5. RESULTS

5.1 Hepatocyte proliferation (mitotic index, Ki-67 positive cells, PCNA)

Following an initial marked increase of hepatocyte mitosis on days 1 and 2 after surgery, the number of mitotic cells clearly decreased at day 7 following partial hepatectomy (Figure 8) with significantly higher values for both treated groups up to the second postoperative day compared to the control group ($p<0.05$). In contrast to this, the control animals showed an almost similar mitotic index on postoperative day 7.

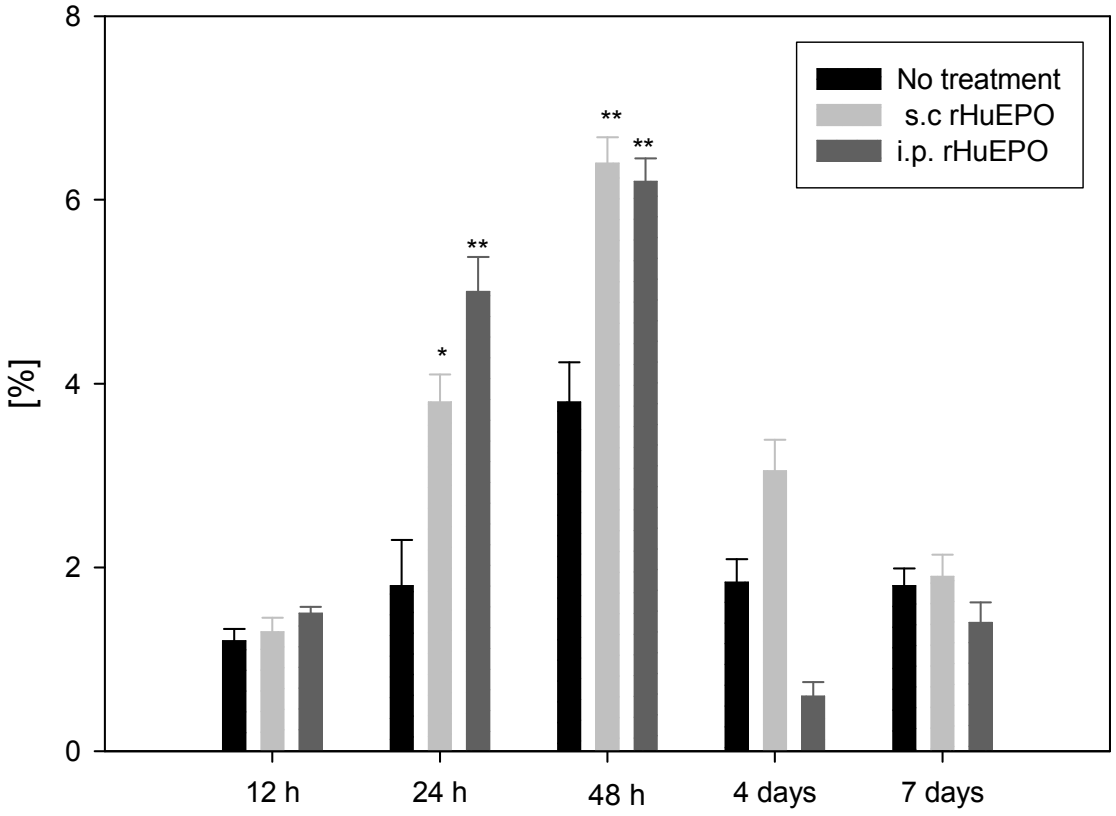

Figure 8.

The mitotic index in the H&E staining was significantly enhanced in rats receiving intraportal venous (i.p.) or subcutaneous (s.c.) rHuEPO treatment compared to untreated controls 24h and 48 hours after hepatectomy. (* = p<0.05; ** = p<0.01)

The highest percentage of Ki-67 positive cells was found on day 2 after surgery in the groups of rats with subcutaneous (group 2) and intraportalvenous (group 3) rHuEPO injection ($p<0.05$) compared to controls. However, group 3 with intraportalvenous administration displayed a significant increase in Ki-67 expression as early as 24 hours after surgery, whereas Ki-67 values remained equally low for subcutaneously treated animals and controls at this point of time. The percentage of Ki-67 positive cells continuously decreased in all groups during the investigated time period and was similar in all groups on days 4 and 7 (Figures 9, 10).

