or placebo in the concentration

mentioned above. They were subjected to a 90% PH 10 days after the injection and followed up for 4 days.

Liver Body Weight Ratio (LBWR),

After the observation period, the remnant, regenerated liver was resected and weighed (A) and total body weight (B) was measured. The acquired data were expressed as percentage of the ratio between remnant liver weight, divided by the total body weight times 100. LBWR (%) = A/B x 100.

Immunohistochemistry for Ki-67

Immunostaining for Ki-67, a marker for cell proliferation, was performed to evaluate the proliferation of hepatocytes. We used an antibody against mouse Ki-67 antigen (Fa. DCS) to evaluate the percentage of hepatocytes that entered the regenerative process after hepatectomy. 'Proliferation index' was defined as the percentage of Ki-67-positive cells counted in 5 periportal (within 100 μm of the portal area) and perivenular (within 100 μm of the central vein) fields of a specimen.

Immunohistochemistry for Vascular Endothelial Growth Factor (VEGF)

The primary antibody was a rabbit anti-VEGF antibody, dilution 1: 600 (Zymed laboratories). VEGF expression was described as weak (+), moderate (++) or strong (+++) in 5 randomly chosen fields of a specimen.

RNA Isolation

Total RNA was isolated from each regenerating liver of each animal using Trizol (Gibco) according to the manufacturer's specification. The quality and integrity of RNA were checked by spectrophotometry and 1% ethidium bromide agarose gel electrophoresis (Gibco).

Complementary DNA Array

A customized cDNA array consisting of 134 rat genes was established as described previously. Briefly, 18 genes were collected from image cDNA clones ordered at the 'Deutsches Ressourcenzentrum für Genomforschung GmbH, Berlin'. The clones were cultivated and plasmid DNA was isolated using Qiagen Mini Prep Kit (Qiagen) and PCR was performed to amplify the products. The other 116 genes were amplified from total rat cDNA using the Omniscript Reverse Transcriptase kit (Qiagen). As a control, glyceraldehyde-3-phosphate dehydrogenase (13 GAPDH) and β-actin gene probes were added.

RT-PCR

One-step RT-PCR was performed using the Rotor-Gene 2000 real-time Amplification System (Corbett Research) and the QuantiTect SYBR Green RT-PCR Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions.

3. Hepatic vein deprivation

Operative technique

The procedures were performed under isoflurane anesthesia. Rats were randomly assigned to one of the following three groups, intending:

- To investigate the stimulatory effect of an anatomic 90% subtotal hepatectomy. Five rats underwent a 90% partial hepatectomy using the method described by Emond et al. and Higgins et al.
- To investigate the impact of hepatic vein deprivation. Five rats received a 70% liver resection with an additional narrowing of the hepatic vein draining the bigger left lobe (70%+ PH). This was done with a 7-0 prolene suture under the microscope always in the middle of the draining vein. The rats were afterwards observed for an additional 10min to check for an immediate change in perfusion of the narrowed lobe. In none of the used animals did we notice an immediate perfusion problem.
- To evaluate a control group, a 70% PH without additional venous outflow obstruction was performed.

In all groups, five rats per group were sacrificed postoperatively at 0, 24, 48, 72, and 120h (n = 75). One hundred and twenty hours was chosen as the latest observation point, as no change in regenerative or functional parameters beyond 120h has been observed.

Except the above mentioned methods the followings were performed:

Galactose elimination capacity

To determine the galactose elimination capacity (GEC), 0.5ml of 50% galactose was administered via the portal vein. Blood was drawn before and every 10min between 20 and 60min after the administration. A bladder puncture was performed at 60min to collect urine. Galactose elimination capacity was calculated as the ratio of the injected amount of galactose (with correction for urinary concentration) to the extrapolated time to zero concentration as described before.

