Semmelweis University

Doctoral School of Pathological Sciences

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

PhD Thesis

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ABBREVIATIONS

ALP	alkaline phosphatase
ALT	alanine aminotransferase
ANOVA	analysis of variance
ASDCL	naphtol-AS-D-Chloracetate-esterase
AST	aspartate aminotransferase
cDNA	complementary deoxyribonucleic acid assay
Ccng1	cyclin G1
DAB	diamino-benzidine
DAPI	4',6-Diamidino-2-phenylindol
DDLT	deceased donor liver transplantation
DNA	deoxyribonucleic acid
ELISA	enzyme-linked immunosorbent assay
EPOS	enhanced polymer one-step staining technique
Flt1	Fms-related tyrosine kinase 1
Fth1	ferritin, heavy polypeptide 1
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
GEC	galactose elimination capacity
GLDH	glutamate dehydrogenase
HE	hematoxylin eosin
HGF	hepatocyte growth factor
HIF1-α	hypoxia-inducible factor-1 alpha protein
ICAM1	intercellular adhesion molecule 1
ICU	intensive care unit
IL-1b	interleukin 1 b
IL-6	interleukin 6
IRAK-M	protein kinase IL-1R-associated kinase-M
LBWR	liver body weight ratio
LDH	lactate dehydrogenase
LDLT	living donor liver transplantation
NF-κB	factor kappa-light-chain-enhancer of activated B cells

OD	optical density
PDGFβ	platelet derived growth factor beta
PH	partial hepatectomy (70%; 90%)
PPAR	peroxisome proliferator-activated receptors
PT	prothrombin time
RNA	ribonucleic acid
RT-PCR	real time polymerase chain reaction
SDS-PAGE	SDS-polyacrylamide gel electrophoresis
SH	subtotal hepatectomy (90%)
STAT3	signal transducer and activator of transcription 3
T3	tri-iodothyronin
TGF-βR1	transforming growth factor beta receptor 1
TNF-α	tumor necrosis factor alpha
TUNEL	terminal transferase-mediated dUTP nick end-labeling
UNOS	United Network of Organ Sharing
US	United States (of America)
VEGF	vascular endothelial growth factor

1. Introduction

1.1. History of liver transplantation

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.

1.1.1. Genesis of liver transplantation

Transplantation of all the major organs except the liver can be traced to the early 1900s. Despite of it, the first report of liver transplantation appeared only in 1955 in a journal called Transplantation Bulletin (1). There are two major concepts in the field of liver transplantation; one is the insertion of hepatic allograft without disturbing the native liver, called auxiliary liver transplantation. The other one is the orthotopic liver transplantation, which is the replacement the new organ after total hepatectomy.

Auxiliary liver concept

In a one-page article C. Stuart Welch of Albany Medical College described the insertion of hepatic allograft in the right paravertebral gutter of dogs, without disturbing the native liver (2). More complete information was published in the following year (3).

The auxiliary livers were revascularized by anastomosing the graft hepatic artery to the recipient aortoiliac system by end-to-end anastomosis, and the graft portal vein to the host inferior vena cava. The upper end of the caval segment of the graft was anastomosed to the recipient vena cava, and the lower end was ligated or sutured. Unlike other kinds of transplanted organs, the auxiliary allografts underwent dramatic shrinkage. The atrophy which began within 3 or 4 days was at that time attributed to liver rejection. The view was consistent with the recurrent dogma of that time, that liver size and regeneration are governed by the volume of portal venous inflow (the "flow hypothesis" of hepatic homeostasis). Because the portal vein of the transplanted extra livers had been provided with an ample amount of systemic blood, the acute allograft atrophy was attributed to immunological factors. A decade passed before it was demonstrated that the liver shrinkage actually was due to the disappearance in vena caval or systemic blood of molecules (especially insulin) that are normally presented to liver in high concentrations in splanchnic venous blood (4-7).

Orthotopic liver transplantation

The concept of liver replacement (orthotopic transplantation) was first mentioned by Jack Cannon in a one-page account of the transplant activities in the surgery department of the newly founded University of California, Los Angeles School of Medicine (8). The species studied was not mentioned (presumably dog) and there was no specific information about the procedure. To facilitate these studies, a new method of total hepatectomy was developed in which the unique feature was preservation of the retro-hepatic inferior vena cava, as we call "piggy back" – technique (9). The first cadaveric liver transplantation was performed by Thomas Starzl in 1963 in Denver. After this failed trial, liver transplantation was successfully performed in humans in July 1967 again by Dr. Starzl. Although rejection was a major concern, many recipients from this early era have survived for more than 20 years using immunosuppression with azathioprine, prednisone, and antilymphocyte globulin (10). For clinical transplantation, the historical beginning was Medawar's recognition that rejection is an immune reaction (11, 12) (Table 1.).

Year	Author	Application
1943	Gibson	Defined the immunologic nature of skin allograft rejection in humans
1955	Welch	First mention of liver transplantation in the literature in a dog study
1960	Medawar	Definition of acquired transplantation tolerance
1960	Starzl	Transplantation in dogs of multiple abdominal viscera
1963	Starzl	World's first three attempts of liver transplantation in humans with maximum survival of 21 d
1968	Starzl	First long-term survival of four patients after liver transplantation
1978	Calne	Introduction of cyclosporine
1984	Bismuth	Reduced-sized adult liver transplanted into a small child
1987	Raia	First living donor liver transplantation
1988	Pichlmayr	Split one donor liver and two graft were used for two recipients
1988	Kalayoglu	Introduction of University of Wisconsin solution for preservation
1992	Starzl	Baboon to human xenotransplantation

Table 1.
Major issues related with liver transplantation.

1.1.2. Technical innovations

Although the increasing numbers of liver transplantation was dominated by improvements in immunosuppression, there were other significant developments, including the modification in the details of both the donor and recipient operations.

Donor procedures

By reason of the organ shortage there are several surgical procedures developed in the last decades, such as reduced size organ transplantation, split liver transplants or living related liver transplantations. In case of deceased donor organ explantation cooling of donor organs is done today by variations of the in situ technique originally developed before the acceptance of brain death conditions but with simple infusion without a bypass (13). These methods (14, 15) allow removal of all thoracic and

abdominal organs, including the liver, without jeopardizing any of individual organs-even with unstable donors, including those whose hearts have ceased to beat. After the cooled organs are removed subsequent preservation usually is performed by simple cooling rather than by sophisticated methods of continuous perfusion that were developed in the 1960s.

Recipient operation

The incidence of biliary duct complications (obstructions, fistula, cholangitis) which had been more than 30% was reduced by the use of choledocho-choledochstomy with a T-stent or if it this was not feasible, by choledocho-jejunostomy to a Roux limb (16). The systematic use of veno-venous bypasses without anticoagulation in adult recipients greatly diminished the occurrence of hemorrhages that were common at one time and posed a nightmare to surgeons. Management of coagulopathies continued to be facilitated by the use of the thromboelastogram to follow the minute to minute clotting changes in the operation room (17). With better control of bleeding, scar formation from multiple upper abdominal operations as well as previous used portosystemic shunts were eliminated as serious adverse factors in major centers. The systematic use of arterial and venous grafts that were introduced in the 1970s (18) eliminated extensive thrombosis of the portal and the superior mesenteric veins as a contraindication of liver transplantation (19) and has facilitated the formation of the arterial blood supply in complex cases. The shortage of appropriate size donors for very small pediatric recipients was greatly improved with the use of partial livers. The introduction of such operations followed the development of sophisticated techniques of hepatic resections for neoplasms (20-23). Implantation of liver fragments has been facilitated by use of piggy back principle, by which the recipient retro-hepatic inferior vena cava is kept intact and the venous outflow of the graft is anastomosed to the cuffs of the host hepatic veins.

1.2. Indications for Liver Transplantation

By the early 1990s liver transplantation has became the accepted last resort for essentially all non-neoplastic end stage liver diseases and for selected patients with otherwise nonresectable hepatic malignancies.

1.2.1. Benign diseases

Parenchymal and cholestatic disorder

By the end of the 1980s, diagnoses that excluded liver transplantation such as diagnose of alcoholic cirrhosis were no longer absolute contraindications. The list of benign diseases treatable by transplantation had become so long (nearly 100) that it was being divided into broad categories (Table 2.) (24, 25).

Disease category		
Parenchymal		
	Postnecrotic cirrhosis	Alcoholic cirrhosis
	Acute liver failure	Budd-Chiari syndrome
	Congenital hepatic fibrosis	Cystic fibrosis
	Neonatal hepatitis	Hepatic trauma
	Others	
Cholestatic		
	Biliary atresia	Primary biliary cirrhosis
	Sclerosing cholangitis	Secondary biliary cirrhosis
	Familiar cholestasis	Others
Inborn Errors of Metabolism		
	Alpha1 antitrypsin deficiency	Wilson's disease
	Tyrosinemia	Cystic fibrosis
	Type I glycogen storage disease	
	Type IV glycogen storage disease	
	Niemann Pick disease	Sea-blue histiocyte syndrome
	Erythropoietic protoporphyria	Crigler Najjer syndrome
	Type I hyperoxaluria	Urea cycle enzyme deficiency
	C protein deficiency	Familial hypercholesterolemia
	Hemophilia A	Hemophilia B
Hepatic malignancies		
	Primary hepatic tumors	
	Metastatic tumors	

Table2.
Etiological classification of liver disease treatable by liver transplantation

Inborn error of metabolism

Products of hepatic synthesis permanently retain the original metabolic specificity of the donor after transplantation. Consequently the correction of inborn errors by liver transplantation can be expected to endure for the life of the graft.

1.2.2. Neoplastic diseases

The earlier practice of proceeding conventional liver transplantation to treat otherwise nonresectable primary or metastatic hepatic cancers resulted in a high rate of recurrence (16, 26, 27). Although in the clinical practice there are several criterias try to rule the allocation of recipient with controlled size of liver tumors (Miland Criteria, San Francisco or Extended Criteria), the results are very controversial. Nevertheless the use of liver transplantation to treat less-advanced cancers has continued almost invariably in combination with adjuvant chemotherapy or other protocols. Certain kinds of neoplasms have better prognosis than others. In an attempt the increase the perimeter of resecability upper abdominal exenteration has been used to treat extensive sarcomas carcinoid tumors and other malignancies that are still regionally confined (28, 29). The excised organs are replaced with hepato-pancreatico-duodenal grafts, or in some case the liver alone.

1.3. Organ shortage

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. By the late 1980s there were enough liver transplant teams to use the available supply of deceased donor organs. Efforts to equitably allocate livers to competing teams began officially, when the United Network of Organ Sharing (UNOS) and European associations (Eurotransplant) introduced a point system based on urgency of need, size match, and logistic consideration (30). Neither the system nor its many modifications has satisfied all of the caregivers, patient advocacy groups and other

stakeholders. However all interested groups including surgeons have tried to increase the pool of available organs.

1.3.1. Needs for the expansion of the donor pool

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 have contributed to a considerable reduction of their mortality while waiting for a liver transplant. Nowadays, the organ shortage is currently focused on adult recipients who are listed in increasing numbers. Adult mortality while waiting for a transplant remains an excess of 10% to 20%. This explains the fast expansion of adult-to-adult living donor liver transplantation. To what extend this situation will be alleviated by the expansion of the full right / full left split liver transplantation (31, 32) is unpredictable because of the limited number of potential adult recipients with a low bodyweight, allowing the safe graft size to body weight ratio, and increasing proportion of older and expanded criteria donors, not allowing safe splitting.

Use of marginal donors

As early as 1986, Makowka and colleagues (33) identified the impending organ shortage and reported the feasibility of using livers from older donors, donors with biochemical or histopathological evidence of liver injury, and those whose terminal management was not suitable of potentially damaging pharmacological agents were administered. Although it was first criticized, this way means of expanding the donor pool became widely accepted once the magnitude of the supply problem was appreciated. Serious and frequently contentious efforts are still being made to define what constitutes a marginal donor and how to decide who gets the liver (34).

Split liver procedures

More efficient use of deceased donor organs has been made possible by sharing one liver between two recipients. The split liver procedure was first reported from

Europe by Rudolf Pichlmayr in 1989 and soon thereafter by Bismuth and Broelsch. The results were inferior at first to those obtained with whole livers (20, 35). But after a learning curve and incorporation the lessons learned from living donors, the results with liver split between adult and pediatric recipients have been comparable to standard deceased donor transplantation of whole organs. This practice provides an alternative for pediatric recipients beside living donors. Moreover splitting of the adult liver into full left and right lobes for transplantation into two adults (or even the sharing of a pediatric liver by two infants or children) could further relieve the organ shortage. This has been done successfully in a small number of adult cases (31, 36). Full implementation of this technique will require restriction of its use to optimal donors, careful assessment of the donor's physiological status before hepatectomy, careful consideration of the logistics involved, and the intelligent application of allocation rules for recipient selection (37).

Living donor transplantation (LDLT)

In an extension of the reduced liver graft procedures developed in deceased donors (20, 35), portions of liver ranging from the lateral segment to the extended right lobe have been removed from volunteer adult donors for transplantation to pediatric recipients. Living donor liver transplantation from an adult liver to a child was first done successfully by Strong and Lynch in Adelaide, Australia (38). The operation for pediatric recipients was subsequently popularized by Christoph Erich Broelsch and associates at the University of Chicago (21), who reported their results at the American Surgical Conference in 1990 along with their experience reduced size deceased donor organs and deceased donor split livers. To obtain an adequate liver mass for recipient body weight in adult to adult living donor transplantation, the size of the transected liver fragment was first increased from the left lateral segment to the full left lobe.

The most common operation is the transplantation of the right lobe. This was first carried out in Japan when unexpected anatomical situation were encountered in the donor (39). Since then more than 1500 right lobe transplantation have been performed in more than 40 American centers with patient and graft survival equivalent to that with

whole organ, deceased donor transplantation or with various kinds of partial liver transplantations including the predecessor adult to child procedure (40, 41).

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 (42, 43). The surgical procedures for LDLT are technically more challenging and LDLT requires a full understanding of the hepatobiliary anatomy. A wide range of complication rates are reported in the literature in donors after LDLT. Donor safety has a major importance in LDLT.

Published reports on donor outcomes indicated a wide range of complication rates that varied between 9% and 67% (44, 45). In the Kyoto University experience 50 complications in 222 right lobe grafts have been encountered, including surgical complications 18.5% and non-surgical complications 3.2% (46). On the other hand, the American Society of Transplant Surgeons reported a donor complication rate of 10%. Thus it seems that donor morbidities have not been adequately reported and true extend of complications may be underestimated. A standardized system for reporting complications to a registry should be developed to allow meaningful data analysis. Donor mortality is also a major concern of LDLT. In the United States at least 3 deaths were confirmed. Another 3 deaths in Europe and 1 in Japan had been reported (47).

As living donation permits transplantation to take place independently from the waiting time or the severity of liver disease, the criteria required for LDLT may be modified when compared to deceased donor liver transplantation (DDLT). Estimation of liver volume needed in individual situations is an important factor in donor selection. Aged liver, steatotic liver and special anatomic variants have the risk of a relatively poor graft quality. Recipient factors such as metabolic load, preoperative latent infections and other organ failures have negative impact on graft survival. The minimally required quantity of graft volume has not been fully clarified, which is one of the major issues of the adult to adult LDLT. Recipients with a small-for-size graft, suffer from graft dysfunction including hyperbilirubinemia, massive ascites, poor

synthetic function that leads to serious conditions such as gastrointestinal bleeding and renal failure.

When a graft size is conversely too large for a recipient e.g. a newborn infant, graft necrosis occurs due to insufficient blood inflow into the graft. As pointed out by Ghobrial and Bussuttil, future application of LDLT will be based on the accurate definition of risks imposed on donors compared with potential benefits to recipients (34, 48). As an example to this statement, the number of adult LDLT declined from approximately 400 in 2001 to 280 in 2002. Such a precipitous reduction may be due to the donor death in US in 2002 which raised increasing concerns for donor safety. While the number of LDLT in the US has declined, the number in Asia as a whole has continued to increase. LDLT accounted for less than 5% of liver transplants in the US but more than 95% of the transplants in Asia excluding mainland China. The overall number of LDLT procedures performed in Asian countries and areas with well-established programs (Japan, Korea, China Hong Kong and China Taiwan) has steadily increased over years (48).

The most extensive experience in LDLT was initially gained in Asia. In countries such as Japan, where the availability of organs from deceased donor is limited, LDLT seems to be the only solution in the treatment of end stage liver diseases. According to the data of Japanese Liver Transplantation Society, the adult to adult LDLT is increasing per year. Cases in children have reached a peak around 100 cases per year. The 1 and 5-year survival rates of all recipients were reported to be 81.8%, and 77%, respectively, while those of recipients of less than 18 years old was 85.6%, and 82.6% respectively. The prognosis of adult recipients was poor when compared to children (46).

In summary, the overall results with good patient and graft survival, together with acceptable donor morbidity and mortality has led to the acceptance of LDLT in the transplant community. To maintain this procedure as a treatment modality in the future, satisfactory risk-benefit analysis and long-term morbidities imposed on living donors should be further investigated.

1.4. Optimize the donor operation outcome in experimental animal models

1.4.1. Pretreatment of the donor with different substances

As we have already shown living donor liver transplantation (LDLT), initially introduced for pediatric recipients with terminal liver disease, is now increasingly used for adult-to-adult liver transplantation. An inherent problem of the latter is that the graft is often only just meeting the volume required for sustaining life in the recipient, but is still a major hepatectomy for the donor. Therefore, every effort should be undertaken to minimize graft injury during harvesting and storage in order to preserve all functional tissue procured (42) as well as liver injury and risk to the donor. 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 – offering likely benefits for the graft without risk to the donor – 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 (49-51) and against hypothermic injury/cold-induced apoptosis (52, 53).

Silibinin

The flavonolignane silymarin, isolated from the fruit of the milk-thistle (Silybinum marianum is shown in Figure 1.), has well-known hepatoprotective properties (54, 55). 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 (53, 56) as well as reperfusion injury and oxidative components of inflammatory injuries (54).

Figure 1.

Silibinin is isolated from the fruit of milk thistle (Silybinum marianum)

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 (57, 58), 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.

1.4.2. Pretreatment of the donors with hormonal substance

The unique regenerating capacity of the liver is important for the donor as well as for the recipient to guarantee a good outcome. Based on the increase in understanding the mechanisms underlying hepatic regeneration (59), a variety of strategies have been developed to bolster liver regenerating, including injection of several growth factors, preconditioning methods, etc. (60-62). Francavilla et al. (63)were able to demonstrate

that exogenous application of tri-iodothyronine (T3) stimulated a liver regeneration response that resembled in timing and in magnitude of deoxyribonucleic acid (DNA) synthesis. It was induced by 40% hepatic resection in rats and Malik et al. (64) showed an enhanced regenerative capacity after partial hepatectomy.

1.4.3. Role of venous outflow on liver regeneration capacity

The function of the remnant liver after extended hepatectomy is critical for the clinical outcome, influencing mainly the postoperative morbidity and mortality (65, 66). 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 (67-69). 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. The functional and regenerative capacity of a partial liver graft and of a liver remnant after hepatectomy are difficult to compare due to the additional influence of cold ischemic time, ischemia reperfusion injury or changes in hemodynamics in portal hypertension post-transplantation. However, it can be supposed that hepatic venous obstruction could have similar effects on liver function and regeneration after partial hepatic resection (70-72).

1.4.4. Extent of donor liver resection and regeneration

Major hepatectomy is primarily used to treat malignant liver disease and in living donor liver transplantation (LDLT). After resection the remaining liver can regenerate to a fully functional organ (73-75). Tissue damage due to the surgical process further decreases the amount of healthy remnant liver tissue leading to an additional reduction in functional liver capacity in the postoperative period (76, 77). This situation can lead to liver insufficiency and, ultimately, to liver failure and death (78, 79). Therefore, patients have to fulfill certain criteria, e.g. the absence of end-stage liver

disease, in order to be eligible for extended hepatectomy (74). The same is true for donors in LDLT. While LDLT is a life saving procedure for the recipient, it is a potentially lethal operation for the donor. Therefore, very stringent criteria have to be fulfilled prior to hepatectomy to ensure donor safety (80-82). For the donor, fast liver regeneration is imperative to reduce the probability of liver insufficiency (83, 84).

Thus, an improvement in regenerative capacity could enhance donor safety and increase the possibility of including individuals who are not eligible for donation due to insufficient liver size (85, 86). As already stated the major concern in major hepatectomy or in the LDLT setting, is efficient regeneration of the remnant or transplanted partial liver, respectively. It has been demonstrated that graft size is of high importance in regenerative processes in the recipient (84). 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% (87). 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 (59, 88-91). In particular, the role of proregenerative cytokines (e.g. interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α)) and the role of transcription factors such as nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) are ambiguous (76, 92-94).

2. Research objectives

Determination of the effect of pre-treatment with α-tocopherol, silibinin and L-glycine on the liver injury, after partial hepatectomy in the rat. We wanted to determine the effect of the donor pre-treatment with these three agents, either alone or in combination, whether they have adverse affect on the donor or they improve graft quality or protect the residual donor liver. The aim of the first study was to analyze the effects of these pre-treatments on the donor in a model of 90% partial hepatectomy in the rat.

- How does pretreatment with α-tocopherol, silibinin, L-glycine and combination of these three compounds influence the survival rate and clinical outcome of the rats?

- Has the pretreatment any effect on transaminases enzyme activities, and synthetic function after 90% liver resection?