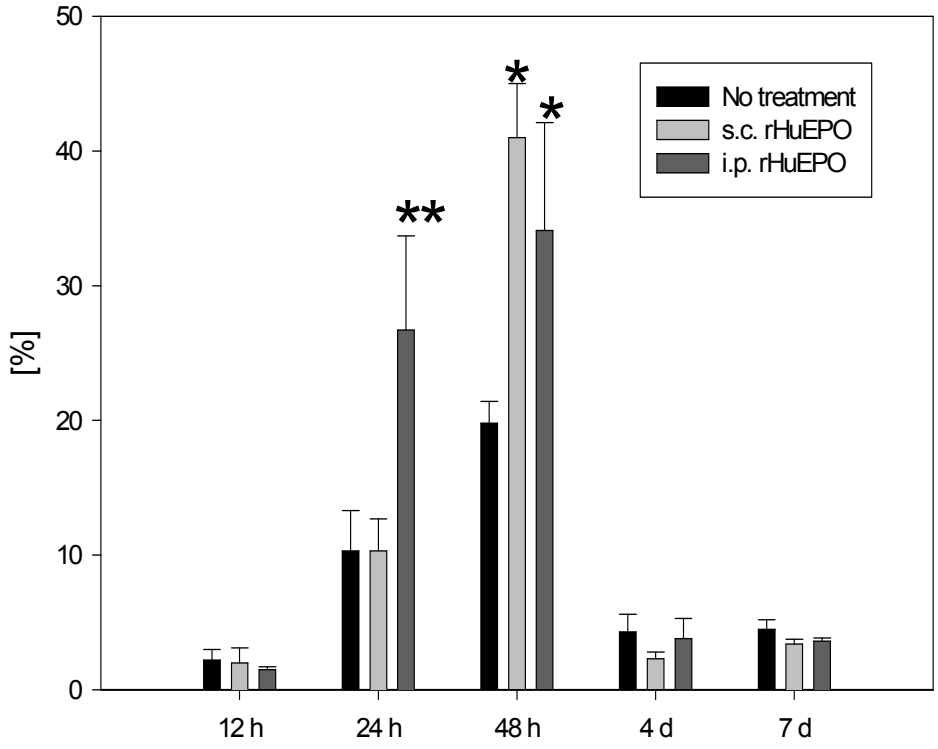

Figure 9.

Number of Ki-67 positive cells significantly increased after rHuEPO administration in both treatment groups (*=<0.05). (intraportalvenous = i.p., subcutaneous = s.c.)