4. Extent liver resection and activation of cytokines and transcription factors

Animals and methods

Six to eight-week-old male Wistar rats were anaesthetized with isoflurane. Seventy percent partial hepatectomy and 90% PH were performed under isoflurane anesthesia as described by Higgins *et al* and Emond *et al*. The rats were divided into 4 groups:

- control group (untreated)
- sham operation
- 70% PH and
- 90% PH

Serum and liver tissue samples were obtained during surgery and 2 h, 12 h, 24 h, 48 h, 72 h and 7 d after resection ($n = 4$ at each time point, per group).

Except the above mentioned methods the followings were performed:

ELISA

NF-κB (NF-κB p65 ELISA KIT), and STAT3 (STAT3 (pY705) ELISA KIT) ELISAs were conducted according to the manufacturer's instructions. Negative and positive controls were included and a standard curve was determined for each assay.

Statistical analysis

All groups assessed for liver injury, liver regeneration and survival included 6 rats per time point, groups for HIF-1α accumulation 4 rats per time point. Assays for liver enzymes, serum bilirubin and prothrombin time were performed in duplicate. Data are expressed as means ± standard deviation. Data obtained from multiple groups were performed using an analysis of variance (ANOVA) with Dunnett post hoc comparisons. A p value of < 0.05 was considered significant.

Results

1. Results of pre-treatment with α-tocopherol, silibinin and L-glycine

The pre-treatments did not adversely affect the preoperative condition of the rats. Average rat weight at the time of the operation was 336.3 ± 38.5 g, with no significant differences between the groups. 90% partial hepatectomy was performed with an average operation time of 23.1 ± 5.0 min and an average duration of the anaesthesia of 36.4 ± 5.6 min; there were no significant differences between the groups.

The resected liver mass was 3.15 ± 0.16 g / 100 g body weight, which in the rats sacrificed immediately after surgery (residual liver weight: 0.33 ± 0.05 g / 100 g body weight) was equivalent to 89.5% resection. Resected liver mass did not differ between the groups.

Survival rate and clinical outcome

Early postoperative survival (≥ 48 hours) was 16 of 18 animals in the control group (no pre-treatment), 17 of 18 animals after pre-treatment with glycine, 15 of 18 animals after pre-treatment with silibinin, 13 of 18 animals after pre-treatment with α-tocopherol, and 14 of 18 animals after the combined pre-treatment. Long-term survival (4 weeks) after 90% partial hepatectomy was 5 of 6 animals in all groups except for the animals pretreated with α-tocopherol or with combined pre-treatment (where it was 4 of 6 animals).

Laboratory markers of liver injury

As parameters of liver cell damage induced by 90% partial hepatectomy, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were determined. Pre-treatment with glycine decreased the release of both transaminases by about 50%. Pre-treatment with silibinin slightly decreased AST and moderately (significantly) decreased ALT, and α- tocopherol and combined pre-treatment slightly, but not significantly decreased the release of the transaminases. Animals pre-treated with glycine, α-tocopherol or silibinin all had significantly decreased serum ALP activities compared to non-pre-treated animals.

Synthetic function of the liver

In the first 3 days after 90% partial hepatectomy some rats were clinically jaundiced, and an increase in postoperative serum bilirubin levels was observed in all rats. Glycine pre-treatment significantly ameliorated this increase, while pre-treatment with α-tocopherol or silibinin (as well as combined pre-treatment) was not beneficial. Prothrombin time (PT) was slightly increased in non-pre-treated animals peaking at 24 h postoperatively and reaching the baseline again after one week. Pre-treatment with glycine did not change this time course but blunted the increase in PT.

Regeneration of the remnant liver mass

When the rats were sacrificed after different periods, liver regeneration (growth of the caudal lobe) was observed starting from postoperative day 2. There were no significant

differences between the groups. (non-resected rats 3.34 ± 0.26 g/100 g rat vs. resected rats 1.39 ± 0.46 to 1.67 ± 0.29 g/100 g rat).