- How dose this method influence the histopathological and inflammatorical changing of the remnant liver?

Determination of the effect of exogenous administration of T3. We wanted to determine whether exogenous administration of T3 leads to a stimulated liver regeneration after 70% partial hepatectomy (PH) and whether this treatment confers a survival advantage after 90% partial hepatectomy.

- Does the admission of T3 influence the overall survival, the serum laboratory parameters of the rats?

- How does the T3 admission influence the liver body weight ratio and proliferation index after major hepatectomy?

- Has it got any effect on vascular endothelial growth factor (VEGF) and regeneratory gene expression?

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

- Is there any change in biochemical parameters after partial damaging the venous outflow compared to liver rejection?

- Does it have any influence on the histopathological and immunhistological parameters?

- How does it change the expression of genes related to liver regeneration?

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.

- Has the extent of liver resection an influence on the regenerative capacity of the remnant liver?

- How does the extent of the liver resection influence the activation of NF-κB, Signal transducer and activator of transcription 3 (STAT3)?

- How does it influence the expression of pro- and anti-regenerative cytokines?

3. Material and methods

3.1 Pre-treatment with α-tocopherol, silibinin and L-glycine

3.1.1. 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 (Bezirksregierung Düsseldorf, Germany). Male 6-8 weeks-old Wistar rats were obtained from Harlan Winkelmann (Borchen, Germany), 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 by gavage 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, dissolved in normal saline; kindly provided by Madaus, Cologne, Germany, and) 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.

3.1.2. Operative procedure

All animals received one shot antibiotic prophylaxis intramuscularly (100 mg/kg body weight mezlocillin, Baypen®; Bayer Healthcare, Leverkusen, Germany) and 5 mg/kg body weight carprofen subcutaneously (Rimadyl®, Pfizer, Karlsruhe, Germany)

at the beginning of the operation. Partial hepatectomy was performed under isofluran anesthesia (O_2 0.5 l/min + 2.5% (*v/v*) Isofluran Curamed; Delta Select, Dreieich, Germany) according to the method of Higgins and Anderson (95).

We have immobilized the rat in supine position; disinfected the skin with alcohol, fixed on the korkplate at the extremities by elastic rings and pins. The abdomen was opened via a transversal incision 1,5cm below the xyphoid process, progressing to both lateral flanks paralleling to rib arch. We have coagulated the superior epigastrical vessels both sides. Elevated the xyphoid with a straight Mosquito clamp, folded the abdominal wall on the right and on the left side and fix with needles. Retracted the intestines to the left side and covered with saline soaked gauze (Figure 2.).

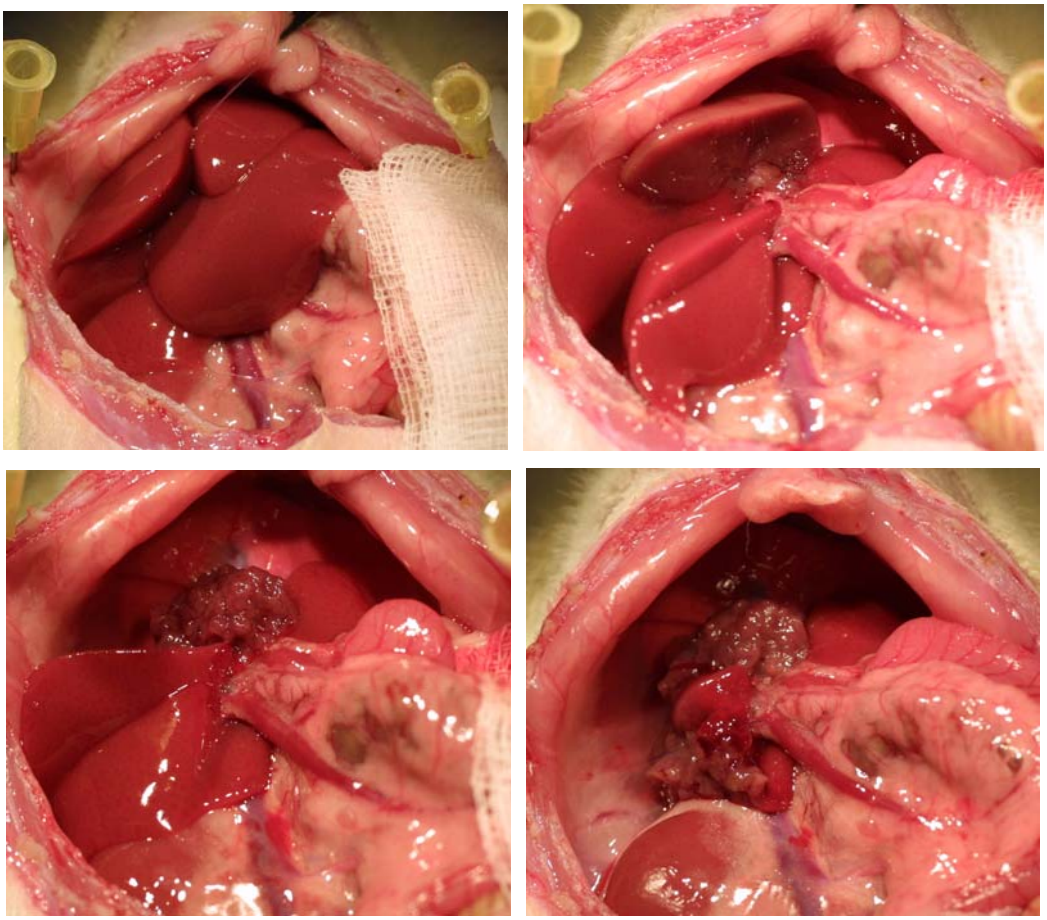


Figure 2.

90% partial hepatectomy was performed on Wistar rats sec Higgins and Anderson.

The “don’t touch technique” was preferred, to avoid any injury on the liver surface, the liver was very carefully handled with wet cotton tips. The liver was freed from its ligaments, started with the dissection of the falciform ligament, the left triangular ligament and all interlobular ligaments which connected the left lateral lobe. A bended Mosquito clamp was inserted below the left lateral lobe, but kept about 5-7mm distance from the lobular vessels. The lobe was severed with scissors above the clamp, as close to it as possible. The tissue below the clamp was ligated with a 6-0 running suture. A Mosquito clamp was inserted into the middle fissure of the median lobes, the left median part was clamped. The lobe was severed with scissors above the clamp, as close to it as possible. The tissue was ligated below the clamp, with a 6-0 running suture. The Mosquito clamp was inserted on the right median lobe, the lobe was severed with scissors above the clamp, as close to it as possible. The tissue was ligated below the clamp, with a 7-0 running suture (Mopylen® 6-0, Resorba, Nürnberg, Germany). 70% partial hepatectomy (PH) is completed. To reach 90% PH the right lateral lobes should be removed, with the same surgical technique, mentioned above.

We have confirmed there is no bleeding in the resection areas. Replaced the intestines into the abdominal cavity, took care to avoid any rotations or injuries. Closed the abdomen with 3-0 absorbable running suture in two layers 3-0 atraumatic, absorbable attached to one 3/8 circle taper needles (PGA Resorba 3-0, Fa. Resorba, PA1145). Put the rat back in the cage.

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. Postoperatively, all rats were fed a standard rat chow ad libitum. On the first postoperative day, rats received carprofen (5 mg/kg body weight) subcutaneously.

3.1.3. Samples

Animals were sacrificed after 0 hours (i.e. immediately after the operation), 6 hours (for hypoxia-inducible factor-1α protein (HIF-1α) accumulation), 12, 24, 48, 72 and 168 hours and 4 weeks after surgery by an intraperitoneal injection of xylazin hydrochloride 2% (0.033 ml / 100 g body weight; Ceva Tiergesundheit GmbH,

Düsseldorf, Germany) and ketamine hydrochloride 10% (0.067 ml / 100 g body weight; Ceva Tiergesundheit, Düsseldorf, Germany). The residual livers were removed, weighed and fixed in buffered 4% formaldehyde or rapidly frozen in liquid nitrogen (without weighing).

Blood samples

Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and total bilirubin were assessed in serum samples using standard assays (DiaSys Diagnostic Systems, Holzheim, Germany). Prothrombin time (PT) was assessed in plasma samples using a standard assay (Neoplastin® Plus, Roche Diagnostics, Mannheim, Germany).

Liver histology

Liver samples fixed in buffered 4% formaldehyde were embedded in paraffin, cut in 5 µm sections and stained with hematoxylin-eosin. Liver degeneration, areas of necrosis and leukocyte infiltration was assessed in these samples. Additional sections were used for naphtol-AS-D-Chloracetate-esterase (ASDCL) staining to assess granulocyte infiltration.

Western blots

Tissue samples were homogenized in the extraction buffer provided with the Nuclear protein extraction kit (Pierce: NE-PER kit, Thermo Scientific, Woburn, MA, USA). Nuclear proteins were extracted according to the manufacturer's instructions. Protein concentrations were determined using the Bio-Rad kit and extracts were stored at -80°C until use. 30 µg of nuclear extracts per lane were subjected to 7.5 % SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto a nitrocellulose membrane (0.2 µM pore size; Schleicher and Schuell, Dassel, Germany). HIF-1α was detected using a rabbit polyclonal antibody against mouse HIF-1α (Novus Biologicals, Littleton, CO, USA). α-tubulin (Sigma) served as a loading control.

Terminal transferase-mediated dUTP nick end-labeling (TUNEL) assay

Paraffin-embedded tissue sections were dewaxed by heating to 60°C for 30 minutes and subsequent washing in xylene. The slices were rehydrated through a graded series of ethanol (100%, 90%, 70%), permeabilised with permeabilisation solution (0.1% Triton X-100, 0.1% sodium citrate) and washed twice in phosphate-buffered saline (PBS). TUNEL reaction mixture was prepared according to protocol (*In Situ* Cell Death Detection Kit, Fluorescein; Roche Diagnostics; Mannheim, Germany). Labeling was conducted as described in the assay manual, samples were subsequently counterstained with DAPI (4',6-Diamidino-2-phenylindol, 10 µg/ml in PBS). 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 Hercules, CA, USA) and the QuantiTect SYBR Green RT-PCR Kit (Qiagen, Hilden, Germany) according to the manufacturers instructions. To account for possible concentration errors, the house keeping gene β-actin served as a reference control. The following primers were used for real-time PCR: intercellular adhesion molecule 1 (ICAM1) (Rn ICAM1 SG QuantiTect Primer Assay, QT00174447) and interleukin-1b (IL-1b) (Rn IL-1b SG QuantiTect Primer Assay, QT00181657) (both Qiagen, Hilden, Germany).

Statistics

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.

3.2. Pre-treatment with tri-iodothyronin

3.2.1. Animals and methods

Male Wistar rats (Charles River Laboratories, Netherlands) weighing 180–220 g were maintained on a commercial pelleted diet and water ad libitum under normal laboratory lighting conditions in our animal facility. All animal study protocols were fully approved by the animal care and use committee. Tri-iodothyronine (Sigma, Taufkirchen, Germany) was dissolved in 0.01 M NaOH at a concentration of 5 mg/ml and administered intraperitoneally at a dose of 4 mg/kg bodyweight in each rat in the early morning hours as described before (96).

- To investigate the stimulatory effect of exogenous tri-iodothyronine 6 rats each group 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 group 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.

3.2.2. Samples

Determination of AST, ALT, GLDH and Bilirubin

At the above-mentioned time points, rats were sacrificed and blood was collected through aortic puncture. Serum of the animals was sampled and analyzed for aspartate aminotransferase (AST), alanine aminotransferase (ALT), glutamate dehydrogenase (GLDH) and total bilirubin.

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, Biogenex, Hamburg, Germany) to evaluate the percentage of hepatocytes that entered the regenerative process after hepatectomy (97). Deparaffinized 3 µm sections of liver were immersed in ethanol and incubated to block endogenous peroxidase activity. Sections were then treated with 10 mM citrate buffer in the water bath at 30°C for 30 min and cooled slowly to room temperature.

The primary antibody was a rabbit monoclonal anti-rat/mouse/human Ki-67 antigen (DCS Diagnostics, Hamburg, Germany; 1:1.200 dilutions). Immunohistochemistry was performed using a biotin-free enhanced polymer one-step staining technique (EPOS-method) with a peroxidase-conjugated polymer backbone coupled with a goat anti-rabbit secondary antibody (DAKO, Hamburg, Germany). The slides were rinsed in diamino-benzidine (DAB, 2 mg/ml) containing 0.02% hydrogen peroxide and were allowed to sit for 5 min or until brown chromagen deposition was observed. Finally, the tissue sections were rinsed in deionized water, counterstained with hematoxylin, and examined microscopically. '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; San Francisco, Calif., USA) and was performed similar to Ki-67. 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, BRL, Eggenstein, Germany) according to the manufacturer's specification. The quality and integrity of RNA were checked by spectrophotometry and 1% ethidium bromide agarose gel electrophoresis (Gibco, BRL, Eggenstein, Germany).

Complementary DNA Array

A customized cDNA array consisting of 134 rat genes was established as described previously (98). 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, Hilden, Germany) 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, Hilden, Germany). The specific primers for each gene were designed to amplify a product between 400 and 500 bp. As a control, glyceraldehyde-3-phosphate dehydrogenase (13 GAPDH) and β-actin gene probes were added. Subsequently, the gene products were spotted on Hybond N^+ nylon membranes (Amersham Pharmacia, Freiburg, Germany) and hybridized overnight at 65°C with 32 P-labeled cDNA prepared from 10 µg total RNA of each rat liver sample. The membranes were stored in a Phosphoimager Cassette (Amersham Biosciences, Freiburg, Germany), exposed for 2–3 days and scanned on the STORM Phosphoimager (Amersham Biosciences, Freiburg, Germany). The data were analyzed using Image Quant software (Amersham Biosciences, Freiburg, Germany) as described (98). Experiments were repeated twice.

RT-PCR

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

instructions. Primers for PCR were designed using Primer3 software (Whitehead Institute for Biomedical Research, Cambridge, UK). The incorporation of SYBR Green into the PCR products was monitored in real time after each PCR cycle resulting in the calculation of the threshold cycle or Ct value, which defines the PCR cycle number at which an exponential growth of PCR product begins. PCR conditions were the following: 30 min 50°C, 15 min 95°C; 35–40 cycles with 20 s 95°C, 20 s 55°C, 40 s 72°C and 15 s 79°C. To account for possible concentration errors, the house keeping gene β-actin served as a reference control. The following primers were used for real-time PCR: β-actin forward 5'-gaggcccagagcaagagag-3', reverse 5'-tggccttagggttcagagg-3'; FLT1 forward 5'-cctgtcactacaaccactcc-3', reverse 5'-caccaatgtgctaaccgtctt-3', PPAR forward 5'-agctcgtgcaggtcatcaa-3', reverse 5'-tgtgcaaatccctgctctc-3', C3 (complement) forward 5'-cctgaccttcaagacaaacca-3', reverse 5'-atgccatcctcacaacacttc-3'.

Statistical Analysis

All data are expressed as means ± SD. The statistical analysis was performed by one-way ANOVA and log-rank test. $p < 0.05$ was considered statistically significant.

3.3. Hepatic vein deprivation

3.3.1. Animals and methods

Ten-week-old male Wistar rats (Charles River Laboratories, The Netherlands) weighing 180–250g were maintained on a commercial pelleted diet and water ad libitum under normal laboratory lighting conditions in our animal facility. All animal study protocols were fully approved by the animal care and use committee.

3.3.2. Operative technique

Operative procedures were performed under isofluran 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. (99).
- 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 left lateral 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 (Figure 3.).
- To evaluate a control group, a 70% PH without additional venous outflow obstruction was performed.

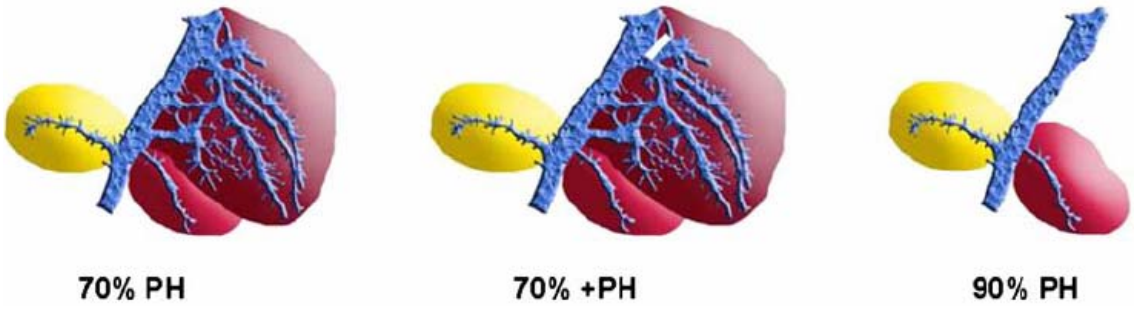


Figure 3.
Graphics of the different surgical procedures. Rats underwent 70% PH or 70% PH and partial suture of the left hepatic vein or 90% PH.

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 (84).

3.3.3. Samples

Overall-Survival

Additional 45 rats underwent either 90% PH, 70%+PH or 70% PH (15 rats each) and were monitored for overall survival.

Determination of the liver enzymes

Aspartate aminotransferase (AST), alanine aminotransferase (ALT), glutamate dehydrogenase (GLDH) and bilirubin were determined in the blood of the animals. At the above-mentioned time points, rats were killed, and blood was collected through aortic puncture. Serum of the animals was sampled and analysed for AST, ALT, GLDH and total bilirubin.

Histopathology and immunohistochemistry for Ki-67

All liver tissue specimens were fixed in 4% formalin, dehydrated through ethanol and xylene and embedded in paraffin wax. 5 um sections were cut and stained with hematoxylin and eosin. For immunohistochemistry, we used an antibody against Ki-67 antigen (Fa. DCS, Biogenex, Hamburg, Germany) to evaluate the percentage of hepatocytes that entered the regenerative process after hepatectomy as described before (83).

"Proliferation index" was defined as the percentage of Ki-67 positive cells counted in five periportal (within 100 μm of the portal area) and perivenular (within 100μm of the central vein) fields of a specimen (97).

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 (100).

RNA isolation

To further elucidate the underlying molecular mechanisms, hepatic gene expression was determined by customised complementary DNA (cDNA) arrays and quantitative real time polymerase chain reaction (RT-PCR). Total RNA was isolated from each regenerating liver of each animal using Trizol (Gibco, BRL, Eggenstein, Germany) according to the manufacturer's specification. The quality and integrity of

RNA were checked by spectrophotometry and 1% ethidium bromide agarose gel electrophoresis (Gibco, BRL).

cDNA array

A customised cDNA array consisting of 134 rat genes was established as described previously (98). Briefly, 18 genes were collected from image cDNA clones ordered at the "Deutsches Ressourcenzentrum für Genomforschung GmbH, Berlin". The other 116 genes were amplified from total rat cDNA using the Omniscript Reverse Transcriptase kit (Qiagen, Hilden, Germany). As a control, 13 GAPDH and β-actin gene probes were added. Subsequently, the gene products were spotted on Hybond N+ nylon membranes (Amersham Pharmacia, Freiburg, Germany) and hybridised overnight at 65°C with P32-labeled cDNA prepared from 10 μg total RNA of each rat liver sample. The membranes were stored in a Phosphoimager Cassette (Amersham Biosciences, Freiburg, Germany), exposed for 2–3days and scanned on the STORM Phosphoimager (Amersham Biosciences). The data were analysed using Image Quant software (Amersham Biosciences) as described (98).

RT-PCR

One-step RT-PCR was performed using the Rotor-Gene 2000 real-time Amplification System (Corbett Research, Sydney, Australia) and the QuantiTect SYBR Green RT-PCR Kit (Qiagen) according to the manufacturer's instructions. The incorporation of SYBR Green into the PCR products was monitored in real time after each PCR cycle resulting in the calculation of the threshold cycle or Ct value that defines the PCR cycle number at which an exponential growth of PCR product begins. PCR conditions were the following: 30min at 50°C, 15min at 95°C; 35–40 cycles with 20s at 95°C, 20s at 55°C, 40s at 72°C and 15s at 79°C. To account for possible concentration errors, the house keeping gene β-actin served as a reference control. The following primers were used for RT-PCR: β-actin (rat, accession number BC063166) forward 5′-gaggcccagagcaagagag- 3′, reverse 5′-tggccttagggttcagagg-3′; for IRAK-M (protein kinase IL-1R-associated kinase-M), transforming growth factor beta receptor1 (TGF-β-R1) and platelet derived growth factor-beta (PDGF-β), we used commercially

available primers by Qiagen (Qiagen, Catalogue nos. QT00455329, QT00190953, and QT00495523).

Statistical analysis

All data are expressed as mean ± SD. The statistical analysis was performed by t test or one-way ANOVA. A $p < 0.05$ was considered statistically significant.

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

3.4.1. Animals and methods

Six to eight-week-old male Wistar rats were anaesthetized with isofluran. 70% partial hepatectomy (PH) and 90% PH were performed under isofluran anesthesia as described by Higgins *et al* (95) and Emond *et al* (99). 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 before abdominal wall closure and 2 h, 12 h, 24 h, 48 h, 72 h and 7 d after resection ($n = 4$ at each time point, per group).

3.4.2. Samples

Liver to body weight ratio

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 a percentage of the ratio between the remnant liver weight divided by the total body weight multiplied by 100. Liver body weight ratio (LBWR, %) = A/B × 100.