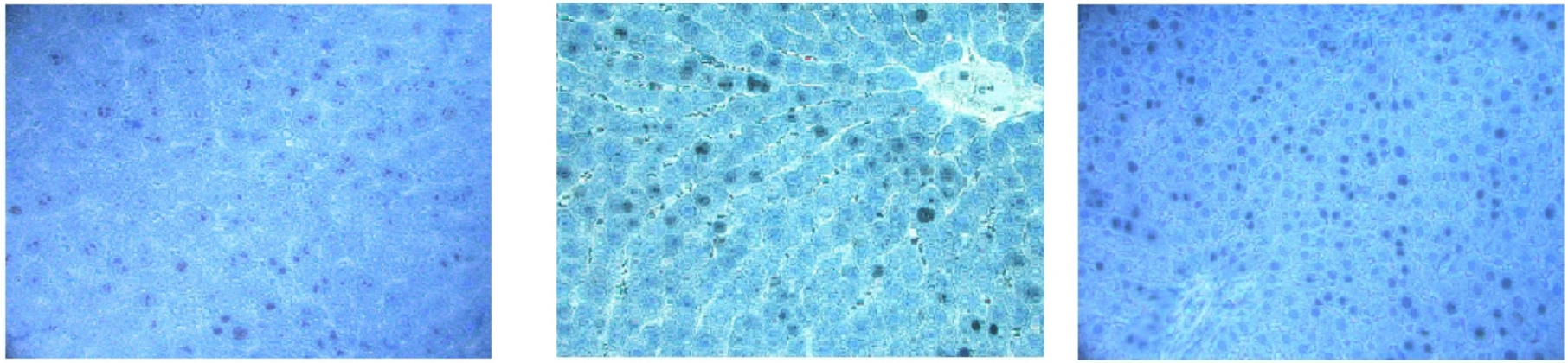

Figure 10. Ki-67 positive cells 48 hours after 70% liver resection in controls (left); s.c. rHuEPO treatment (middle); and intraportal venous (right) rHuEPO treatment (photographed with blue colour filter for better discrimination). (Ki-67 positive cells marked by "*", magnification 200 x each)

PCNA labeling confirmed the data of the Ki-67 staining with a significantly increased number of positive cells in rats treated with rHuEPO i.p. 12, 24 and 48 hours after surgery; for the s.c. treated animals PCNA labelled cells displayed a less impressive increase with significance only on day 1 (Figures 11, 12). Astonishingly, after a general decrease of PCNA positive cells on day 4 a non-significant increase could be observed on day 7 with emphasis on controls and s.c. treated rats.