Histological and immunhistochemical results

In glycine pre-treated animals, histology did not show necrotic areas, although some fatty changes could also be observed in the regenerating livers at later time points. ASDCL staining of remnant liver sections of non-pre-treated rats revealed occasional infiltrating granulocytes in the perisinusoidal areas, and staining of residual liver tissue in the glycine group hardly differed from normal liver tissue (7.1 ± 2.5 granulocytes per field of vision in remnant livers of non-pre-treated rats and 5.9 ± 4.3 granulocytes in remnant livers of glycine-pre-treated animals; $p > 0.05$). Early postoperative apoptosis, as assessed by TUNEL staining, was decreased in glycine-pre-treated animals (27 ± 9 TUNEL-positive cells, mainly hepatocytes, per 10 fields of vision in liver sections of non-pre-treated animals and 5 ± 4 TUNEL-positive cells in liver sections of glycine-pre-treated animals at 24 hrs postoperatively; $p < 0.01$), while delayed apoptosis (48 hrs) did not differ between glycine-pre-treated and non-pre-treated animals.

Activation of inflammatory factors

Immediately after the operation as well as 12 hrs postoperatively, neither in the residual livers of non-pre-treated rats nor in those of glycine-pre-treated rats an induction of the inflammatory cytokine IL-1-β could be observed (RT-PCR; values were below those of non-operated rats). In contrast to this, early induction of the adhesion molecule ICAM-1 was observed after 90% liver resection, and this induction was also blunted by glycine-pre-treatment.

Activation of HIF-1 α

Anaesthetized rats showed little accumulation of the hypoxia-inducible factor 1α (HIF- 1α). In sham operated rats (laparotomy and mobilization of the liver), HIF-1α accumulation was decreased and HIF-1α was barely detectable. In contrast, 90% liver resection led to strong accumulation of HIF-1α. Glycine pretreatment significantly reduced this accumulation.

2. Results of pre-treatment with tri-iodothyronin

Impact of T3 on Liver Body Weight Ratio (LBWR)

24 h after 70% PH, rats treated with T3 showed a LBWR of 1.9 ± 0.12%, which was statistically higher than untreated rats with a LBWR of 1.65 ± 0.19% ($p = 0.049$). Similar results could be demonstrated for 90% hepatectomized rats. Animals treated with a single injection of T3 had a LBWR of 1.57 ± 0.15% compared to 1.2 ± 0.14 in rats with placebo treatment only ($p = 0.025$).

Impact of T3 on Proliferation Index (Ki-67)

As expected, there were a significantly higher proportion of hepatocytes proliferating in hepatectomized rats than in the quiescent liver. The proliferation index increased to 78.6 ± 9.46 after T3 stimulation compared to 41.30 ± 19.92 in control injected rats 24 h after 70% PH ($p < 0.001$). 4 days after 90% PH, 68.32 ± 18.38% of all hepatocytes were proliferating in T3-stimulated rats, but only 42.76 ± 14.73% in rats with a control injection ($p < 0.001$).

Impact of T3 on VEGF Expression

In animals treated with T3 we saw a higher expression of VEGF, which was described as strong (+++) in the fields investigated, while we saw only weak (+) to moderate (++) expression in placebo treated animals.

Impact of T3 on Overall Survival

To assess the effect of T3 on survival, we had to use a model of subtotal hepatectomy. After performing 90% SH, the survival over 96 h was assessed. While only 7 of 20 animals died during the observation period in T3-treated rats, 11 placebo- treated rats died during the observation period. Treated rats showed a tendency towards a higher postoperative survival compared to rats which received placebo only. However, statistical analysis showed no significant differences ($p = 0.1318$).

Impact of T3 on Gene Expression

To further elucidate the underlying mechanisms of action for the modulatory effects of T3 on liver regeneration in our model, we established a customized complementary DNA array for 134 genes known to be involved in liver regeneration. 24 h after 70% PH, there was no difference in gene expression of treated or untreated rats compared to sham operated rats. Furthermore, we did not detect a significant difference in gene induction, when both groups

were compared with each other. 90% hepatectomized rats treated with a single injection of T3 showed a statistical significant overexpression of Fms-related tyrosine kinase 1 (Flt1), peroxisome proliferator-activated receptors (PPAR), and complement 3 (C3) compared to untreated rats, which could be confirmed by RT-PCR.