Determination of the liver enzymes and liver function

Serum concentrations of liver related enzymes (ALT, AST, LDH and serum bilirubin) were assessed using commercially available enzyme activity tests [ALAT (GPT) FS (IFCC mod.); ASAT (GOT) FS (IFCC mod.); Bilirubin Auto Direct FS; LDH FS IFCC; DiaSys Diagnostic Systems; Holzheim, Germany].

RNA isolation from liver tissue

Samples of liver tissue (approximately 2-5 g) were placed in 5 mL Trizol and homogenized with an Ultra Turrax (Janke & Kunkel, Staufen, Germany). One ml aliquots of the homogenized samples were used for RNA isolation using chloroform. RNA precipitation was performed using 100% isopropanol. The dry RNA pellet was resuspended in 100 µL Rnase-free water and purified with Rneasy® Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. RNA concentration in the samples was measured by optical density (OD) 260, purity was determined by OD 260/280.

Protein isolation

Samples of liver tissue (approximately 2-5 g) were placed in 3 ml lysis buffer (10 mmol/L HEPES, 10 mmol/L NaCl, 0.1 mmol/L EDTA, 1 mmol/L DTT, 0.4% NP-40) and homogenized with an Ultra Turrax. One mL aliquots of the homogenized tissue were centrifuged at 4°C, 800 *g* for 5 min. Supernatants were centrifuged again at 4°C, 20 000 *g* for 30 min and the protein concentration was measured using a Bradford assay (BioRad, Munich, Germany). This fraction was referred to as the cytosolic fraction. The pellet obtained by centrifugation was resolved in 350 µL extraction buffer (20 mmol/L HEPES, 400 mmol/L NaCl, 1 mmol/L EDTA, 1 mmol/L DTT, 0.2% NP-40) and repeatedly vortexed over a 30 min period. After centrifugation at 4°C, 20 000 *g* for 20 min, the supernatant was collected and the protein concentration measured with the Bradford assay. This fraction was referred to as the nucleic fraction. Proteinase

inhibitors (Complete Mini EDTA free, Roche) were added to the extraction and lysis buffers 30 min prior to use.

RT-PCR

Changes in mRNA expression were analyzed by quantitative RT-PCR using the iCycler system (Bio-Rad, Munich, Germany). RT-PCR was performed with the QuantiTect SYBR Green RT-PCR kit (Qiagen, Hilden, Germany) to determine the cytokines involved in liver regeneration (TNF-α, IL-6, hepatocyte growth factor (HGF), TGF-α, and TGF-β; Quantitect Primer Assays, Qiagen, Hilden, Germany). Each PCR was performed using a total 30 μL volume of a mixture containing 2 μL of total RNA (20 ng to 200 ng). β-actin expression was chosen for normalization. The quantification was performed using the Pfaffl method (101) by calculating copy numbers from the Ct-value for each gene per sample. β-actin mRNA levels were calculated in the same manner and the relation of target gene copies/100 000 copies of β-actin are given. The data are shown as the mean of four separate experiments.

ELISA

NF-κB (NF-κB p65 ELISA KIT, BioSourceTM CA, USA), and STAT3 [STAT3 (pY705) ELISA KIT, BioSourceTM, CA, USA] ELISAs were conducted according to the manufacturer's instructions. Negative and positive controls were included and a standard curve was determined for each assay. Sample size of the nucleic protein extract was 10 μL. Normalization was carried out by calculating relative protein concentrations.

TUNEL staining

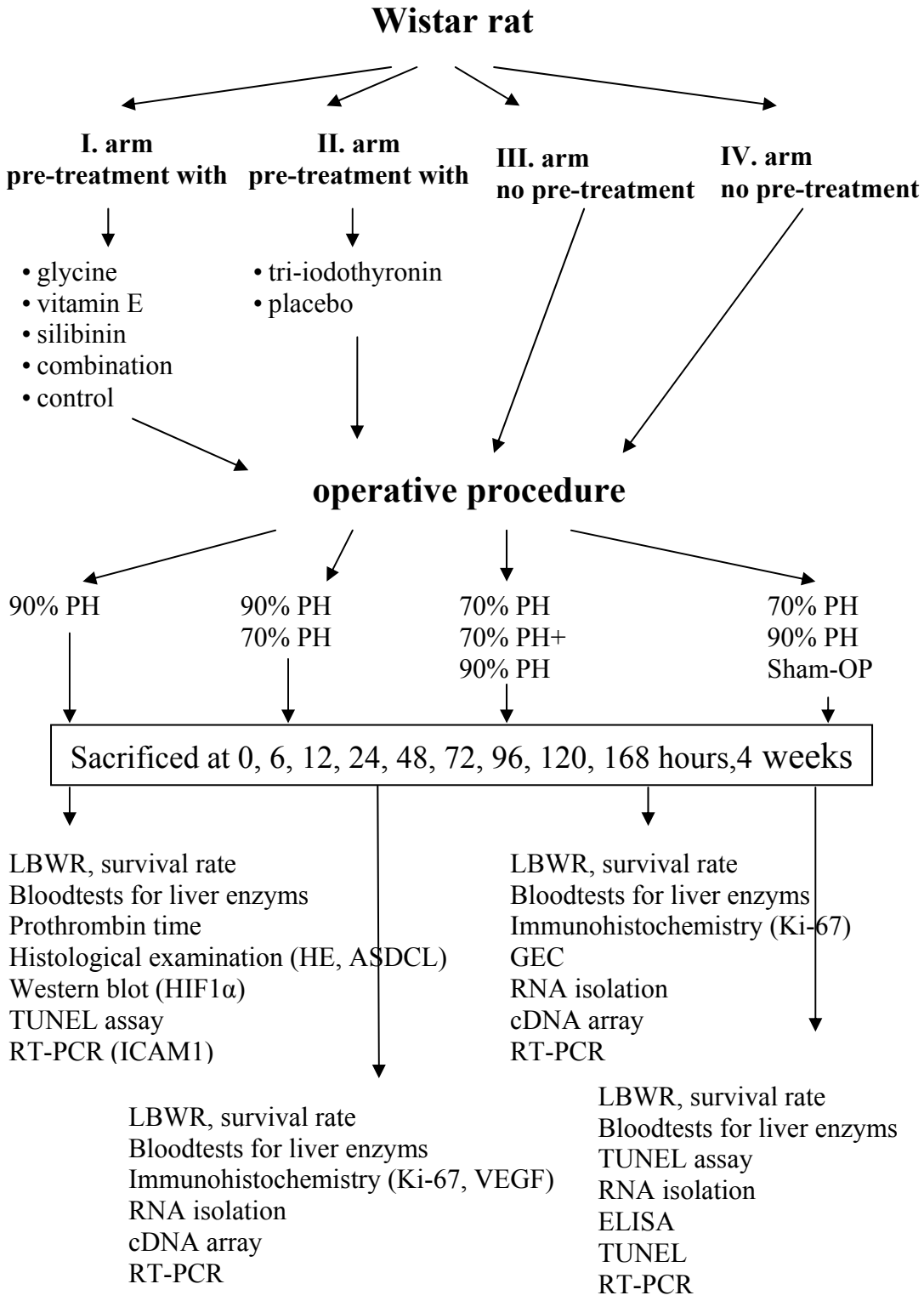
Sections of paraffin-embedded tissue were dewaxed by heating to 60°C for 30 min and subsequent washing in xylene. The slices were rehydrated through a grade series of ethanol (100%, 90%, 70%), permeabilized with permeabilization solution (0.1% Triton X-100, 0.1% sodium citrate) and washed twice in PBS. Positive controls were incubated with DNase I for 10 min at room temperature prior to labeling. TUNEL reaction mixture was prepared according to protocol (*In Situ* Cell Death Detection Kit, Fluorescein; Roche, Germany). Labeling was conducted as described in the assay manual, samples were subsequently embedded in ProLong® Gold antifade reagent with

DAPI (Invitrogen, CA, USA). Labeled cells per 10 fields of vision were counted on a fluorescence microscope and absolute numbers were compared.

Statistical analysis

Data are shown as mean ± SE. Differences between any two groups were determined by the Wilcoxon test for ELISA, qrt-PCR, TUNEL-assay and bilirubin. $P < 0.05$ was considered to be statistically significant. For differences between any two groups regarding the serum parameters ALT, AST and LDH the variance test was used and $F < 0.05$ was considered statistically significant.

3.5. Summarized study design



4. Results

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

4.1.1 Animals and operative procedure

The pre-treatments did not adversely affect the preoperative condition of the rats, although the rats pre-treated with α-tocopherol by gavage were subjected to some stress during the application of the substances.

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 anesthesia of 36.4 ± 5.6 min; there were no significant differences between the groups (Table 3.).

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 (Table 3.).

4.1.2. 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) (Table 3.).

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, although these differences were not significant.

	Pre-treatment with				
	no pre-treatment	vitamin E	silibinin	glycine	combination
Rat weight at the time of operation (g)	305.1 ± 40.2	348.2 ± 42.9	356.1 ± 60.9	339.1 ± 53.1	348.1 ± 43.3
Duration of anesthesia (min)	38 ± 5	37 ± 6	36 ±5	37 ± 6	35 ± 3
Duration of operation (min)	23 ± 5	23 ± 5	23 ± 3	24 ± 6	23 ± 3
Size of resection / 100 g rat (g)	3.04 ± 0.33	2.94 ± 0.42	2.97 ± 0.48	2.98 ± 0.34	3.04 ± 0.47
Survival rate ≥ 48 h	16 / 18	13 / 18	15 / 18	17 / 18	14 / 18
Survival rate after 4 weeks	5 / 6	4 / 6	5 / 6	5 / 6	4 / 6
Increase in the animal weight after 4 weeks (g)	50.9 ± 17.2	14.7 ± 26.8	39.7 ± 16.4	63.0 ± 40.8	38.8 ± 9.6
Remnant liver weight / 100 g rat after 48 hours	0.74 ± 0.10	0.84 ± 0.21	0.80 ± 0.14	0.86 ± 0.19	0.74 ± 0.17
Remnant liver weight / 100 g rat after 4 weeks	1.52 ± 0.32	1.61 ± 0.20	1.64 ± 0.27	1.67 ± 0.29	1.39 ± 0.46

Table 3.
Operation-related data, survival and changes of the liver and body weight after 90% PH.

Male Wistar rats were pre-treated or not with α-tocopherol , glycine, silibinin, and the combination of these three pre-treatments. Thereafter, 90% PH was performed under isofluran anesthesia. Body weight and weight of the resected liver were determined at the time of the operation. At different postoperative time points, rats were sacrificed and body weight and remnant liver weight were determined. Values shown are means ± S.D. of 6-24 experiments (6 per time point, i.e. 0, 12, 24, 48, 72 h and 4 weeks postoperatively).

4.1.3. 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. In the control group (90% resection, no pre-treatment) serum transaminase activities peaked at 12 hours after surgery, declined thereafter and returned

to baseline levels at 72 hours postoperatively. 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 (Figure 4, Table 4.).

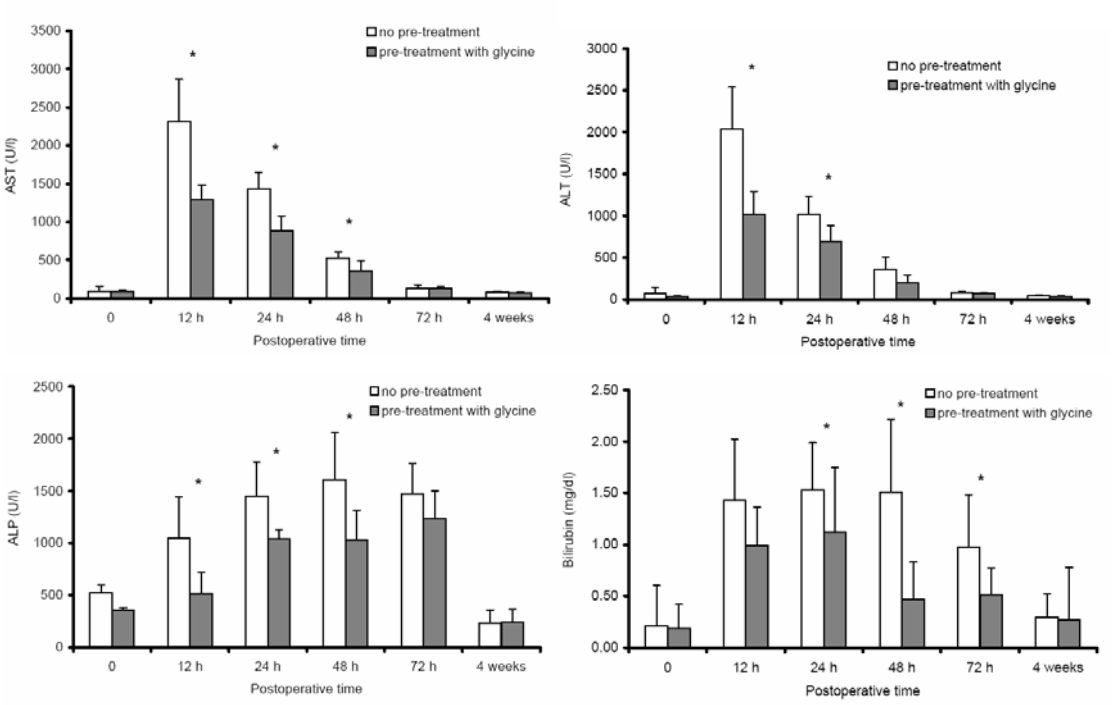


Figure 4.

Laboratory parameters of liver injury and liver function after 90% PH, with and without pre-treatment with glycine.

*Male Wistar rats were pre-treated (black bars) or not (open bars) with glycine. Thereafter, 90% partial hepatectomy was performed under isofluran anesthesia. At different postoperative time points rats were sacrificed and blood samples were collected. Serum AST, ALT, ALP activities and serum levels of total bilirubin were determined using standard assays. Values shown are means ± S.D. of 6 experiments (for every time point). * Significantly different from control group (partial resection, no pre-treatment); $p < 0.05$.*

Alkaline phosphatase (ALP) release after surgery showed a delayed peak after 48 hours. Animals pre-treated with glycine, α-tocopherol or silibinin all had significantly decreased serum ALP activities compared to non-pre-treated animals. With combined pre-treatment, serum ALP activities were also decreased, but this did not reach significance.

		Pre-treatment with					
	No hepatectomy	*Postopera -tive time*	no pre-treatment	vitamin E	silibinin	glycine	combination
AST (U/l)	166 ± 51	*12h*	2311 ±556	2148 ± 719	1808 ± 641	1292 ± 192*	2015 ± 691
ALT (U/l)	48 ± 18	*12h*	2038 ± 500	1608 ± 315	1445 ± 205*	1013 ± 278*	1309 ± 607
ALP (U/l)	310 ± 68	*48h*	1602 ± 461	879 ± 604*	1169 ± 419*	1028 ± 288*	1037 ± 537
Bilirubin (mg/dl)	0.07 ± 0.00	*72h*	0.98 ± 0.71	1.28 ± 0.82	0.47 ± 0.68*	0.51 ± 0.36*	0.37 ± 0.44*
PT (sec)	15.0 ± 0.5	*12h*	22.8 ± 2.0	25.8 ± 1.0*	23.9 ± 1.7	22.1 ± 0.8	24.7 ± 1.0*
PT (sec)	14.4 ± 1.9	*48h*	21.3 ± 2.2	24.7 ± 5.1	21.1 ± 2.5	19.3 ± 0.8*	19.5 ± 1.7*

Table 4.
Laboratory parameters in the postoperative course after 90% PH.
*Male Wistar rats were pre-treated or not with α-tocopherol, glycine, silibinin and the combination of these three pre-treatments. Thereafter, 90% PH was performed under isofluran anesthesia. At different postoperative time points, rats were sacrificed and blood samples were collected. Serum AST, ALT, and ALP activities and serum levels of total bilirubin as well as prothrombin time (PT) were determined using standard assays. Values shown are means ± S.D. of 6 experiments (for every time point). * Significantly different from control group (no pre-treatment); $p < 0.05$.*

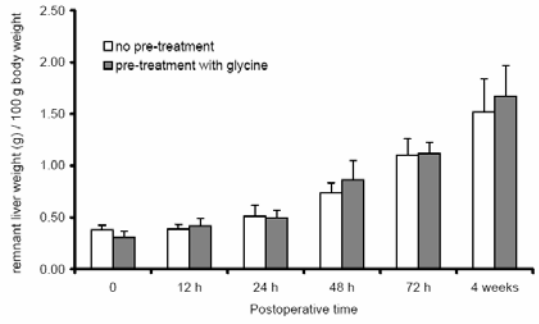
4.1.4. Synthetic function of the liver

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 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. Silibinin pre-treatment did not change PT significantly, while α-tocopherol pretreatment enhanced the increase in PT slightly at all time points and

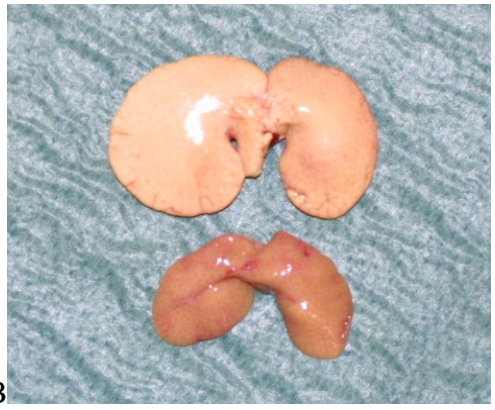
significantly after 12 hours. Combined pre-treatment appeared to have adverse effects early, but beneficial effects at later time points. (Table 4.)

4.1.5. Regeneration of the remnant liver mass

When the rats underwent 90% partial hepatectomy and 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. In all groups liver weight had not yet reached the values of non-resected rats 4 weeks after resection (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) (Figure 5.).



A



B

Figure 5.

Liver regeneration after 90% PH, with and without pre-treatment with glycine.

Male Wistar rats were pre-treated (black bars) or not (open bars) with glycine. Thereafter 90% PH was performed under isofluran anesthesia. At different postoperative time points, rats were sacrificed and remnant liver weight was determined. A: Remnant liver weight is given in relation to body weight (b.w.). Values shown are means ± S.D. of 6 experiments (for every time point). B: Rat's caudal lobe after 48 hours and at the time of the operation after 90% PH.

4.1.6. Histological and immunhistochemical results

HE staining of sections of the residual liver tissue of 90% hepatectomized animals without pre-treatment revealed necrotic fields already at the early (12 hours) time points (Figure 6A), and some microvesicular steatosis at later time points (data not shown). In

glycine pre-treated animals, in contrast, histology did not show necrotic areas (Figure 6B), although some fatty changes could also be observed in the regenerating livers at later time points. Naphtol-AS-D-Chloracetate-esterase (ASDCL) staining of remnant liver sections of non-pre-treated rats revealed occasional infiltrating granulocytes in the perisinusoidal areas (Figure 6C), 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; $p > 0.05$) (Figure 6D).

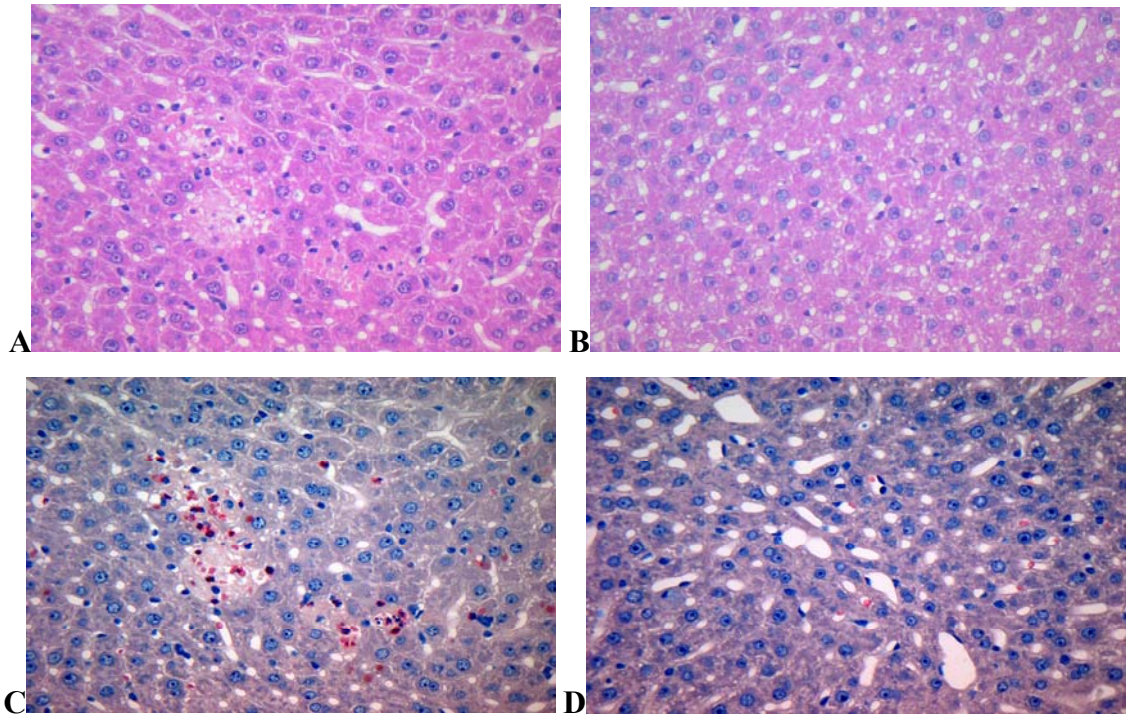


Figure 6.