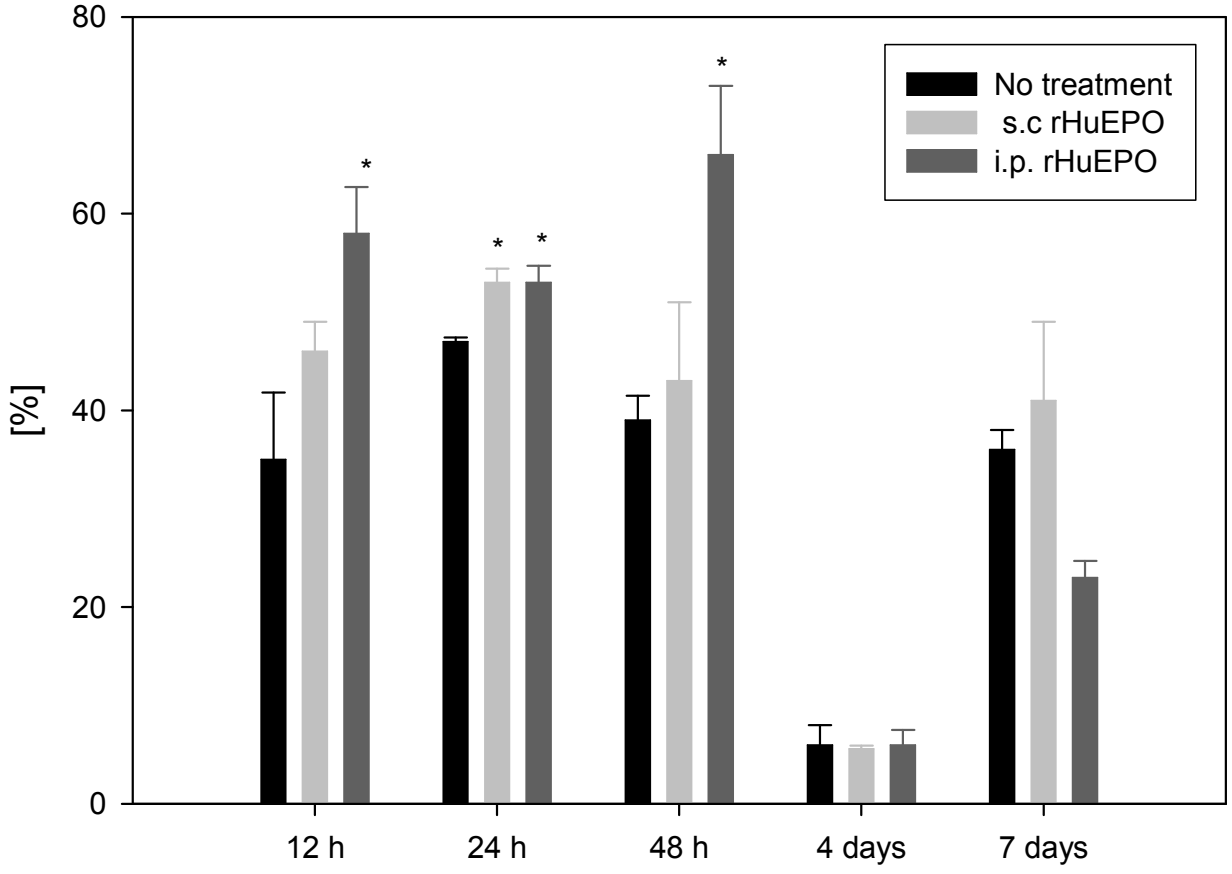

Figure 11.

PCNA staining confirmed the increased regeneration in the rHuEPO groups (*=<0.05) with significantly increased PCNA positive cells after 12h, 24h and 48h. (intraportal vein = i.p. subcutaneous = s.c.)

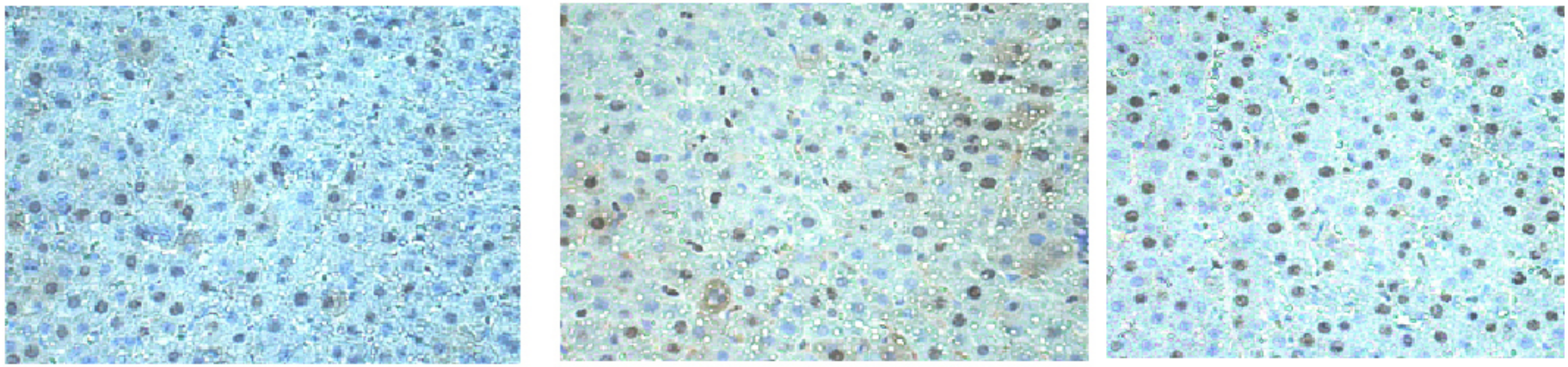

Figure 12. PCNA positive cells 24 hours after liver resection in controls (left); s.c. rHuEPO treatment (middle), and intraportal venous (right) rHuEPO treatment. (positive cells marked by “*”, magnification 200 x each)

5.2 Hypoxia induced factor

HIF (hypoxia induced factor) mRNA expression was significantly higher among control animals 3 hours after partial liver resection than in EPO treated rats (p= 0.048); both groups displayed equal amounts of HIF mRNA 6 hours after surgery (Figure 13).