3. Results of impact of hepatic vein deprivation

Histopathology and immunohistochemistry for Ki-67

At 120h, we observed an almost equal proliferation index in both groups with 6.6 ± 3 and 7.5 ± 2%, respectively. In 70% PH, we observed a rapid increase during the first 24–48h and a slow decrease afterwards. Nevertheless, the regenerative response was still higher than in 70%+ PH. Regarding those rats that received a classic 90% PH, the liver showed high mitotic rates. This was also confirmed by immunohistochemistry with Ki-67. The proliferation index showed similar kinetics in rats, which underwent a classic or a 70%+ PH with only a slow proliferation during the first 24h, a rapid increase at 48h, and a decline at 72 and 120h. Rats, which received a classic 90% PH (group A), showed an increased proliferation index at 12 and 24h, but this did not reach statistical significance compared to animals with a 70%+ PH (group B; $p > 0.05$).

Galactose elimination capacity

The GEC is a further liver function test, in which the elimination rate of administered galactose by the liver is determined. There was no difference in GEC between 90% PH (7.96mg min^{-1} g^{-1}) compared to 70%+ PH (8.46mg min^{-1} g^{-1}). In rats with 70% PH, a significantly higher GEC (11.74mg min^{-1} g^{-1}) was measured compared to 90% PH ($p < 0.001$) and 70%+ PH ($p < 0.002$).

Gene expression analysis

To further elucidate the underlying mechanisms of action for the modulatory effects of hepatic vein deprivation on liver regeneration and functionality in our model, we established a customised complementary DNA array for genes known to be involved in liver regeneration. Of the 134 genes chosen, we found 14 genes (TGF-β, Ftl, TNF- α, TGF- β receptor1, FLT1, VEGF-δ, PPAR, NFkB-α, IRAK-M, PDGF-α, C3, Cyclin G1 (Ccng1), Ferritin, heavy polypeptide 1 (Fth1) and V-jun) to be modulated during the observation period compared to untreated control rats. Interestingly though, the expression kinetics did not differ significantly

between the two groups. Randomly chosen, we were able to confirm our findings for TGF-β receptor1, PDGF-β and Irak-M by quantitative RT-PCR.

Overall survival

Mortality rate was 0% in 70% PH, 25% in 70% + PH and 26% in 90% PH. In 90% PH and 70%+PH, deaths occurred always between 48 and 72h. There were no deaths observed beyond 120h.

4. Results of the impact of different extent of hepatectomies on cytokine and transcription factor expression.

Liver regeneration

The overall mean of liver body weight ratio LBWR was 4.06% ± 0.35% in control and sham-operated animals. After 70% resection, animals showed a continuous increase in LBWR over 7 d starting from 0.74% ± 0.06% at the time of surgery, and reaching 2.70% ± 0.15% 7 d postoperatively. The earliest significant increase in LBWR occurred between 2 h (0.88% ± 0.15%) and 12 h (1.39% ± 0.07%) with $p = 0.006$.

Serum levels of liver enzymes

AST and ALT were significantly raised in the 70% resected animals compared to sham-operated rats. Peak levels were found for both enzymes at 12 h postoperatively (AST, 12 h: 1055 ± 55 for 70% and 2204 ± 739 for 90%, $F = 0.011$; ALT, 12 h: 753 ± 110 for 70% and 1706 ± 725 for 90%, $p = 0.011$). LDH after 70% resection did not differ significantly from sham animals except at 7 d postoperatively. LDH 7 d after 70% resection was 2060 U/I while the level in shamoperated animals was 890 U/I ($p = 0.033$).