Liver histology of the residual liver after 90% PH, with and without glycine pretreatment.

HE staining without pre-treatment revealed areas of confluent necrosis within the lobulus and showed occasional accumulation of neutrophils after 12 hours (A). Histology of liver remnants in glycine pre-treated animals did not markedly differ from the histological appearance of normal liver tissue (B). With ASDCL staining in untreated rat remnant liver sections (C) numerous infiltrating granulocytes could be seen in the sinusoidal areas. Staining of liver remnants in the glycine group did not differ markedly difference to the normal liver tissue (D). Original magnification: x 400.

Early postoperative apoptosis, as assessed by TUNEL staining, was less in glycine-pre-treated animals than in the non pretreated ones (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.

4.1.7. Activation of inflammatory factors

Immediately after the operation and 12 hrs postoperatively an induction of the inflammatory cytokine IL-1-β could be observed in the residual livers of non-pre-treated rats as well as of glycine-pre-treated rats (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 (Figure 7).

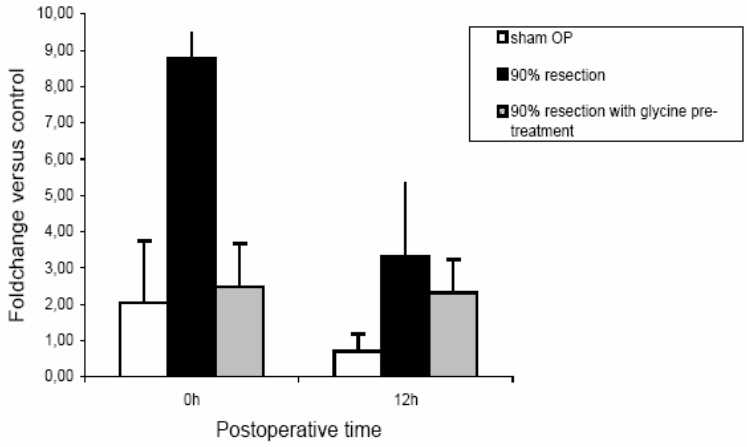


Figure 7.

Induction of ICAM-1 after 90% PH.

ICAM-1 induction was assessed by RT-PCR in liver tissue of non-operated control animals and of sham-operated animals, and in the residual liver after 90% PH immediately after the operation (0 h) and 12 h postoperatively. Animals subjected to 90% PH were either not pre-treated or were pre-treated with glycine. ICAM-1 mRNA levels are given as fold change compared to non-operated controls. n=6.

4.1.8. 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 (Figure 8.).

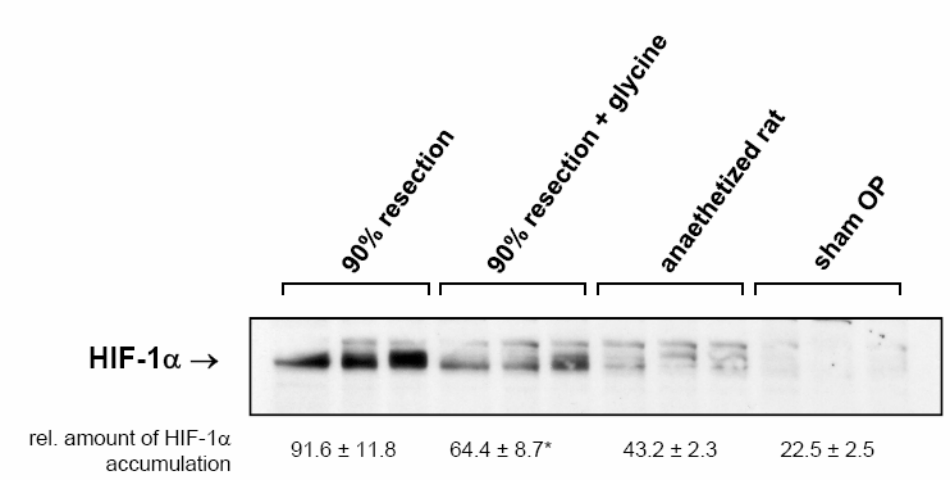


Figure 8.

Effects of 90% PH with and without glycine pretreatment on the accumulation of HIF-1α.

*Male Wistar rats were pre-treated or not with glycine. Thereafter, 90% PH was performed under isofluran anesthesia. Controls include anesthesia only (anaesthetized rat) and sham operation (sham OP), i.e. laparotomy + dissection of the liver without actual liver resection. Six hours after the end of the operation, samples of liver tissue were obtained and immediately frozen in liquid nitrogen. The accumulation of the hypoxia-inducible factor-1α (HIF-1α) was assessed by Western blot. Quantitative values given are means ± S.D. of 4 experiments. * Significantly different from the values of liver-resected animals without glycine pretreatment; $p < 0.05$.*

4.2 Results of pre-treatment with tri-iodothyronin

4.2.1. 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) (Figure 9.).

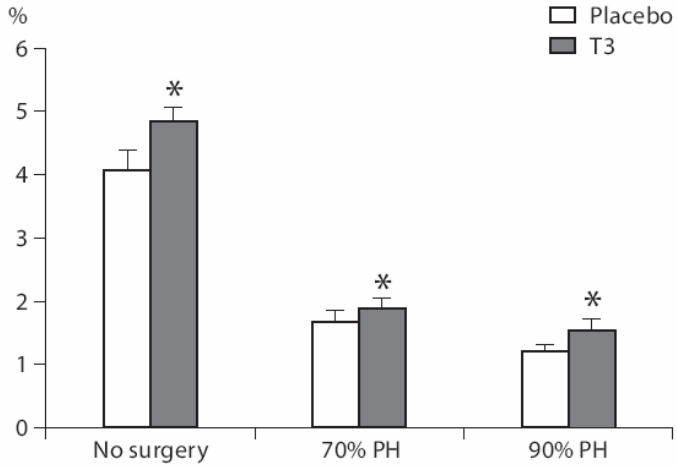


Figure 9.

Influence of T3 on the LBWR.

Liver body weight ratio after 70% PH and 90% PH. 6 rats each were included per group. As an additional control we incorporated rats which were treated by T3 or placebo, but did not receive a liver resection.

4.2.2. 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 with 41.30 ± 19.92 in placebo injected rats

(control group) 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 placebo injection ($p < 0.001$) (Figure 10.).

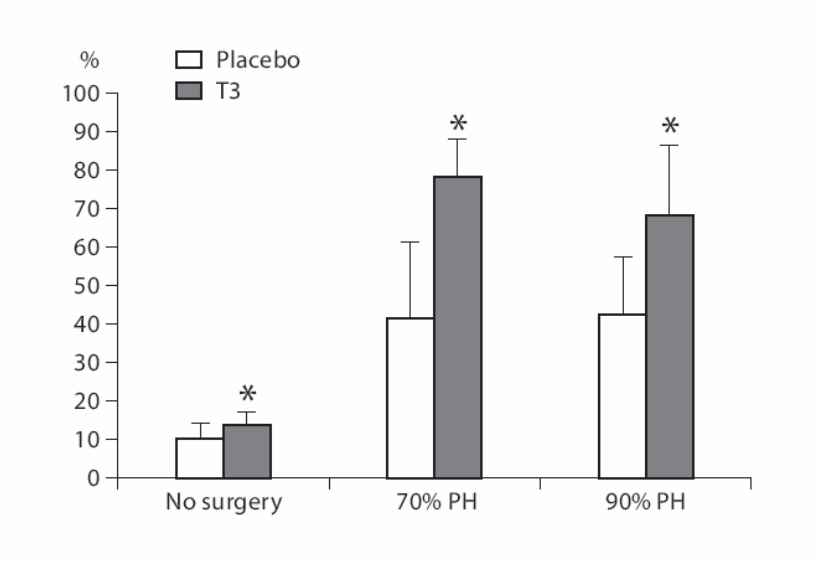


Figure 10.

Impact of T3 on the hepatocyte proliferation.

Hepatocyte proliferation after 70% PH and 90% PH. 'Proliferation index' was defined as the percentage of Ki-67-positive cells counted in 5 periportal and perivenular fields of a specimen. 500 hepatocytes were counted each.

4.2.3. 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 (Figure 11.).

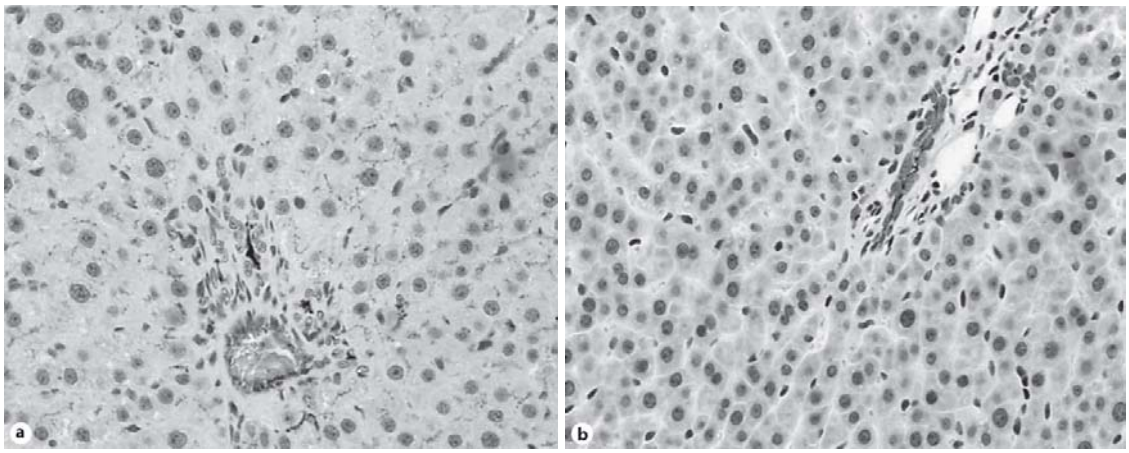


Figure 11.

VEGF expression after partial hepatectomy.

*VEGF expression after 90% PH. VEGF expression was assessed in rat livers by VEGF protein staining. Representative pictures of VEGF staining in rat livers 96 h after 90% PH treated with T3 (**a**) or placebo (**b**) are shown.*

4.2.4. Impact of T3 on Serum Parameter

We were not able to detect a significant difference regarding the serum parameters (AST, ALT and GLDH) in rats treated with T3 compared with untreated rats. Even though there was a marked increase of GLDH in untreated rats after 70% PH, this did not reach statistical significance ($p > 0.05$). The same observations were made for bilirubin (Figure 12.).

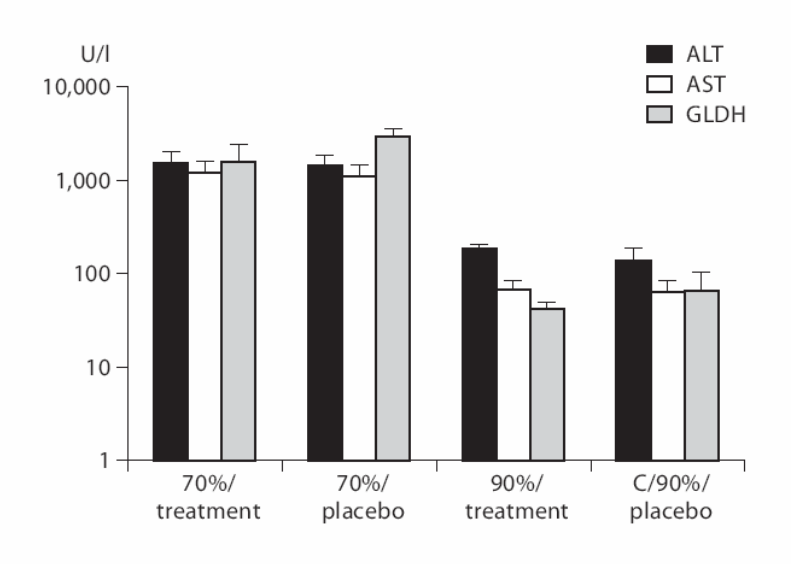


Figure 12.

Serum parameters.

Effects of pre-treatment with tri-iodothyronin on the rats. After the administration of placebo or T3 70% PH or 90%PH was performed. After 24 hours rats were sacrificed and blood samples were taken. ALT, AST and GLDH levels were determined. No statistical differences could be observed.

4.2.5. Impact of T3 on Overall Survival

Independently from T3 stimulation, there wasn't any postoperative mortality after 70% PH in our hands. 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 ($p = 0.1318$) (Figure 13).

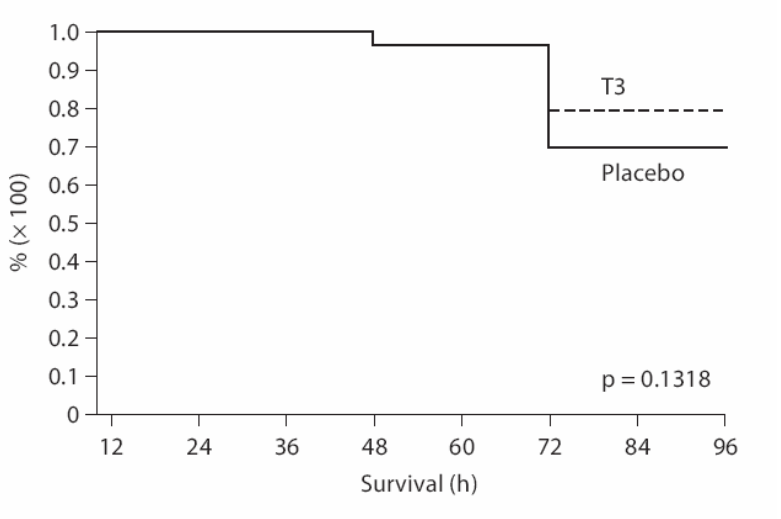


Figure 13.

Overall survival after 90% PH (log-rank).

T3-treated animals showed a slightly improved overall survival 96 h after 90% SH, even though this did not reach statistical significance.

4.2.6. 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 (Table 5.).

T3 vs. placebo RT-PCR	Fold Change	
Flt-1	2.6	8.7*
PPAR-α	2.0	3.1*
C3	2.3	5.8*

Table 5.
Modulation of hepatic gene expression after 90% partial hepatectomy by T3 vs. placebo treatment.
A single injection of T3 showed a statistical significant overexpression of Flt1, PPAR, and C3 compared to untreated rats, which could be confirmed by RT-PCR

Data for the 3 genes that were reinduced by T3 treatment are shown as fold change compared to placebo and sham-operated controls (means of all animals)

4.3. Results of impact of hepatic vein deprivation

4.3.1. 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 (Figure 14.).

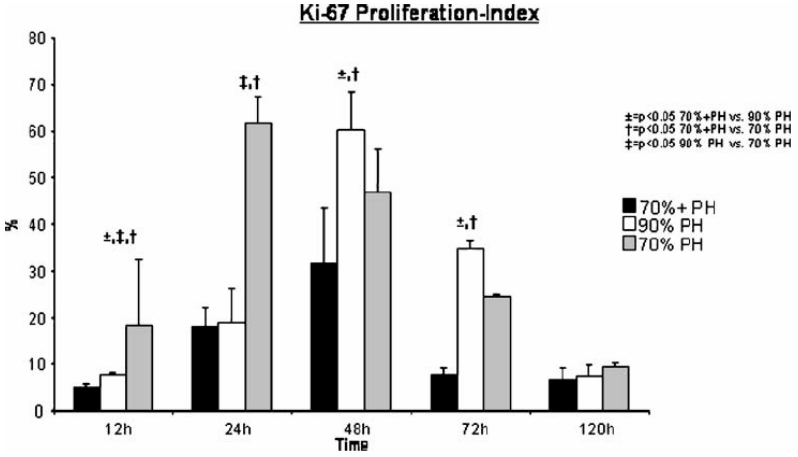


Figure 14.
Hepatocyte proliferation after PH.
Hepatocyte proliferation was assessed in rat livers during the first 5 days after PH by Ki-67 staining. Animals were treated with a 90% PH, 70%+ PH or a 70% PH. A rapid proliferation can be observed in 70% hepatectomized rats. In 90% hepatectomized rats, the proliferation is delayed. Interestingly, the proliferation capacity in 70% PH is still higher than in rats, which underwent a 70%+ PH.

Hematoxylin and eosin stained tissue specimen of all groups varied. Regarding those rats that received a classic 90% PH, the liver showed high mitotic rates (Figure 15a.). 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; $p > 0.05$). After 48h, however, 60.4 ± 8% of cells

stained positive for Ki-67 in group A compared to 31.75 ± 11% in group B. At 72h, there were still 34.75 ± 5% of all hepatocytes proliferating in group A, compared to only 7.68 ± 1% in 70%+ PH ($p < 0.003$). HE section of the same animals with 70%+ PH showed a perivenular swelling of the hepatocytes with clumped strands of eosinophilic cytoplasmic material (Figure 15b) compared with those without restriction of the hepatic outflow (Figure 15c).

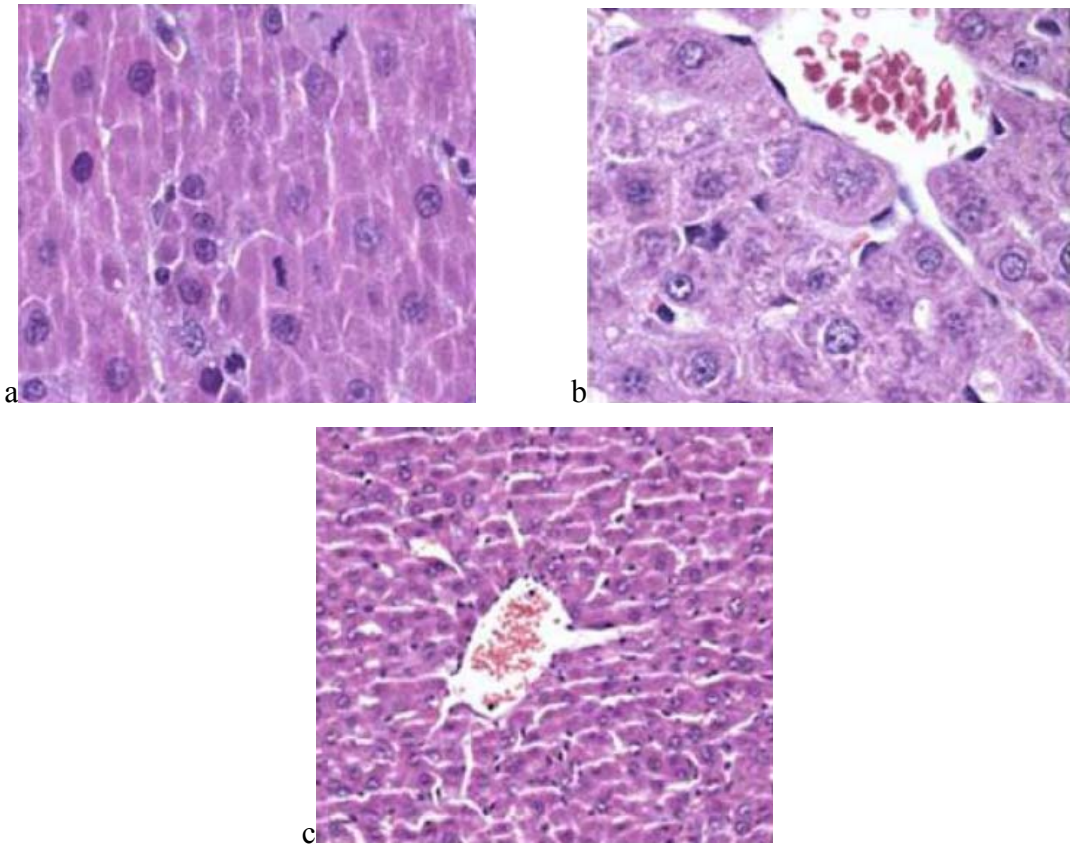


Figure 15.
Histopathology and immunohistochemistry after restriction of hepatic outflow.
High mitotic rate in an animal with 90% PH (a, H&E, ×1,000). Tissue specimen of one liver after 70%+ PH (b, H&E, ×1,000). Perivenular hepatocytes are enlarged showing clumping of eosinophilic cytoplasmic material representing intermediate filaments compared with rats after 70% PH (c, H&E, ×400)

4.3.2. Biochemical markers of liver cell damage

To assess the liver cell damage and the impairment of liver function induced by PH, AST, ALT, GLDH and bilirubin were determined. In 90% PH and 70+ PH resected animals, the highest serum concentrations of AST, ALT, GLDH and bilirubin were

detected 12–48h after surgery, declined thereafter and leaned towards baseline levels at 120h. In most instances, the highest parameters were observed in animals, which had received a classic 90% PH, but this did not reach statistical significance, when compared to animals with a 70%+ PH ($p > 0.05$, Figure 16.). Seventy percent partial hepatectomized rats did not show such a significant increase after 12–24h after surgery. Compared to the abovementioned animals, the serum parameters of AST, ALT, GLDH and bilirubin were lower compared to 90% and 70+ PH rats throughout the observation period. These results were statistically significant at 12 and 24h ($p < 0.05$).