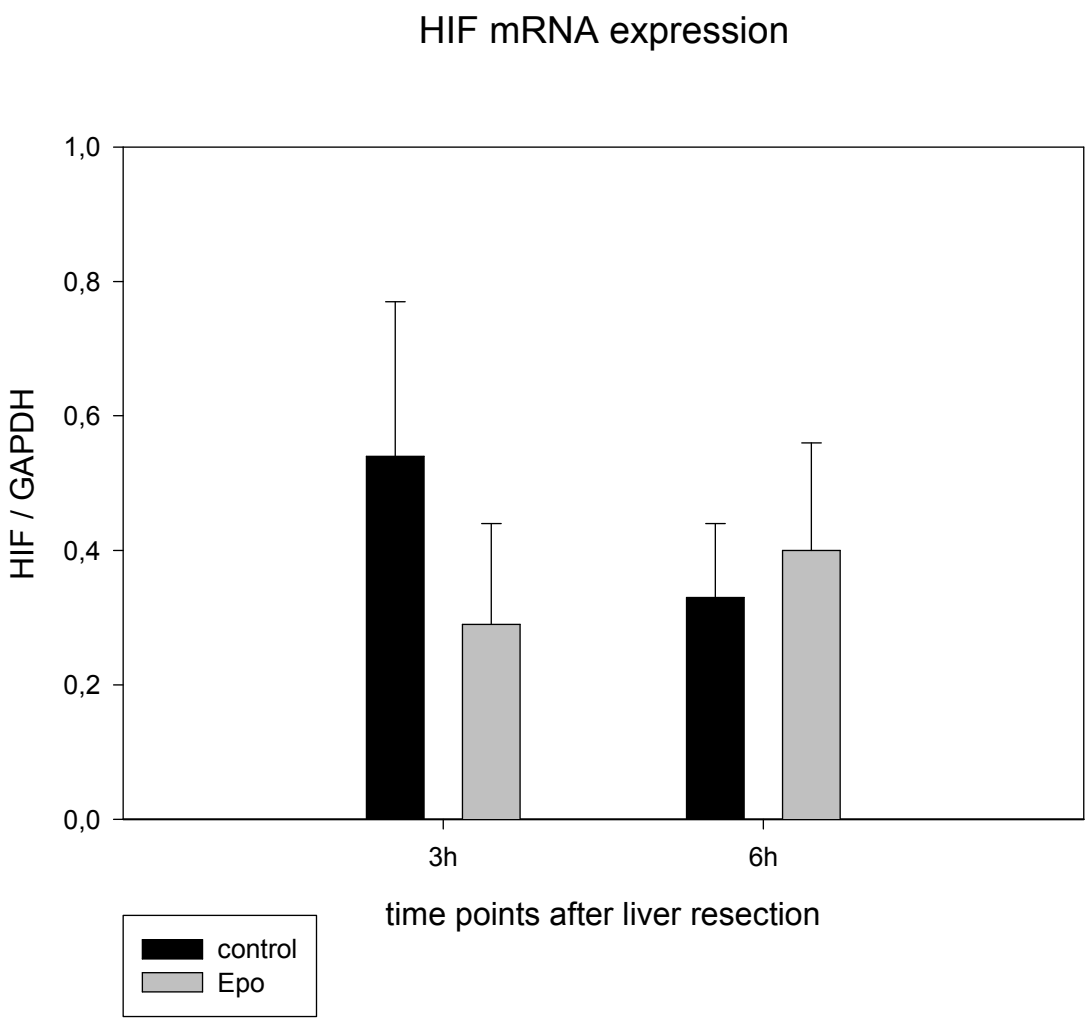

Figure 13.

HIF mRNA expression 3 and 6 hours after partial liver resection in hepatic tissue (RT-PCR) (Epo = intraportalvenous application; 4,000 IU /kg); * p< 0.05

5.3 Transforming growth factor β

TGF-ß (transforming growth factor beta) mRNA was expressed slightly more intensively in the control group 3 hours after resection, yet this did not reach statistical significance. 6 hours post intervention this difference vanished (Figure 14).