Activation of NF-κB and STAT3

As described in the literature, NF-κB activation was observed after 70% PH during the early phase of regeneration (0 h: 273.33 ± 24.45 pg, $P = 0.024$; 2 h: 285.34 ± 36.49 pg, $p = 0.009$) and 12 h postoperatively (313.21 ± 17.22 pg, $p = 0.001$). NF-κB remained activated until 7 d after surgery in this group. After 90% PH, however, NF-κB activation was delayed until 24 h after the operation. NF-κB was significantly activated in the 90% PH group 24h after surgery (475.66 ± 144.29, $p = 0.048$) with a peak at 48 h (747.18 ± 146.36 pg, $p = 0.02$). NF-κB activation was comparable in both groups at day 7. Because we utilized a STAT3 (pY705) ELISA, only phosphorylated STAT3 was measured in the assay. Activation of

STAT3 occurred during surgery in both the 70% (16-fold) and 90% (3-fold) resections. Two hours after surgery, STAT3 activation increased significantly in the 70% PH (138-fold) and in the 90% PH group (197-fold), decreasing thereafter and reaching preoperative levels 24 h after surgery. The differences between the two groups did not reach statistical significance.

Expression of pro- and anti-regenerative cytokines

In the group with 70% PH, 6 h after resection a rise in TNF-α expression was detected compared to controls, reaching a maximum after 24 h and decreasing thereafter to preoperative levels. In contrast, a significant rise in TNF-α expression was not detected after 90% PH. For IL-6, a biphasic expression pattern occurred in 70% PH with high levels of expression at 2 h and 12 h postoperatively, while after 90% PH a significant up-regulation was only detected at 2 h after surgery. Postoperatively, HGF expression increased steadily reaching a maximum at 12 h after surgery and returning to preoperative levels after 24 h in both groups. A significant increase in early postoperative TGF-α expression was only detected after PH (12 h). At later time points, TGF-α expression was down-regulated in this group while it increased up to 7 d after resection in 90% PH. We detected a slight up-regulation in TGF-β expression in both resection groups at early time points (2 h, 6 h) with a strong peak at 12 h postoperatively which was detectable only in the 70% PH group (8.25-fold compared to controls). Thereafter, TGF-β expression returned to control levels.

Determination of apoptotic activity

Control animals had a TUNEL index (percentage of TUNEL-positive cells) of approximately 0.12%. After 70% PH, the rate of apoptosis reached a peak directly after surgery (0.44%), followed by a decrease to 0.27% at 24 h and to 0.20% at 48 h and returned to control levels at 7 d (0.15%). After 90% PH, however, the apoptotic peak was delayed until 24 h after surgery, declining to 0.18% at 48 h. In contrast to 70% PH, a second apoptotic peak (0.63%) was detected at 7 d in this group.

Conclusion

- The pre-treatments with glycine, vitamin-E and silibinin did not adversely affect the preoperative condition or the overall survival of the rats, although the rats pre-treated with α-tocopherol by gavage were subjected to some stress during the application of the substances. The weight gain of the rats in the postoperative period was slightly higher in the glycine group than in the control group, slightly lower with silibinin or

combined pretreatment and markedly lower in the α-tocopherol group. We also detected slightly toxic effect of vitamin-E, although the chosen dosage could be too high.

- Pre-treatment with glycine decreased the release of transaminases AST, ALT and ALP by about 50%, which shows lower hepatic injury after partial hepatectomy. These effect were significant without nominell ischaemia and reperfusion. We detected the protective effect of glycine pre-treatment also in the histological examination, and TUNEL assay –with the decreased rate of apoptotic activity.
- Glycine effects blunted induction of adhesion molecule ICAM-1 and activation of HIF-1 α was observed after 90% liver resection.

In summary, pre-treatment with dietary glycine significantly reduced liver injury after 90% partial hepatectomy in the rat in a model without nominal ischemia/reperfusion. Thus, pre-treatment of donors with glycine before the operative procurement of livers in living donation might be worthwhile in order to decrease the injury of the remnant liver (and possibly also of the graft). In addition, this glycine pre-treatment might also be considered for patients undergoing major hepatectomy, e.g. in tumor surgery.