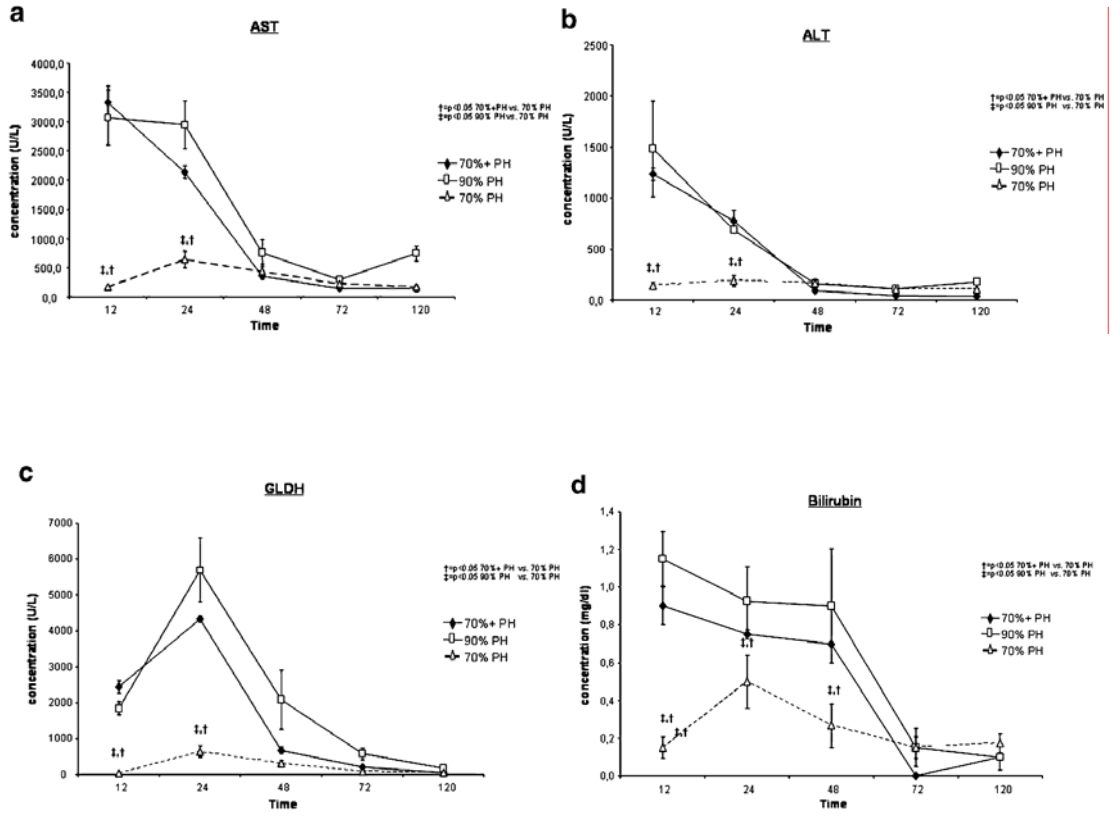


Figure 16.
Biochemical markers for liver cell damage.
There was no difference between 90% PH and 70%+ PH. Seventy percent partial hepatectomized rats though had significantly lower serum parameters.

4.3.3. 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 ($p < 0.002$, Table 6).

Operation	Body weight (g)	GEC (mg/min/g)
90% PH	225.5 ± 5.8	7.96 ± 0.34
70%+ PH	221 ± 10.2	8.46 ± 0.40*
70% PH	230.2 ± 7.5	11.74 ± 0.56**

Table 6.
Body weight and GEC after PH in the rat.
*Galactose elimination capacity (GEC) which is the elimination rate of administered galactose by the liver is determined after 72h in rats after 90 % PH to 70%+ PH in comparison to rats, which received a 70%(*p<0.001, **p<0.002)*

4.3.4. Gene expression analysis

To further elucidate the underlying mechanisms of action for the modulatory effects of hepatic venous flow 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 (Table 7.).

Gene Array / RT-PCR	90% PH	70%+ PH
IRAK-M	2.3/2.9	2.9/3.7
TGF-beta R1	0.9/0.3	0.9/0.3
PDGF-beta	2.8/2.9	2.0/1.0

Table 7.

Modulation of hepatic gene expression in rats after 90% PH and 70%+ PH for three randomly chosen genes IRAK-M, TGF-β–R1, PDGF-β. Shown are only the fold changes at 24h of the genechip expression analysis and their confirmation by RT-PCR.

4.3.5. 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.

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

4.4.1. 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 $P = 0.006$ (Figure 17.).

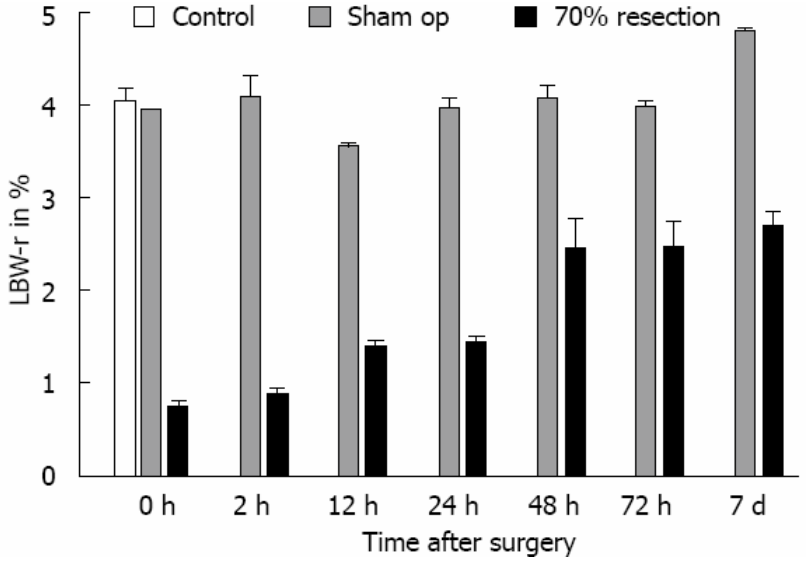


Figure 17.
Liver-body-weight-ratio of control animals, sham-operated and 70% resected animals given in g per 100 g body weight. Controls had a LBWR of 4.04% ± 0.15%.

4.4.2. Serum levels of liver enzymes

AST and ALT were significantly raised in the 70% resected animals compared to sham-operated rats (Figure 18 A, B.). 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%, $F = 0.011$). Serum levels of both enzymes diminished over time, reaching control levels 7 d after surgery. LDH after 70% resection did not differ significantly from sham animals except at 7 d postoperatively (Figure 18 C.). LDH 7 d after 70% resection was 2060 U/I while the

level in sham operated animals was 890 U/I (F = 0.033). Seventy percent resection did not lead to a significant increase in bilirubin serum levels when compared to sham-operated animals (Figure 18 D.).

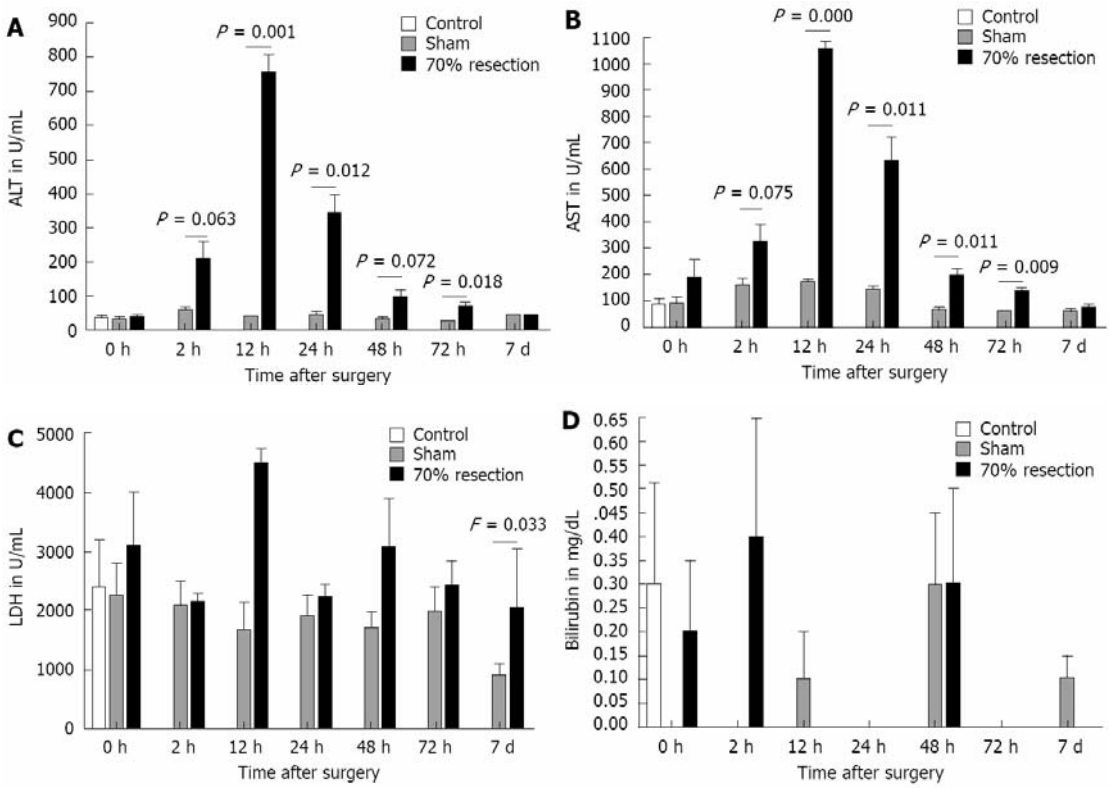


Figure 18.
Serum levels of liver related enzymes after sham operation and 70% PH.
Each given in U/L and mg/dL for bilirubin, respectively. A: ALT, base level in serum was 36.2 ± 5.2 U/mL; B: AST in controls was 84.0 ± 31.1 U/mL in serum; C: LDH, basal release of LDH into serum was 2416 ± 1088 U/mL; D: Bilirubin, with a baseline serum level of 0.3 ± 0.3 mg/dL.

4.4.3. 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 (Figure 19 A.).

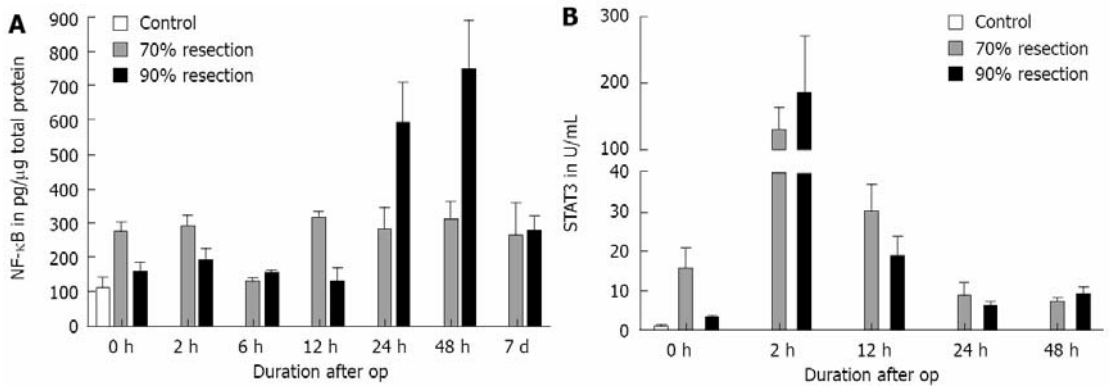


Figure 19.
ELISA results of nucleic protein extracts.
***A**: Active NF-κB. Baseline activation was 111 pg/μg total protein; **B**: Phosphorylated STAT3 (tyrosine 705) in total cellular protein. Activation in control animals was 4.5 U/mL. Protein was isolated from regenerating rat liver after 70% or 90% resection, respectively, at different time points after surgery. All data are normalized to an internal standard and are shown as mean values of four separate experiments. Significance is given versus control group.*

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 (Figure 19 B.).

4.4.4. 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 (Figure 20 A.). 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 (Figure 20 B.). Postoperatively, HGF expression increased steadily reaching a maximum at 12 h after surgery and returning to preoperative levels after 24 h in both groups (Figure 20 C.).

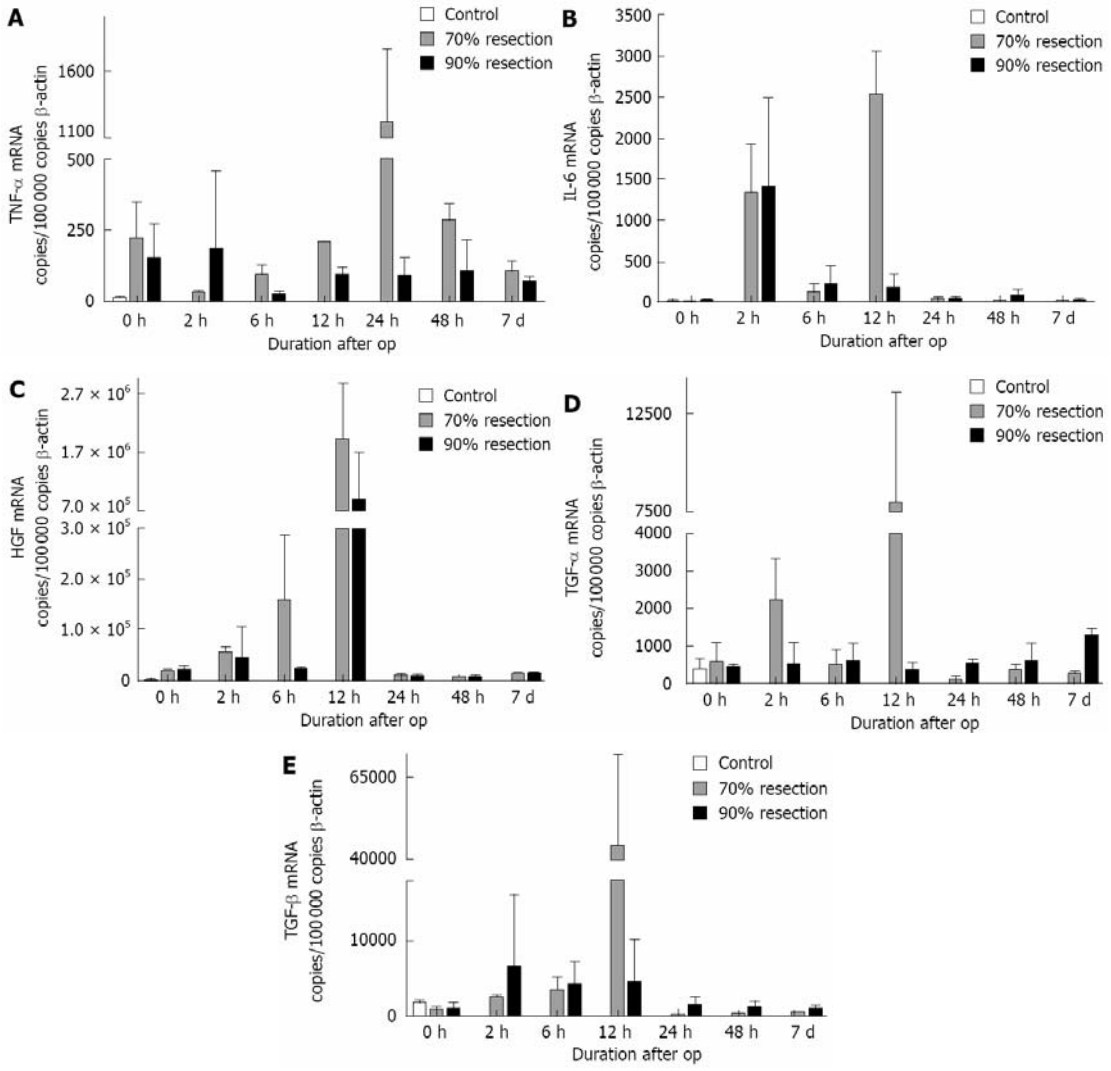


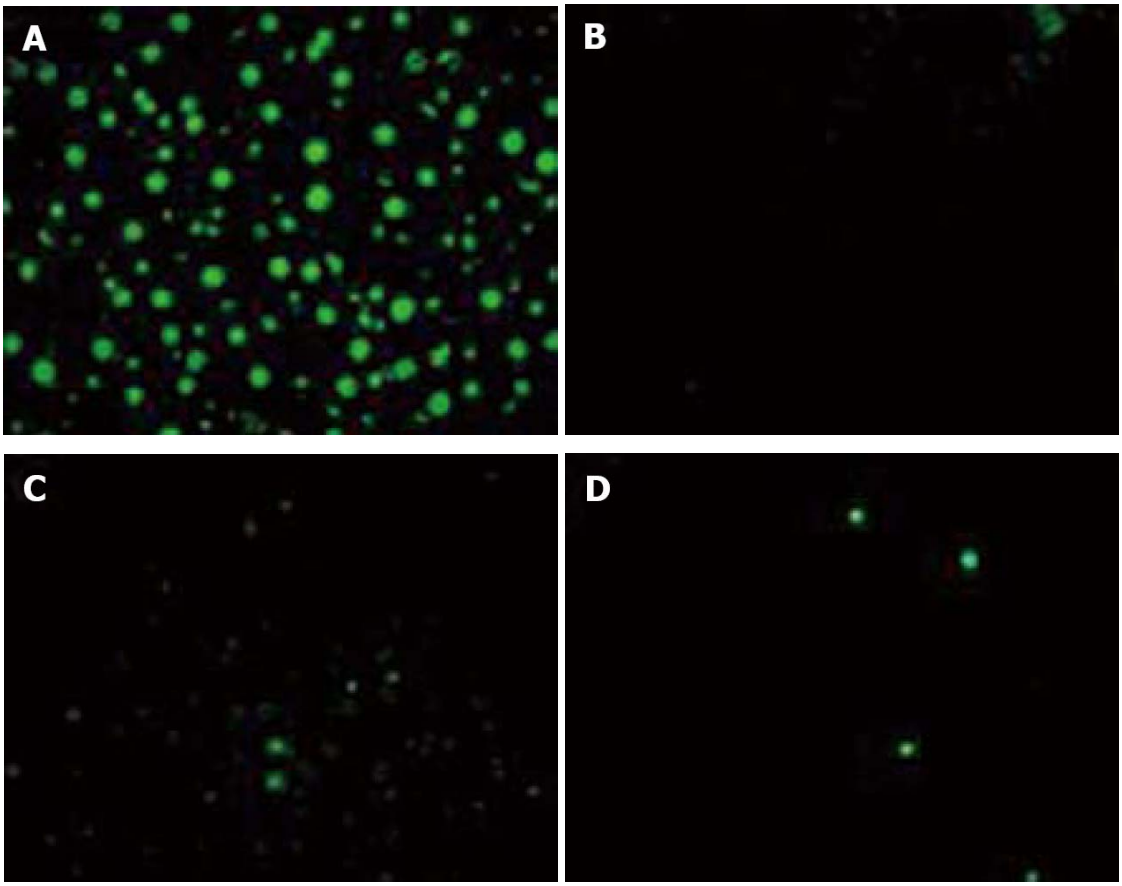
Figure 20.
mRNA was isolated from regenerating liver tissue of rats at different time points after 70% or 90% resection, respectively.
***A**: TNF-α (< 50 copies/100 000 copies b-actin); **B**: IL-6 (< 15 copies/100 000 copies b-actin); **C**: HGF (50 000 copies/100 000 copies b-actin); **D**: TGF-α (< 2500 copies/100 000 copies b-actin); **E**: TGF-β (< 5500 copies/100 000 copies – β-actin). Measurement of cytokine and growth-factor expression was performed by quantitative real-time (rt) PCR. Copy numbers of each gene were calculated from ct values. Data shown are the mean of four separate experiments with standard error of mean. All statistical significances were calculated against control animals. Baseline expression of each gene is given in parentheses.*

A significant increase in early postoperative TGF-α expression was only detected after 70% 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 (Figure 20 D.). 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 (Figure 20 E.).

4.4.5. 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 (Figures 21, 22.).