- Animals treated with a single injection of tri-iodothyronin had a higher level of liver body weight ratio, which shows an icreased regenerative activity compared rats with placebo treatment only. These data were supported by a significantly higher proportion of hepatocytes proliferating (proliferation index, Ki-67) in hepatectomized rats than in the quiescent liver. In animals treated with T3 we saw a higher expression of VEGF, which declares an icreased neovascularisation activity.
- Pre-treatment with tri-iodothyronin did not significantly influence the overall survival rate and the level of hepatic injury. We could not detect a significant difference regarding the serum parameters (AST, ALT and GLDH) in rats treated with T3 compared to untreated rats. Furthermore, we did not detect a significant difference in gene induction.

In conclusion, we have shown that T3-treated rats have an improved liver regeneration following 70% PH and 90% SH. This may partly be due to its effects on neovascularization as demonstrated by immunohistochemistry. Therefore, treatment with T3 may represent a promising strategy to optimize liver regeneration in the setting of LDLT or after massive resection of the liver, especially due to its excellent general practicability.

- Deprivation of the venous outflow showed similar kinetics of the proliferation index in rats, which underwent a classic or a 70%+ PH. Rats, which received a classic 90% PH, showed an increased proliferation index, but this did not reach statistical significance compared to animals with a 70%+ PH.
- Histological investigation of the HE section of the same animals with 70%+ PH showed a perivenular swelling of the hepatocytes with clumped strands of eosinophilic cytoplasmic material compared with those without restriction of the hepatic outflow. There were statistical difference in the level of hepatic injury and liver function compared the groups of 70PH+ and 90% determing the transaminase levels, and galactose elimination capacity, and gene expression but there were difference compared to classic 70% PH.

I*n conclusion, in an animal model, we could demonstrate the influence of venous outflow obstruction on the functional and regenerative capacity of the liver after PH. Liver regeneration seems to be mainly driven by loss of volume and by the vascularisation of the remnant rather than by functionality. These results suggest that reconstruction of dissected large hepatic veins should be considered in resections with a small functioning remainder volumes subjected to venous congestion.*

- The overall mean of liver body weight ratio and level of transaminases were icreased after 70% PH compared to sham operated and controll rats.
- As described in the literature, NF-κB activation was observed after 70% PH during the early phase of regeneration and activation of STAT3 occurred during surgery in both the 70% and 90% resections.
- In the group with 70% PH, a rise in TNF-α, IL-6, HGF expression was detected compared to controls. After 70% PH, the rate of apoptosis reached a peak directly after surgery compared with 90% PH where this peak was detected only 24h later with TUNEL assay.

In conclusion, our data suggest that the molecular events involved in liver regeneration are significantly influenced by the extent of resection, as subtotal hepatectomy leads to delayed activation of NF-κB and suppression of proregenerative cytokines compared to partial hepatectomy. Therefore, strategies to improve the activation of proregenerative transcription factors and the early production of proregenerative cytokines may improve clinical outcome after extended hepatectomy.

Publications

Publications for the dissertation based on

1. **Tamas Benko**, Stilla Frede, Yanli Gu, Jan Best, Hideo Andreas Baba, Jörg Friedrich Schlaak, Herbert de Groot, Joachim Fandrey, and Ursula Rauen. Glycine pretreatment ameliorates liver injury after partial hepatectomy in the rat. J Invest Surg. In Press. UIVS-2009-0047.R1 *IF: 1,05*

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1. J Best, **T Benkö**, M Goralski, P Grünewald, M Trippler, M Bockhorn, G Gerken, U Rauen, JF Schlaak. Analysis of the transcriptional response after partial hepatectomy – use of customized gene arrays. German Association of the Study of the Liver, Leipzig, Germany 2006.

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