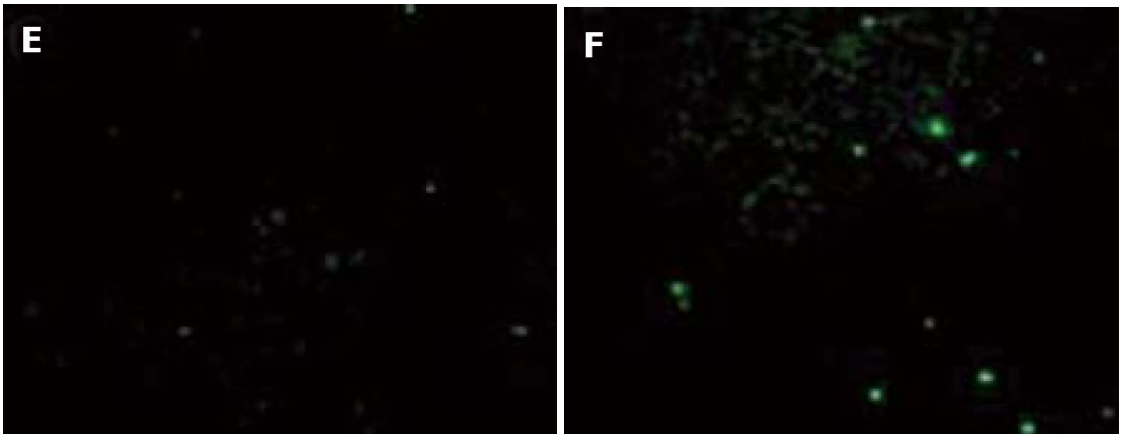


Figure 21.
Representative images of TUNEL staining in liver tissue from the following groups.
***A**: Positive control; **B**: Negative control; **C**: 70% resection, 24 h after surgery; **D**: 90% resection, 24 h after surgery; **E**: 70% resection 7 d after surgery; **F**: 90% resection, 7 d after surgery.*

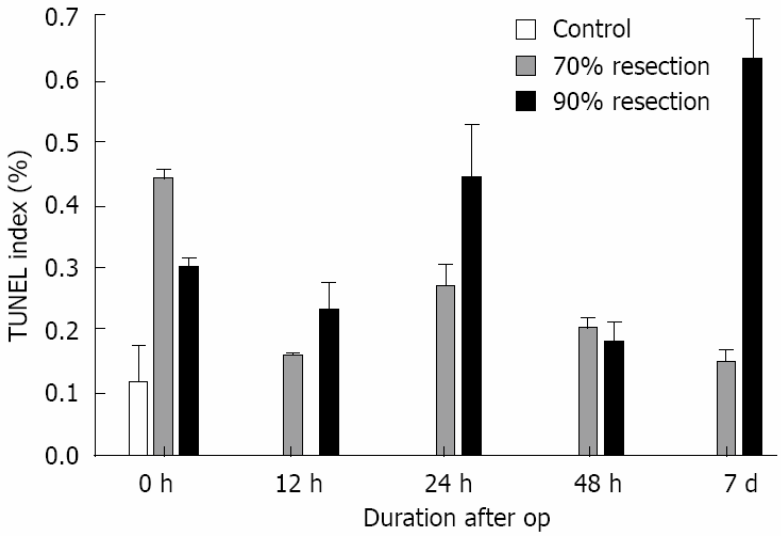


Figure 22.
Results of TUNEL staining from paraffin embedded tissue sections.
Paraffin embedded tissue slides from regenerating rat liver were dewaxed, washed and stained using the TUNEL method. The slides were covered in anti-fade medium with DAPI and analysed with a fluorescence microscope. False positive results were ruled out by comparison of the images with DAPI counterstaining (not shown). The TUNEL index is the number of TUNEL-positive cells divided by the total cell number. Cells were counted in ten fields of vision per section. Numbers shown are mean of four separate experiments in each group. TUNEL index for control animals (no resection) was approximately 0.12%.

5. Discussion

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

Many patients die each year on the waiting list before a suitable graft becomes available. To enlarge the graft pool, living donor liver transplantation is now performed in an increasing number of centers. However, if the recipient is an adult, this requires a major hepatectomy in the donor, causing substantial liver injury and leaving relatively little remaining liver mass in the donor and thus subjects the donor to a risk of hepatic failure. Measures to decrease the risk for the donor are therefore urgently needed. Here, we show that pre-treatment with glycine drastically decreased tissue injury after 90% partial hepatectomy as evidenced by decreased transaminase release, improved histology proven injury and improved parameters of liver function (Figure 4., 6., Table 4.). In the established model of 90% partial hepatectomy in rats (95), elevated serum activities of AST, ALT and ALP (Figure 4., Table 4.) in the postoperative course after partial hepatectomy, with the transaminases peaking at 12 hours, suggest that a transient liver cell injury occurs during/after the operation. The serum enzyme levels correlated with the histological demonstration of marked necrosis (Figure 6.).

Manipulation of the liver has been shown to cause priming or activation of Kupffer cells (102-104). Activation of Kupffer cells has been suggested to enhance the production of vasoactive mediators, which cause constriction of intrahepatic vessels, thus disturbing hepatic microcirculation, leading to hypoxia (102). Excess portal perfusion offers an alternative mechanism to explain hepatic injury after extended hepatectomy because portal blood is necessarily directed through a small remnant, leading to a state of relative portal hyperperfusion (105), which could lead to shear stress-induced injury to the sinusoidal endothelium and possibly also to activation of Kupffer cells. Destruction of the sinusoidal endothelium is likely to lead to microcirculatory disturbances and secondary hypoxic hepatocyte injury. In addition, injury can occur as a consequence of exposure of parenchymal cells to toxic substances normally cleared by sinusoidal lining cells (106). In human beings, exposure of the graft

to portal hyperperfusion has been shown to result in hepatocyte ballooning, centrolobular necrosis and parenchymal cholestasis (107), changes that we also observed in the histological samples after 90% hepatectomy in the rat (Fig). In the current study, glycine strongly ameliorated this injury, biochemically as well as histologically (Figure 4., 6., Table 4.).

Glycine can be administered in the diet without side effects. This amino acid has been shown to be very efficient in preventing cell death due to hypoxia or metabolic inhibition in a variety of cell types, including renal tubular cells and hepatocytes (58, 108-113). In hepatocytes, this protection appears to be due to inhibition of hypoxia-induced sodium influx (108, 110). This inhibition has been attributed to an action of glycine on hepatocellular chloride channels (108, 110); however, inhibition of a sodium-conducting unspecific membrane pore forming under hypoxic conditions – as has also been shown for other cell types (109, 111) – appears more likely (110). In addition, glycine has been suggested to be anti-inflammatory, decreasing the activation of neutrophils and macrophages, like the one of Kupffer cells (57, 104, 114, 115). Thus, glycine has been shown to decrease Kupffer cell activity after manipulation/harvesting and after rat and human liver transplantation (102, 103, 116). This is thought to be due to the action of glycine on a glycine-gated chloride channel in Kupffer cells (117), leading to hyperpolarization and thus inhibiting activation.

HIF-1 is a hypoxia-inducible heterodimeric transcription factor consisting of HIF-1α and HIF-1β (118-121). Its oxygen-regulated subunit HIF-1α is readily hydroxylated under aerobic conditions by prolyl hydroxylases. Hydroxylated HIF-1α binds the von Hippel-Lindau protein and is subsequently degraded by the proteasome. Under hypoxic conditions, oxygen-dependent hydroxylation is reduced and HIF-1α evades degradation. The accumulating non-hydroxylated HIF-1α binds to constitutively expressed HIF-1β forming the active transcription factor HIF-1 which then leads to the expression of diverse genes induced by hypoxia such as erythropoietin and diverse genes involved in inflammatory reactions. Besides by hypoxia, HIF-1 can also be activated by inflammatory stimuli (122, 123) and upregulation/activation of HIF-1α has also been observed following mechanical stretch (124, 125). In this study, where

nominally no ischemia/reperfusion process was present (no portal ligation/Pringle maneuver was used during the procedure); handling of the liver lobes during liver preparation/removal of the ligamental adhesions and during the suturing of the resection surfaces might lead to activation of Kupffer cells. Interestingly, prominent HIF-1α accumulation was observed in rats with 90% liver resection but not in sham-operated animals that were also exposed to manipulation of the liver (albeit less severe, i.e. without resection and suturing). Thus, tissue hypoxia within the liver may have contributed to HIF-1α accumulation after 90% liver resection. The inhibition of HIF-1α accumulation by glycine might be due in part to inhibition of Kupffer cell activation, but inhibition of hypoxic cell injury and subsequent microcirculatory disturbances (and thus of further hypoxia) by glycine might also contribute to this effect. Some HIF-1α accumulation was already observed in control rats that were anaesthetized but not operated. This is most likely due to some respiratory/cardiovascular depression during anesthesia, giving rise to a certain degree of hypoxia. In sham operated animals, this HIF-1α accumulation was not observed (although the animals received the same anesthesia), most likely because the surgical stimulus and the subsequent catecholamine release antagonized respiratory/cardiovascular depression. There was only little evidence for an anti-inflammatory effect of glycine, as only little granulocyte infiltration was observed in both non-pre-treated and glycine-pretreated animals. Although glycine is likely to inhibit Kupffer cells and although the cytokines IL-6 and TNF-α, for which Kupffer cells are a major source, are considered necessary for regeneration (126), glycine pre-treatment did not have adverse effects on liver regeneration (Figure 5., Table 3.); thus, it appears to be an efficient and safe protective intervention.

α-Tocopherol is an important radical scavenger that protects cell membranes against lipid peroxidation (127, 128). The short-term preoperative supplementation of α-tocopherol in patients undergoing partial liver resection has been shown to reduce the duration of intensive unit care (ICU) significantly (50). However, in the current study α-tocopherol showed some toxicity (Table 4.), which might be due to the very high dose applied.

The flavonolignane silymarin, isolated from the fruit of the milk-thistle (Silybinum marianum), has well-known hepatoprotective properties (54, 55). 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. The therapeutic effects of both silymarin and silibinin have been related to their antioxidant, membrane stabilizing and cell regeneration-promoting actions. In vitro, silibinin is a strong inhibitor of macrophage and especially of Kupffer cell activation (129, 130). Beneficial effects were also seen here, i.e. serum transaminase and ALP activities after 90% partial hepatectomy were decreased after pre-treatment with silibinin (Table 4.). However, this effect was not as pronounced as with glycine, suggesting that glycine exerts part of its effects independently of Kupffer cells, e.g. directly on hepatocytes (likely by inhibiting hypoxic injury, see above). The most important finding was, that the effects of silibinin and glycine were not additive, when used in the combined, triple pre-treatment.

Although some of the effects of these two compounds might be overshadowed by the toxicity elicited by the high dose of α-tocopherol, the results presented in Table 4., where the triple pre-treatment was inferior to glycine alone, suggest that pre-treatment with glycine alone is the preferable option.

5.2. The effect of exogenous administration of tri-iodothyronin on the liver regeneration after partial hepatectomy

We have demonstrated that the exogenous administration of T3 confers a survival advantage after massive resection of the liver. The survival advantage seems to be induced due to an increase of the proliferative response following PH. After 70% as well as after 90% PH, we observed a statistically significantly increased LBWR in T3 compared to placebo treated animals. The LBWR, however, is not as specific, since several parameters like edema, postoperative course, etc. may modulate the LBR. Objective hepatocyte proliferation can only be demonstrated by Ki-67 immunostaining (131). Animals treated with a single injection of T3 had a higher Ki-67 proliferation index following 70% as well as 90% PH than untreated animals. Malik et al. (64)

postulated from their studies that T3 activates hepatocyte proliferation through a different pathway than surgical resection. Cells proliferating after PH are predominantly found in the periportal area, after T3 stimulation most cells are in the midzonal area. We could observe similar results in our immunohistochemical studies using Ki-67 instead of BrdU. On the transcriptional level, Pibiri et al. (132) identified an earlier expression of cyclin D1 in T3 stimulated rats compared with PH only, confirming the hypothesis, that there were different mechanisms regulating T3-induced proliferation than proliferation seen after PH.

LeCouter et al. (133) have demonstrated the importance of VEGF during liver regeneration mediated through its receptor VEGF-R1 (Flt-1). Injury such as resection leads to the secretion of VEGF A, which binds to its receptors VEGF-R1 (Flt-1) and VEGF-R2 (KDR/Flk-1). Endothelial cells then proliferate and release growth factors such as IL-6 and hepatocyte growth factor (HGF). Over-expression of Flt-1 is therefore a possible prerequisite for regeneration. To further elucidate the molecular basis for these findings, we investigated the modulation of gene expression mediated by T3 administration. Following 90% PH, we found a complex modulation of several genes involved in liver regeneration, of which 3 (Flt-1, PPAR, and C3) showed a statistical difference compared to the control and to each other (table). Despite detecting a higher expression of the Flt-1, we did not detect over-expression of VEGF in T3-treated rats neither after 70% PH or 90% PH on the transcriptional level compared to sham or placebo treated rats. Even when we used different time points ranging from 3 to 168 h, we did not detect over-expression of VEGF. This might of course be due to insensitivity of the array or due to only temporary over-expression not detected by our chosen time points. The two other over-expressed genes have also been described to be involved in liver regeneration (134, 135). Interestingly though, T3-treated rats showed a strong expression of VEGF as demonstrated by immunohistochemistry, compared to only moderate or weak expression in placebo-treated rats. Therefore, stimulation of liver regeneration in T3-treated rats might be in part through its effects on neovascularization.

Despite an improved regeneration capacity and overall survival, we did not notice any difference in markers of liver cell damage (AST, ALT, GLDH, bilirubin) when T3 was administered. This led us to conclude that T3 administration seems not to have a protective role after PH.

5.3. The role of the venous outflow deprivation after major hepatectomy in the rat

We could show that a reduced hepatic venous outflow of the liver remnant leads to a significant impairment of liver function and ultimately to a significantly reduced survival after PH. This is suggested by the significantly higher galactose elimination capacity (GEC) after 70% PH compared to 70%+PH. Furthermore, the fact that there was no difference between 70%+ and 90% PH in the GEC indicates that severe venous outflow obstruction of parts of the remnant is functionally almost equal to resection of this liver part. In addition to its importance for liver function, we could also confirm that hepatic venous deprivation has an impact on liver regeneration. We observed a significantly increased regenerative response after 90% PH compared to animals which received a 70%+ PH only. This means that increase of liver volume after PH is related to volume of the residual liver after PH rather than to liver function. Several authors have already demonstrated that the magnitude of response of liver regeneration correlates with the size of resected parenchyma (59, 136-138).

Based on our data, we hypothise that liver regeneration is driven by the loss of volume and by the vascularisation of the remnant rather than its function, as we noticed a suppressed regeneration capacity in hepatectomised rats with additional venous outflow impairment. This hypothesis is confirmed at the transcriptional level, as there was no difference in the kinetics and magnitude of hepatic gene expression between the 90% PH and 70%+ PH. In addition to the suppression of liver regeneration, venous congestion leads to increased liver cell damage and liver cell necrosis as shown by the trend towards higher levels of liver enzymes after 70%+ PH compared with 70% hepatectomised rats only. These higher levels of liver enzymes might be due to the histomorphological changes seen at 72h in 70%+PH, which showed a perivenular

swelling of the hepatocytes with clumped strands of eosinophilic cytoplasmic material (Figure 16.) compared with those without restriction of the hepatic outflow. The liver cell damage seen in 70%+PH was similar to rats with 90% PH. Possibly alterations in congested lobes equal that of small for size syndromes. For the clinical practice, the results of our experimental study could be of importance in several respects. As already mentioned, with the development of right-lobe living donor liver transplantation, the importance of optimal venous outflow for liver function and regeneration has become more and more evident although never proven (68, 139). Our experimental data are in particular in accordance to clinical observations of Maema et al. (67, 140) who found an impaired volume regeneration in split livers with partial venous disruption. In addition to living donor liver transplantation, our experimental data might also be of interest in the cases of extended hepatectomy, especially in central or extended left liver resections (141).

Certainly, pathophysiological pathways of venous congestion after PH in an experimental and clinical setting are difficult to compare, i.e. due to the existence or the development of hepatic venous collaterals. However, it is likely that venous congestion might be the one contributing risk factor to operative morbidity and mortality in extended hepatectomies with an otherwise large enough remnant volume. This is also suggested by recent studies, which have shown that liver insufficiency is still a major problem in left trisegmentectomies. In these resections, despite a distinct larger liver remnant, morbidity and mortality is still considerably higher compared to right trisectionectomies (142). Certainly, liver insufficiency after hepatic resection is not a function of a single but multiple factors, one of which is bile leakage. However, with regards to the great anatomical variation of the middle hepatic vein, which sometimes drains large parts of the postero-lateral segments, one may speculate that impairment of venous outflow, even if intraoperatively not evident, could be one of the main contributing factor to morbidity and mortality in extended left hepatectomies.

Therefore, in accordance with the findings of this experimental study, in the clinical setting, it might be reasonable to reconstruct dissected large hepatic veins, especially in cases with a small remnant subjected to considerable venous deprivation (142).

5.4. The activation of transcription factors and cytokines after different extent of hepatectomies in a rat model

We analyzed the molecular events in liver tissue after subtotal hepatectomy (expression of proregenerative cytokines, activation of transcription factors and apoptosis) which are, in contrast to partial hepatectomy, not well understood. Our data indicate that activation of proregenerative genes like TGF-α and IL-6 is stronger after 70 % PH compared with 90% PH. TNF-α which is also involved in liver regeneration, is induced by 70% PH but not by 90%. In addition, HGF expression was higher in 70% than in 90% PH. This was associated with stronger activation of NF-κB in 70% PH during the early phase of regeneration. Finally, the apoptotic peak was delayed until 24 h after surgery and had a biphasic course in 90% PH. In 70% PH, apoptosis had a monophasic course and peaked directly after surgery.

These data are in accordance with other experiments from our group, in which we observed higher regenerative capacities and better liver function tests (ALT, AST, bilirubin) after 70% PH compared with 90% PH. The time course of liver regeneration differed significantly between 70% PH and 90% PH. Animals with 70% resection showed a significant increase in LBWR as early as 12 h after surgery. In the 70% PH group, liver weight reached 65% of sham-operated controls 7 d after surgery. In contrast, after 90% resection, LBWR started to increase 24 h after surgery and reached only 30% of the liver weight compared to controls at day 7. The time course in both groups for AST and ALT was similar although the release of both enzymes into serum was significantly higher after 90% PH, 12, 24 and 48 h postoperatively. LDH levels were similar in both groups until 12 h after surgery where the increase was more prominent in the 90% PH group than in the 70% PH group. At all later time points, LDH decreased in animals with 90% resection compared to controls and the 70% group. Serum bilirubin in the 70% resection group did not differ from the sham group. In animals with 90% resection we found a significant increase in bilirubin from 12 h to 72 h postoperatively. These findings show that damage to liver cells is increased after 90% hepatectomy compared to 70% resection.

Genes related to the initial phase of regeneration, such as TNF-α and IL-6, which prime hepatocytes into a state in which they are susceptible to growth factors (90, 91), were expressed at later time points in our model. Nonetheless, both cytokines were expressed at lower levels or were delayed after 90% PH compared to 70% PH. As described previously (88, 143-145), growth factors relevant to liver regeneration (HGF and TGF-β) were upregulated in 70% PH, whereas no or only a reduced induction was observed after 90% PH. This suggests that the expression of the factors relevant to the regeneration of liver tissue is influenced by the extent of resection. For TGF-β we detected a distinct peak at 12 h after 70% PH but not after 90%. Since TGF-β has antiregenerative properties (143), high expression levels would be expected to counteract regeneration in this group. On the other hand, regeneration is a very tightly regulated process (88), which implies that high expression of TGF-β could lead to attenuation of ongoing regenerative processes in the 70% PH group, whereas the regenerative stimuli which induce TGF-β expression are lacking in the 90% PH group. This concept is supported by a very tight downregulation of TGF-β in the 70% hepatectomy group at later time points after surgery. TUNEL indices were significantly raised directly after PH.

Although we found a decrease in apoptosis over time, TUNEL indices remained elevated compared with controls. Thus, the data indicate that apoptosis occurs earlier and in parallel with regeneration in animals with 70% resection. In contrast to this, animals with 90% hepatectomy showed slightly higher TUNEL indices at earlier time points and a strong increase in apoptosis 7 d after surgery. This could on the one hand, indicate that there was more tissue damage, which led to a higher number of apoptotic cells during the early postoperative phase. On the other hand, the significant rise in TUNEL indices 7 d after surgery leads to the conclusion that the main phase of remodelling occurs at later time points. Our data suggest that liver regenerative processes after 90% PH are impaired by reduced or delayed activation of proregenerative factors compared to 70% PH.

Activation of NF-κB as well as expression of important cytokines and growth factors depends on the amount of resected liver tissue. The underlying mechanisms are

not yet clear, although they may be associated with the liver's ability to regenerate and to reduce tissue to fit current requirements (90, 145). It is still unclear, which role NF-κB plays during the regenerative process in the setting of extended hepatectomy. A continuous activation was detected at low levels after 70% PH while a delayed and more pronounced activation could be seen after 90% PH. While basal activation of NF-κB may be sufficient and important for regeneration and structural reformation after 70% resection (146), the strong NF-κB activation in animals with 90% hepatectomy may also favor inflammatory processes in the damaged tissue, which counteract the restoration of a functional liver (93, 147, 148).

6. 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 effects were significant without actual 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 actual 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 increased regenerative activity compared with the placebo treated rats. These data were supported by a significantly higher proportion of hepatocytes proliferation (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 increased neovascularization 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 with animals underwent 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 was a 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 no difference compared to classic 70% PH.

In 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 liver rather than by it's function. 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 increased after 70% PH compared with sham operated and control 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.

7. Summary

Background: Protection of the liver during the living donation is desirable to ensure that the remnant liver is able to maintain sufficient function. The aim was to analyze the effects of extended hepatectomy on liver regeneration in rats with or without pre-treatments with α-tocopherol, silibinin and/or L-glycine, exogenous application of tri-iodothyronine (T3). In these extended liver resections the impact of venous outflow impairment and its consequences for liver regeneration and function was investigated.

Methods: Male Wistar rats were pre-treated with L-glycine, α-tocopherol and/or silibinin and T3 thereafter, 70% or 90% partial hepatectomy or a 70% liver resection with narrowing of the hepatic outflow of an additional 20% parenchyma (70%+ PH) was performed. Untreated and sham-operated animals served as controls. Liver function, liver body weight ratio (LBWR), hepatic proliferation (Ki-67), apoptosis (TUNEL assay) and transcription factors (NF-κB, Stat3; ELISA) or cytokines (VEGF, HGF, TNF-a, IL-6, TGF-a, TGF-b) involved in liver regeneration were assessed by immunohistochemistry and by customized cDNA arrays and quantitative RT-PCR.

Results: Glycine pre-treatment decreased transaminase release, serum ALP activity and serum bilirubin levels ($p<0.05$). Prothrombin time was reduced, and histologically, liver injury was also decreased in the glycine group. Liver resection induced HIF-1α accumulation and it was decreased by glycine pretreatment. T3-treated rats showed an increased LBWR and Ki-67 index after 70%PH and 90% PH, T3-treated rats had an increased expression of VEGF which was associated with a higher expression of its receptor Flt-1, which reached statistical significance compared to placebo-treated rats ($p<0.05$). After 70% PH, NF-κB activation was detected 12 h after surgery, while there was no activation after 90% PH. These findings correlated with delayed induction of regenerative genes after 90% PH.

Conclusion: The decrease of liver injury after pre-treatment with glycine suggests that glycine pre-treatment might be beneficial for living liver donors. Exogenous administration of T3 ameliorates liver regeneration after 70% PH and 90% PH, possibly due to stimulation of angiogenesis. Venous outflow obstruction leads to an impairment of liver regeneration and liver function. The molecular events involved in liver regeneration are significantly influenced by the extent of resection, which is associated with delayed activation of NF-κB and suppression of proregenerative cytokines.

8. Összefoglalás

Bevezetés: Élődonoros májátültetés esetén kiemelt fontosságú a donor maradék májszövetének védelme és regenerációja. Célunk az volt hogy az irodalomból ismert ágensek (L-Glycin, α-tocopherol, silibinin, tri-iodothyronine {T3}) külön vagy együttes hatását vizsgáljuk a regenerációra állatkísérletes modellen végzett kiterjesztett májreszekciót követően. Májreszekció során fontos a vénás kiáramlás megóvása, ennek károsodását is vizsgáltuk az általunk kifejlesztett modellen a májregenerációs kapacitás függvényében. **Anyag és módszer:** Hím Wistar patkányokat kezeltünk L-glycin, α-tocopherol és/vagy silibininnel és T3-al, ezt követően végeztük el a 70% vagy 90% részleges májreszekciót (PH) illetve egy csoporton a 70% májreszekciót kiegészítettük egy vena hepatica-t beszűkítő öltéssel mely további kb. 20%-os parenchyma károsodást okozott. Kontroll csoportként kezeletlen, illetve ál-operált állatok szerepeltek. A májfunkció laborvizsgálatát végeztük, máj-testsúly tömegarányt (LBWR), májsejtproliferációt (Ki-67), apoptózist (TUNEL assay) transzkripciós faktorok, cytokinek, és a regenerációban résztvevő gének expresszióját vizsgáltuk különböző módszerekkel. (NF-κB, Stat3; ELISA; VEGF, HGF, TNF-a, IL-6, TGF-a, TGF-b ; cDNA arrays és quantitative RT-PCR). **Eredmények:** A glycin kezelés a transzaminázok az alkalikus foszfatáz és a szérum bilirubin szintjét szignifikánsan csökkenti májreszekciót követően. Csökkent a prothrombin idő és a szövettanilag igazolt májszövetkárosodás is. A reszekció által indukált HIF-1α akkumuláció is jelentősen csökkent A T3 kezelt patkányoknál magasabb LBWR értékeket és Ki 67 arányt mértünk 70% PH and 90% PH-t követően, mellyel összefügg a magasabb VEGF expressziós aktivitás is. 12 órával a 70% PH-t követően mért NF-κB aktiváció szignifikánsan magasabb volt mint a 90%-os reszekció után, melyet alátámasztanak a kiterjesztettebb reszekciót követően mért gátolt illetve késleltetett regenerative génexpressziós értékek. **Következtetés:** A glycin és tri-iodothyronin kezelést követően észlelt csökkent májkárosodást jelző vizsgálatok illetve fokozott regenerációs és angiogenetikus aktivitás azt mutatja, hogy ezen kezelések jótékony hatással bírnak a donor maradék májszövetére. A vénás kiáramlás károsodása visszaveti a májregenerációt. Az ezzel kapcsolatos molekuláris változások függenek a májreszekció mértékétől, ami az NF- κB és más cytokinek késleltetett aktivációjának következménye.

9. Publications

Publications for the dissertation based on

- **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
- Bockhorn M, Frilling A, **Benko T**, Best J, Sheu SY, Trippler M, Schlaak JF, Broelsch CE. Tri-iodothyronine as a stimulator of liver regeneration after partial and subtotal hepatectomy. Eur Surg Res. 2007;39(1):58-63.
- Bockhorn M, **Benkö T**, Opitz B, Sheu SY, Sotiropoulos GC, Schlaak JF, Broelsch CE, Lang H. Impact of hepatic vein deprivation on liver regeneration and function after major hepatectomy. Langenbecks Arch Surg. 2007 Sep 12;
- Sowa JP, Best J, **Benko T**, Bockhorn M, Gu Y, Niehues EM, Bucchi A, Benedetto-Castro EM, Gerken G, Rauen U, Schlaak JF. Extent of liver resection modulates the activation of transcription factors and the production of cytokines involved in liver regeneration. World J Gastroenterol. 2008 Dec 14;14(46):7093-100.

Other publications

- P Sótonyi, É Keller, J Járay, B Nemes, **T Benko**, A Kovács, A Torakán, I Rajs.A light stabilizer Tinuvin 770-induced toxic injury of adult rat cardiac myocytes. Forensic Science International, 2001;119:322-27.
- Bockhorn M, **Benkő T**, Opitz B, Schlaak JF, Broelsch CE, Lang H. Einfluss des Lebervenenausflusses auf die regeneration und funktion der leber nach erweiterter Teilentfernung. Chirurgisches Forum 2007 für experimentelle und klinische Forschung. Forumband 36. German
- **Benko T**, Fehérvári I, Rácz K, Friedrich O, Gálfy I, Török S, Remport A, Járay J, Bodor E, Szabolcs Z. Az első sikeres kombinált szív- és vesetranszplantáció Magyarországon. Orv Hetil. 2008 Jan 27;149(4):147-52.

- Kóbori L , Németh T, Nagy P, Dallos G, Sótonyi P, Fehérvári I, Nemes B, Görög D, Patonai A, Monostory K, Doros A, Sárváry E, Fazakas J, Gerlei Zs, **Benkő T**, Piros L, Járay J, De Jong KP. Experimental results and clinical impact of using autologous rectus fascia sheath for vascular replacement. *Acta Vet Hung. 56(3), 2008, pp411-420*
- Heuer M, **Benkö T**, Cicinnati VR, Kaiser GM, Sotiropoulos GC, Baba HA, Treckmann JW, Broelsch CE, Paul A. Effect of low-dose rapamycin on tumor growth in two human hepatocellular cancer cell lines. Transplant Proc. 2009 Jan-Feb;41(1):359-65.

Oral presentations

- **Benkő T**, et al: Gastrointestinalis vérzések ügyeleti időben. Fiatal Sebészek Kongresszusa Nyíregyháza, 2002
- **Benkő T**, Doros A, Fehérvári I, Görög D, Nemes B, Járay J, Kóbori L. Intraoperatív és percutan rádiófrekvenciás thermoablatio alkalmazása májdaganatos betegeinken. Magyar Sebésztársaság 58. Kongresszusa Budapest, 2006. Magyar Sebészet vol.59., 2006 augusztus
- **Benkő T**, Doros A, Fehérvári I, Görög D, Nemes B, Járay J, Kóbori L. Intraoperatív és percutan rádiófrekvenciás thermoablatio alkalmazása májdaganatos betegeinken. Hepatológia 2007, Bükfürdő
- **Benkő T**, H de Groot, U Rauen. Glycin előkezelés hatása patkánymájreszekciót követően. Hepatológia 2007, Bükfürdő.
- **Benkő T**, Doros A, Fehérvári I, Görög D, Nemes B, Máthé Z, Piros L, Sárváry E, Gerlei Z, Monostory K, Járay J, Kóbori L. Az oxidatív stressz és az artériás vérellátás szerepe májtranszplantációt követően. Magyar Transzplantációs Társaság IX. Kongresszusa. Siófok 2007.
- **Benkő T**, Doros A, Fehérvári I, Görög D, Nemes B, Járay J, Kóbori L. Intraoperatív és percutan rádiófrekvenciás thermoablatio alkalmazása májdaganatos betegeinken. MST Pest Megyei Szekciójának Tudományos Ülése. Budapest 2007.

- **Benkő T**, Doros A, Fehérvári I, Görög D, Nemes B, Máthé Z, Piros L, Sárváry E, Gerlei Z, Monostory K, Járay J, Kóbori L. Az oxidatív stressz és az artériás vérellátás szerepe májtranszplantációt követően. Hepatológia 2008, Bükfürdő
- **Benkő T,** Sápy Z, Doros A, Piros L, Tóth Sz, Járay J, Kóbori L. Májdaganatok kezelésének sebészeti határai – esetbemutatás. Figamu III. Kongresszusa 2008, Balatonalmádi
- **Benkő Tamás**, Maximilian Bockhorn, Kóbori László, Cristoph Broelsch, Hauke Lang. A véna hepatica szerepe a májregenerációban illetve a májműködésben kiterjesztett reszekciót követően. Hepatológia 2009, Bükfürdő
- **Benkő Tamás**, Maximilian Bockhorn, Kóbori László, Cristoph Broelsch, Hauke Lang. A véna hepatica szerepe a májregenerációban illetve a májműködésben kiterjesztett reszekciót követően. PhD napok 2009, Budapest
- **T Benkő**, et al. Exulcerated malignant breast cancer. European Society of Surgery Kongresszusa Budapest, 2002
- **T Benkő**, et al. Missed pathological diagnose, due to incorrect clinical information Fiatal Sebészek Angolnyelvű Kazuisztikai Fóruma Győr, 2004
- **T Benkő**, H de Groot*, U Rauen. Glycine pretreatment ameliorates liver injury after partial hepatectomy in rats. Eastern European Workshop for Pediatric Hepatology. Visegrad 2007.
- **Tamas Benko**, László Kóbori, Hideo Andreas Baba, Jenő Járay, Herbert de Groot, Ursula Rauen. Glycine ameliorates liver injury after partial hepatectomy in rat. A Japán-Magyar Sebésztársaság 3. Tudományos Ülése. Budapest 2008.
- **T Benkő**, et al: Effekte verschiedener Vorbehandlungen bei der Leberteilresektion an der Ratte. 11. Workshop „Mechanismen der Zell- und Gewebeschädigung“ Xanten, 2005.

Posters

- 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.

- **T Benkő**, H Baba, H de Groot, U Rauen. Effekte einer Glycin-Vorbehandlung bei der Leberteilresektion an der Ratte. *17.* Workshop for Experimental and Clinical Livertransplantation and Hepatology. Transplantationsmedizin; ISSN0946-9648, Suppl. I-2006
- J Best, **T Benkö**, M Goralski, P Grünewald, M Trippler, M Bockhorn, G Gerken, U Rauen, JF Schlaak. Hepatische Genexpression nach Leberteilresektion – Etablierung eines Inhouse-cDNA-Makroarrays. 17. Workshop for Experimental and Clinical Livertransplantation and Hepatology. Transplantationsmedizin; ISSN0946-9648, Suppl. I-2006
- L Kóbori, T Németh, P Nagy, B Füle, Sz Toth, G Dallos, **T Benkő**, L Piros, D Görög, M Sloof, J Jaray, K De Jong: Experimental vascular graft for liver transplantation. *The Second Scientific meeting of the Japan-Hungarian Surgical Society*
- **T Benkő**, A Doros, I Fehérvári, D Görög, B Nemes, Z Máthé, L Piros, E Sárváry, Z Gerlei, K Monostory, J Járay, L Kóbori. Role of oxidative stress and arterial blood supply after liver transplantation. Transplant International. September 2007 - Vol. 20 Issue s2.
- M Heuer, **T Benko**, VR Cicinnati, GC Sotiropoulos, HA Baba, M Hertl, A Frilling, CE Broelsch. Effect of Low Dose Rapamycin on Tumor Growth in Two Human Hepatocellular Cancer Cell Lines. *American Transplant Congress, Toronto, Canada 2008. American Journal of Transplantation Juni 2008, Suppl.*
- **T Benkő**, H Baba, L Kóbori, H de Groot, U Rauen. Glycine ameliorates liver injury after partial hepatectomy in rats. Transplant International. April 2009, Vol22, Suppl-1.
- **T Benkő**, H Baba, L Kóbori, H de Groot, U Rauen. Glycine ameliorates liver injury after partial hepatectomy in rats. ESOT 2009, Paris

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11. References

1. Welch C. A note on transplantation of the whole liver in dogs. Transplantation Bulettin 1955;2:54.
2. Welch CS. [Liver graft.]. Maroc Med 1955;34:514-515.
3. Goodrich EO, Jr., Welch HF, Nelson JA, Beecher TS, Welch CS. Homotransplantation of the canine liver. Surgery 1956;39:244-251.
4. Marchioro TL, Porter KA, Dickinson TC, Faris TD, Starzi TE. Physiologic Requirements For Auxiliary Liver Homotransplantation. Surg Gynecol Obstet 1965;121:17-31.
5. Starzl TE, Francavilla A, Halgrimson CG, Francavilla FR, Porter KA, Brown TH, Putnam CW. The origin, hormonal nature, and action of hepatotrophic substances in portal venous blood. Surg Gynecol Obstet 1973;137:179-199.
6. Starzl TE, Marchioro TL, Rowlands DT, Jr., Kirkpatrick CH, Wilson WE, Rifkind D, Waddell WR. Immunosuppression After Experimental And Clinical Homotransplantation Of The Liver. Ann Surg 1964;160:411-439.
7. Starzl TE, Porter KA, Putnam CW. Intraportal insulin protects from the liver injury of portacaval shunt in dogs. Lancet 1975;2:1241-1242.
8. Cannon B, Murray JE. Plastic surgery: tissue and organ homotransplantation. N Engl J Med 1956;255:900-904.
9. Starzl TE, Bernhard VM, Benvenuto R, Cortes N. A new method for one-stage hepatectomy for dogs. Surgery 1959;46:880-886.
10. Groth CG, Brent LB, Calne RY, Dausset JB, Good RA, Murray JE, Shumway NE, et al. Historic landmarks in clinical transplantation: conclusions from the consensus conference at the University of California, Los Angeles. World J Surg 2000;24:834-843.
11. Medawar P. The Nobel lectures in immunology. Immunological tolerance 1960. Scand J Immunol 1991;33:337-344.
12. Starzl TE. History of clinical transplantation. World J Surg 2000;24:759-782.
13. Marchioro TL, Huntley RT, Waddell WR, Starzl TE. Extracorporeal Perfusion For Obtaining Postmortem Homografts. Surgery 1963;54:900-911.

14. Starzl TE, Hakala TR, Shaw BW, Jr., Hardesty RL, Rosenthal TJ, Griffith BP, Iwatsuki S, et al. A flexible procedure for multiple cadaveric organ procurement. Surg Gynecol Obstet 1984;158:223-230.
15. Starzl TE, Miller C, Broznick B, Makowka L. An improved technique for multiple organ harvesting. Surg Gynecol Obstet 1987;165:343-348.
16. Starzl TE, Iwatsuki S, Van Thiel DH, Gartner JC, Zitelli BJ, Malatack JJ, Schade RR, et al. Evolution of liver transplantation. Hepatology 1982;2:614-636.
17. Kang YG, Martin DJ, Marquez J, Lewis JH, Bontempo FA, Shaw BW, Jr., Starzl TE, et al. Intraoperative changes in blood coagulation and thrombelastographic monitoring in liver transplantation. Anesth Analg 1985;64:888-896.
18. Starzl TE, Halgrimson CG, Koep LJ, Weil R, 3rd, Taylor PD. Vascular homografts from cadaveric organ donors. Surg Gynecol Obstet 1979;149:737.
19. Stieber AC, Zetti G, Todo S, Tzakis AG, Fung JJ, Marino I, Casavilla A, et al. The spectrum of portal vein thrombosis in liver transplantation. Ann Surg 1991;213:199-206.
20. Bismuth H, Houssin D. Reduced-sized orthotopic liver graft in hepatic transplantation in children. Surgery 1984;95:367-370.
21. Broelsch CE, Emond JC, Whitington PF, Thistlethwaite JR, Baker AL, Lichtor JL. Application of reduced-size liver transplants as split grafts, auxiliary orthotopic grafts, and living related segmental transplants. Ann Surg 1990;212:368-375; discussion 375-367.
22. Starzl TE, Bell RH, Beart RW, Putnam CW. Hepatic trisegmentectomy and other liver resections. Surg Gynecol Obstet 1975;141:429-437.
23. Starzl TE, Iwatsuki S, Shaw BW, Jr., Waterman PM, Van Thiel D, Diliz HS, Dekker A, et al. Left hepatic trisegmentectomy. Surg Gynecol Obstet 1982;155:21-27.
24. Starzl TE, Demetris AJ, Van Thiel D. Liver transplantation (2). N Engl J Med 1989;321:1092-1099.
25. Starzl TE, Demetris AJ, Van Thiel D. Liver transplantation (1). N Engl J Med 1989;321:1014-1022.

26. Iwatsuki S, Gordon RD, Shaw BW, Jr., Starzl TE. Role of liver transplantation in cancer therapy. Ann Surg 1985;202:401-407.
27. Starzl TE, Marchioro TL, Vonkaulla KN, Hermann G, Brittain RS, Waddell WR. Homotransplantation Of The Liver In Humans. Surg Gynecol Obstet 1963;117:659-676.
28. Alessiani M, Tzakis A, Todo S, Demetris AJ, Fung JJ, Starzl TE. Assessment of five-year experience with abdominal organ cluster transplantation. J Am Coll Surg 1995;180:1-9.
29. Starzl TE, Todo S, Tzakis A, Podesta L, Mieles L, Demetris A, Teperman L, et al. Abdominal organ cluster transplantation for the treatment of upper abdominal malignancies. Ann Surg 1989;210:374-385; discussion 385-376.
30. Starzl TE, Gordon RD, Tzakis A, Staschak S, Fioravanti V, Broznick B, Makowka L, et al. Equitable allocation of extrarenal organs: with special reference to the liver. Transplant Proc 1988;20:131-138.
31. Gundlach M, Broering D, Topp S, Sterneck M, Rogiers X. Split-cava technique: liver splitting for two adult recipients. Liver Transpl 2000;6:703-706.
32. Humar A, Ramcharan T, Sielaff TD, Kandaswamy R, Gruessner RW, Lake JR, Payne WD. Split liver transplantation for two adult recipients: an initial experience. Am J Transplant 2001;1:366-372.
33. Makowka L, Gordon RD, Todo S, Ohkohchi N, Marsh JW, Tzakis AG, Yokoi H, et al. Analysis of donor criteria for the prediction of outcome in clinical liver transplantation. Transplant Proc 1987;19:2378-2382.
34. Busuttil RW, Tanaka K. The utility of marginal donors in liver transplantation. Liver Transpl 2003;9:651-663.
35. Broelsch CE, Whitington PF, Emond JC, Heffron TG, Thistlethwaite JR, Stevens L, Piper J, et al. Liver transplantation in children from living related donors. Surgical techniques and results. Ann Surg 1991;214:428-437; discussion 437-429.
36. Rogiers X, Malago M, Gawad K, Jauch KW, Olausson M, Knoefel WT, Gundlach M, et al. In situ splitting of cadaveric livers. The ultimate expansion of a limited donor pool. Ann Surg 1996;224:331-339; discussion 339-341.

37. Marcos A, Olzinski AT, Ham JM, Fisher RA, Posner MP. The interrelationship between portal and arterial blood flow after adult to adult living donor liver transplantation. Transplantation 2000;70:1697-1703.
38. Strong RW, Lynch SV, Ong TH, Matsunami H, Koido Y, Balderson GA. Successful liver transplantation from a living donor to her son. N Engl J Med 1990;322:1505-1507.
39. Yamaoka Y, Washida M, Honda K, Tanaka K, Mori K, Shimahara Y, Okamoto S, et al. Liver transplantation using a right lobe graft from a living related donor. Transplantation 1994;57:1127-1130.
40. Marcos A, Fisher RA, Ham JM, Shiffman ML, Sanyal AJ, Luketic VA, Sterling RK, et al. Right lobe living donor liver transplantation. Transplantation 1999;68:798-803.
41. Wachs ME, Bak TE, Karrer FM, Everson GT, Shrestha R, Trouillot TE, Mandell MS, et al. Adult living donor liver transplantation using a right hepatic lobe. Transplantation 1998;66:1313-1316.
42. Malago M, Burdelski M, Broelsch CE. Present and future challenges in living related liver transplantation. Transplant Proc 1999;31:1777-1781.
43. Shneider BL, Emre S. Pediatric liver transplantation: past, present, and future. Liver Transpl 2006;12:511-513.
44. Grewal HP, Shokouh-Amiri MH, Vera S, Stratta R, Bagous W, Gaber AO. Surgical technique for right lobe adult living donor liver transplantation without venovenous bypass or portocaval shunting and with duct-to-duct biliary reconstruction. Ann Surg 2001;233:502-508.
45. Pomfret EA, Pomposelli JJ, Lewis WD, Gordon FD, Burns DL, Lally A, Raptopoulos V, et al. Live donor adult liver transplantation using right lobe grafts: donor evaluation and surgical outcome. Arch Surg 2001;136:425-433.
46. Tanaka K. Progress and future in living donor liver transplantation. Keio J Med 2003;52:73-79.
47. Kawasaki S, Makuuchi M, Matsunami H, Hashikura Y, Ikegami T, Nakazawa Y, Chisuwa H, et al. Living related liver transplantation in adults. Ann Surg 1998;227:269-274.

48. Ghobrial RM, Busuttil RW. Future of adult living donor liver transplantation. Liver Transpl 2003;9:S73-79.
49. Bailey SM, Reinke LA. Antioxidants and gadolinium chloride attenuate hepatic parenchymal and endothelial cell injury induced by low flow ischemia and reperfusion in perfused rat livers. Free Radic Res 2000;32:497-506.
50. Bartels M, Biesalski HK, Engelhart K, Sendlhofer G, Rehak P, Nagel E. Pilot study on the effect of parenteral vitamin E on ischemia and reperfusion induced liver injury: a double blind, randomized, placebo-controlled trial. Clin Nutr 2004;23:1360-1370.
51. Giakoustidis D, Papageorgiou G, Iliadis S, Kontos N, Kostopoulou E, Papachrestou A, Tsantilas D, et al. Intramuscular administration of very high dose of alpha-tocopherol protects liver from severe ischemia/reperfusion injury. World J Surg 2002;26:872-877.
52. Rauen U, de Groot H. Cold-induced release of reactive oxygen species as a decisive mediator of hypothermia injury to cultured liver cells. Free Radic Biol Med 1998;24:1316-1323.
53. Rauen U, Polzar B, Stephan H, Mannherz HG, de Groot H. Cold-induced apoptosis in cultured hepatocytes and liver endothelial cells: mediation by reactive oxygen species. Faseb J 1999;13:155-168.
54. de Groot H, Rauen U. Tissue injury by reactive oxygen species and the protective effects of flavonoids. Fundam Clin Pharmacol 1998;12:249-255.
55. Saller R, Meier R, Brignoli R. The use of silymarin in the treatment of liver diseases. Drugs 2001;61:2035-2063.
56. Rauen U, Reuters I, Fuchs A, de Groot H. Oxygen-free radical-mediated injury to cultured rat hepatocytes during cold incubation in preservation solutions. Hepatology 1997;26:351-357.
57. Wheeler MD, Ikejema K, Enomoto N, Stacklewitz RF, Seabra V, Zhong Z, Yin M, et al. Glycine: a new anti-inflammatory immunonutrient. Cell Mol Life Sci 1999;56:843-856.
58. Zhong Z, Jones S, Thurman RG. Glycine minimizes reperfusion injury in a low-flow, reflow liver perfusion model in the rat. Am J Physiol 1996;270:G332-338.
59. Fausto N. Liver regeneration. J Hepatol 2000;32:19-31.

60. Bedirli A, Kerem M, Pasaoglu H, Erdem O, Ofluoglu E, Sakrak O. Effects of ischemic preconditioning on regenerative capacity of hepatocyte in the ischemically damaged rat livers. J Surg Res 2005;125:42-48.
61. Eipel C, Glanemann M, Nuessler AK, Menger MD, Neuhaus P, Vollmar B. Ischemic preconditioning impairs liver regeneration in extended reduced-size livers. Ann Surg 2005;241:477-484.
62. Phaneuf D, Chen SJ, Wilson JM. Intravenous injection of an adenovirus encoding hepatocyte growth factor results in liver growth and has a protective effect against apoptosis. Mol Med 2000;6:96-103.
63. Francavilla A, Carr BI, Azzarone A, Polimeno L, Wang Z, Van Thiel DH, Subbotin V, et al. Hepatocyte proliferation and gene expression induced by triiodothyronine in vivo and in vitro. Hepatology 1994;20:1237-1241.
64. Malik R, Habib M, Tootle R, Hodgson H. Exogenous thyroid hormone induces liver enlargement, whilst maintaining regenerative potential--a study relevant to donor preconditioning. Am J Transplant 2005;5:1801-1807.
65. Redaelli CA, Wagner M, Krahenbuhl L, Gloor B, Schilling MK, Dufour JF, Buchler MW. Liver surgery in the era of tissue-preserving resections: early and late outcome in patients with primary and secondary hepatic tumors. World J Surg 2002;26:1126-1132.
66. Shoup M, Gonen M, D'Angelica M, Jarnagin WR, DeMatteo RP, Schwartz LH, Tuorto S, et al. Volumetric analysis predicts hepatic dysfunction in patients undergoing major liver resection. J Gastrointest Surg 2003;7:325-330.
67. Liu CL, Zhao Y, Lo CM, Fan ST. Hepatic venoplasty in right lobe live donor liver transplantation. Liver Transpl 2003;9:1265-1272.
68. Malago M, Testa G, Frilling A, Nadalin S, Valentin-Gamazo C, Paul A, Lang H, et al. Right living donor liver transplantation: an option for adult patients: single institution experience with 74 patients. Ann Surg 2003;238:853-862; discussion 862-853.
69. Nakamura T, Tanaka K, Kiuchi T, Kasahara M, Oike F, Ueda M, Kaihara S, et al. Anatomical variations and surgical strategies in right lobe living donor liver transplantation: lessons from 120 cases. Transplantation 2002;73:1896-1903.

70. Povoski SP, Fong Y, Blumgart LH. Extended left hepatectomy. World J Surg 1999;23:1289-1293.
71. Vauthey JN, Pawlik TM, Abdalla EK, Arens JF, Nemr RA, Wei SH, Kennamer DL, et al. Is extended hepatectomy for hepatobiliary malignancy justified? Ann Surg 2004;239:722-730; discussion 730-722.
72. Yigitler C, Farges O, Kianmanesh R, Regimbeau JM, Abdalla EK, Belghiti J. The small remnant liver after major liver resection: how common and how relevant? Liver Transpl 2003;9:S18-25.
73. Chen MF, Jeng LB. Partial hepatic resection for hepatocellular carcinoma. J Gastroenterol Hepatol 1997;12:S329-334.
74. Kassahun WT, Fangmann J, Harms J, Hauss J, Bartels M. Liver resection and transplantation in the management of hepatocellular carcinoma: a review. Exp Clin Transplant 2006;4:549-558.
75. Kountouras J, Boura P, Lygidakis NJ. Liver regeneration after hepatectomy. Hepatogastroenterology 2001;48:556-562.
76. Tian Y, Graf R, Jochum W, Clavien PA. Arterialized partial orthotopic liver transplantation in the mouse: a new model and evaluation of the critical liver mass. Liver Transpl 2003;9:789-795.
77. Tian Y, Jochum W, Georgiev P, Moritz W, Graf R, Clavien PA. Kupffer cell-dependent TNF-alpha signaling mediates injury in the arterialized small-for-size liver transplantation in the mouse. Proc Natl Acad Sci U S A 2006;103:4598-4603.
78. Dahm F, Georgiev P, Clavien PA. Small-for-size syndrome after partial liver transplantation: definition, mechanisms of disease and clinical implications. Am J Transplant 2005;5:2605-2610.
79. Zhong Z, Schwabe RF, Kai Y, He L, Yang L, Bunzendahl H, Brenner DA, et al. Liver regeneration is suppressed in small-for-size liver grafts after transplantation: involvement of c-Jun N-terminal kinase, cyclin D1, and defective energy supply. Transplantation 2006;82:241-250.
80. Moreno Gonzalez E, Meneu Diaz JC, Garcia Garcia I, Loinaz Segurola C, Jimenez C, Gomez R, Abradelo M, et al. Live liver donation: a prospective

analysis of exclusion criteria for healthy and potential donors. Transplant Proc 2003;35:1787-1790.

81. Pacheco-Moreira LF, Enne M, Balbi E, Halpern M, Peixoto A, Cerqueira A, Moreira E, et al. Selection of donors for living donor liver transplantation in a single center of a developing country: lessons learned from the first 100 cases. Pediatr Transplant 2006;10:311-315.

82. Yokoi H, Isaji S, Yamagiwa K, Tabata M, Nemoto A, Sakurai H, Usui M, et al. The role of living-donor liver transplantation in surgical treatment for hepatocellular carcinoma. J Hepatobiliary Pancreat Surg 2006;13:123-130.

83. Bockhorn M, Goralski M, Prokofiev D, Dammann P, Grunewald P, Trippler M, Biglarnia A, et al. VEGF is important for early liver regeneration after partial hepatectomy. J Surg Res 2007;138:291-299.

84. Bockhorn M, Schollmann S, Opitz B, Sotiropoulos GC, Sheu SY, Niehaus E, Trippler M, et al. Vascular endothelial growth factor does not improve liver regeneration and survival after 90% subtotal liver resection. Hepatol Res 2007;37:353-359.

85. Florman S, Miller CM. Live donor liver transplantation. Liver Transpl 2006;12:499-510.

86. Lechler RI, Sykes M, Thomson AW, Turka LA. Organ transplantation--how much of the promise has been realized? Nat Med 2005;11:605-613.

87. Chijiiwa K, Saiki S, Tanaka M. Serum interleukin-6 and hepatocyte growth factor levels in patients after hepatectomy. Hepatogastroenterology 2002;49:467-471.

88. Fausto N, Campbell JS, Riehle KJ. Liver regeneration. Hepatology 2006;43:S45-53.

89. Fausto N, Laird AD, Webber EM. Liver regeneration. 2. Role of growth factors and cytokines in hepatic regeneration. Faseb J 1995;9:1527-1536.

90. Pahlavan PS, Feldmann RE, Jr., Zavos C, Kountouras J. Prometheus' challenge: molecular, cellular and systemic aspects of liver regeneration. J Surg Res 2006;134:238-251.

91. Qin SW, Zhao LF, Chen XG, Xu CS. Expression pattern and action analysis of genes associated with the responses to chemical stimuli during rat liver regeneration. World J Gastroenterol 2006;12:7285-7291.
92. Coelho MC, Tannuri U, Tannuri AC, Mello ES, dos Santos NA. Expression of interleukin 6 and apoptosis-related genes in suckling and weaning rat models of hepatectomy and liver regeneration. J Pediatr Surg 2007;42:613-619.
93. Luedde T, Assmus U, Wustefeld T, Meyer zu Vilsendorf A, Roskams T, Schmidt-Supprian M, Rajewsky K, et al. Deletion of IKK2 in hepatocytes does not sensitize these cells to TNF-induced apoptosis but protects from ischemia/reperfusion injury. J Clin Invest 2005;115:849-859.
94. Yuceturk H, Yagmurdur MC, Gur G, Demirbilek M, Bilezikci B, Turan M, Karakayali H, et al. Role of heparin on TNF-alpha and IL-6 levels in liver regeneration after partial hepatic resection. Eur Surg Res 2007;39:216-221.
95. Higgins GM, Anderson RM. Experimental pathology of the liver 1: Restoration of the liver of the white rat following partial surgical removal. Arch Pathol 1931;12:186-202.
96. Malik R, Mellor N, Selden C, Hodgson H. Triiodothyronine enhances the regenerative capacity of the liver following partial hepatectomy. Hepatology 2003;37:79-86.
97. Gerdes J, Schwab U, Lemke H, Stein H. Production of a mouse monoclonal antibody reactive with a human nuclear antigen associated with cell proliferation. Int J Cancer 1983;31:13-20.
98. Schlaak JF, Hilkens CM, Costa-Pereira AP, Strobl B, Aberger F, Frischauf AM, Kerr IM. Cell-type and donor-specific transcriptional responses to interferon-alpha. Use of customized gene arrays. J Biol Chem 2002;277:49428-49437.
99. Emond J, Capron-Laudereau M, Meriggi F, Bernuau J, Reynes M, Houssin D. Extent of hepatectomy in the rat. Evaluation of basal conditions and effect of therapy. Eur Surg Res 1989;21:251-259.
100. Tygstrup N. Determination of the hepatic elimination capacity (Lm) of galactose by single injection. Scand J Clin Lab Invest Suppl 1966;18:118-125.
101. Pfaffl MW. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res 2001;29:e45.

102. Schemmer P, Enomoto N, Bradford BU, Bunzendahl H, Raleigh JA, Lemasters JJ, Thurman RG. Activated Kupffer cells cause a hypermetabolic state after gentle in situ manipulation of liver in rats. Am J Physiol Gastrointest Liver Physiol 2001;280:G1076-1082.
103. Schemmer P, Schoonhoven R, Swenberg JA, Bunzendahl H, Raleigh JA, Lemasters JJ, Thurman RG. Gentle organ manipulation during harvest as a key determinant of survival of fatty livers after transplantation in the rat. Transpl Int 1999;12:351-359.
104. Schemmer P, Schoonhoven R, Swenberg JA, Bunzendahl H, Thurman RG. Gentle in situ liver manipulation during organ harvest decreases survival after rat liver transplantation: role of Kupffer cells. Transplantation 1998;65:1015-1020.
105. Marcos A. Split-liver transplantation for adult recipients. Liver Transpl 2000;6:707-709.
106. Sato Y, Tsukada K, Hatakeyama K. Role of shear stress and immune responses in liver regeneration after a partial hepatectomy. Surg Today 1999;29:1-9.
107. Troisi R, de Hemptinne B. Clinical relevance of adapting portal vein flow in living donor liver transplantation in adult patients. Liver Transpl 2003;9:S36-41.
108. Carini R, Bellomo G, Grazia De Cesaris M, Albano E. Glycine protects against hepatocyte killing by KCN or hypoxia by preventing intracellular Na+ overload in the rat. Hepatology 1997;26:107-112.
109. Dong Z, Patel Y, Saikumar P, Weinberg JM, Venkatachalam MA. Development of porous defects in plasma membranes of adenosine triphosphate-depleted Madin-Darby canine kidney cells and its inhibition by glycine. Lab Invest 1998;78:657-668.
110. Frank A, Rauen U, de Groot H. Protection by glycine against hypoxic injury of rat hepatocytes: inhibition of ion fluxes through nonspecific leaks. J Hepatol 2000;32:58-66.
111. Nishimura Y, Lemasters JJ. Glycine blocks opening of a death channel in cultured hepatic sinusoidal endothelial cells during chemical hypoxia. Cell Death Differ 2001;8:850-858.
112. Weinberg JM. The effect of amino acids on ischemic and toxic injury to the kidney. Semin Nephrol 1990;10:491-500.

113. Zhang K, Weinberg JM, Venkatachalam MA, Dong Z. Glycine protection of PC-12 cells against injury by ATP-depletion. Neurochem Res 2003;28:893-901.
114. Schemmer P, Bradford BU, Rose ML, Bunzendahl H, Raleigh JA, Lemasters JJ, Thurman RG. Intravenous glycine improves survival in rat liver transplantation. Am J Physiol 1999;276:G924-932.
115. Zhong Z, Wheeler MD, Li X, Froh M, Schemmer P, Yin M, Bunzendaul H, et al. L-Glycine: a novel antiinflammatory, immunomodulatory, and cytoprotective agent. Curr Opin Clin Nutr Metab Care 2003;6:229-240.
116. Schemmer P, Mehrabi A, Kraus T, Sauer P, Gutt C, Uhl W, Buchler MW. New aspects on reperfusion injury to liver--impact of organ harvest. Nephrol Dial Transplant 2004;19 Suppl 4:iv26-35.
117. Ikejima K, Qu W, Stachlewitz RF, Thurman RG. Kupffer cells contain a glycine-gated chloride channel. Am J Physiol 1997;272:G1581-1586.
118. Fandrey J, Gorr TA, Gassmann M. Regulating cellular oxygen sensing by hydroxylation. Cardiovasc Res 2006;71:642-651.
119. Mole DR, Ratcliffe PJ. Cellular oxygen sensing in health and disease. Pediatr Nephrol 2008;23:681-694.
120. Semenza GL. Regulation of mammalian O2 homeostasis by hypoxia-inducible factor 1. Annu Rev Cell Dev Biol 1999;15:551-578.
121. Wenger RH. Cellular adaptation to hypoxia: O2-sensing protein hydroxylases, hypoxia-inducible transcription factors, and O2-regulated gene expression. Faseb J 2002;16:1151-1162.
122. Frede S, Berchner-Pfannschmidt U, Fandrey J. Regulation of hypoxia-inducible factors during inflammation. Methods Enzymol 2007;435:405-419.
123. Zhou J, Brune B. Cytokines and hormones in the regulation of hypoxia inducible factor-1alpha (HIF-1alpha). Cardiovasc Hematol Agents Med Chem 2006;4:189-197.
124. Milkiewicz M, Doyle JL, Fudalewski T, Ispanovic E, Aghasi M, Haas TL. HIF-1alpha and HIF-2alpha play a central role in stretch-induced but not shear-stress-induced angiogenesis in rat skeletal muscle. J Physiol 2007;583:753-766.
125. Milkiewicz M, Haas TL. Effect of mechanical stretch on HIF-1{alpha} and MMP-2 expression in capillaries isolated from overloaded skeletal muscles:

laser capture microdissection study. Am J Physiol Heart Circ Physiol 2005;289:H1315-1320.

126. Selzner N, Selzner M, Tian Y, Kadry Z, Clavien PA. Cold ischemia decreases liver regeneration after partial liver transplantation in the rat: A TNF-alpha/IL-6-dependent mechanism. Hepatology 2002;36:812-818.
127. Hagymasi K, Kocsis I, Lugasi A, Feher J, Blazovics A. Extrahepatic biliary obstruction: can silymarin protect liver function? Phytother Res 2002;16 Suppl 1:S78-80.
128. Halliwell B, Gutteridge JMC. Free radicals in biology and medicine. 1999:200-219.
129. Dehmlow C, Erhard J, de Groot H. Inhibition of Kupffer cell functions as an explanation for the hepatoprotective properties of silibinin. Hepatology 1996;23:749-754.
130. Dehmlow C, Murawski N, de Groot H. Scavenging of reactive oxygen species and inhibition of arachidonic acid metabolism by silibinin in human cells. Life Sci 1996;58:1591-1600.
131. Taniguchi E, Sakisaka S, Matsuo K, Tanikawa K, Sata M. Expression and role of vascular endothelial growth factor in liver regeneration after partial hepatectomy in rats. J Histochem Cytochem 2001;49:121-130.
132. Pibiri M, Ledda-Columbano GM, Cossu C, Simbula G, Menegazzi M, Shinozuka H, Columbano A. Cyclin D1 is an early target in hepatocyte proliferation induced by thyroid hormone (T3). Faseb J 2001;15:1006-1013.
133. LeCouter J, Moritz DR, Li B, Phillips GL, Liang XH, Gerber HP, Hillan KJ, et al. Angiogenesis-independent endothelial protection of liver: role of VEGFR-1. Science 2003;299:890-893.
134. Markiewski MM, Mastellos D, Tudoran R, DeAngelis RA, Strey CW, Franchini S, Wetsel RA, et al. C3a and C3b activation products of the third component of complement (C3) are critical for normal liver recovery after toxic injury. J Immunol 2004;173:747-754.
135. Skrtic S, Carlsson L, Ljungberg A, Linden D, Michalik L, Wahli W, Oscarsson J. Decreased expression of peroxisome proliferator-activated receptor alpha and

liver fatty acid binding protein after partial hepatectomy of rats and mice. Liver Int 2005;25:33-40.

136. Court FG, Wemyss-Holden SA, Dennison AR, Maddern GJ. The mystery of liver regeneration. Br J Surg 2002;89:1089-1095.

137. Michalopoulos GK, DeFrances M. Liver regeneration. Adv Biochem Eng Biotechnol 2005;93:101-134.

138. Zimmermann A. Liver regeneration: the emergence of new pathways. Med Sci Monit 2002;8:RA53-63.

139. Lo CM, Fan ST, Liu CL, Chan JK, Lam BK, Lau GK, Wei WI, et al. Minimum graft size for successful living donor liver transplantation. Transplantation 1999;68:1112-1116.

140. Maema A, Imamura H, Takayama T, Sano K, Hui AM, Sugawara Y, Makuuchi M. Impaired volume regeneration of split livers with partial venous disruption: a latent problem in partial liver transplantation. Transplantation 2002;73:765-769.

141. Ku Y, Tominaga M, Sugimoto T, Iwasaki T, Fukumoto T, Takahashi T, Suzuki Y, et al. Preoperative hepatic venous embolization for partial hepatectomy combined with segmental resection of major hepatic vein. Br J Surg 2002;89:63-69.

142. Lang H, Sotiropoulos GC, Brokalaki EI, Radtke A, Frilling A, Molmenti EP, Malago M, et al. Left hepatic trisectionectomy for hepatobiliary malignancies. J Am Coll Surg 2006;203:311-321.

143. Fausto N. Growth factors in liver development, regeneration and carcinogenesis. Prog Growth Factor Res 1991;3:219-234.

144. Lowes KN, Croager EJ, Olynyk JK, Abraham LJ, Yeoh GC. Oval cell-mediated liver regeneration: Role of cytokines and growth factors. J Gastroenterol Hepatol 2003;18:4-12.

145. Mangnall D, Bird NC, Majeed AW. The molecular physiology of liver regeneration following partial hepatectomy. Liver Int 2003;23:124-138.

146. Yang WJ, Zhang QY, Yu ZP, Song QT, Liang HP, Xu X, Zhu GB, et al. Effects of nuclear factor-kappaB on rat hepatocyte regeneration and apoptosis after 70% portal branch ligation. World J Gastroenterol 2005;11:6775-6779.

147. Campbell JS, Riehle KJ, Brooling JT, Bauer RL, Mitchell C, Fausto N. Proinflammatory cytokine production in liver regeneration is Myd88-dependent, but independent of Cd14, Tlr2, and Tlr4. J Immunol 2006;176:2522-2528.
148. McAllister-Lucas LM, Ruland J, Siu K, Jin X, Gu S, Kim DS, Kuffa P, et al. CARMA3/Bcl10/MALT1-dependent NF-kappaB activation mediates angiotensin II-responsive inflammatory signaling in nonimmune cells. Proc Natl Acad Sci U S A 2007;104:139-144.