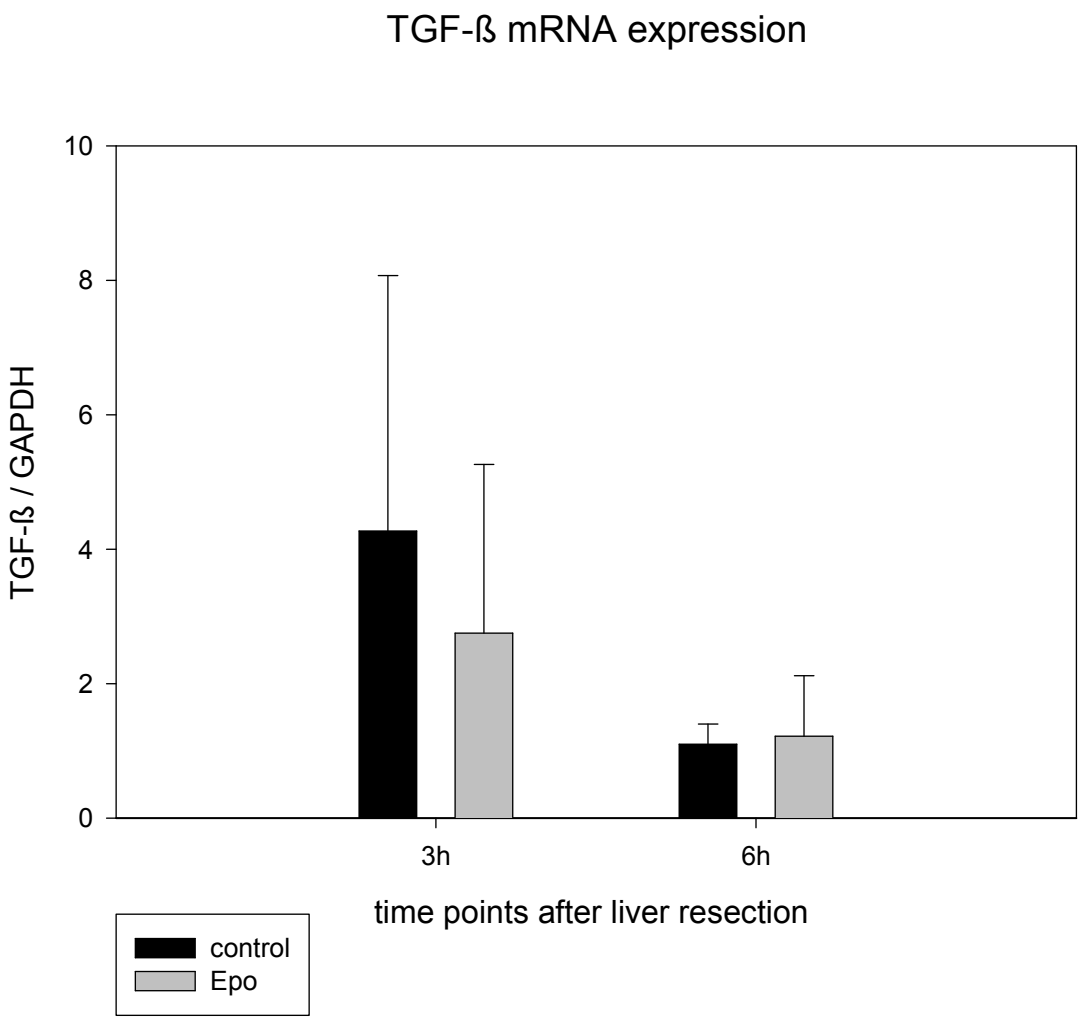

Figure 14.

TGF-ß mRNA expression 3 and 6 hours after partial liver resection in hepatic tissue (RT-PCR); (Epo = intraportalvenous application; 4,000 IU /kg)

5.4 Signal transducing activator 3

Differences in STAT-3 mRNA expression between groups could NOT be shown at any time point (Figure 15).

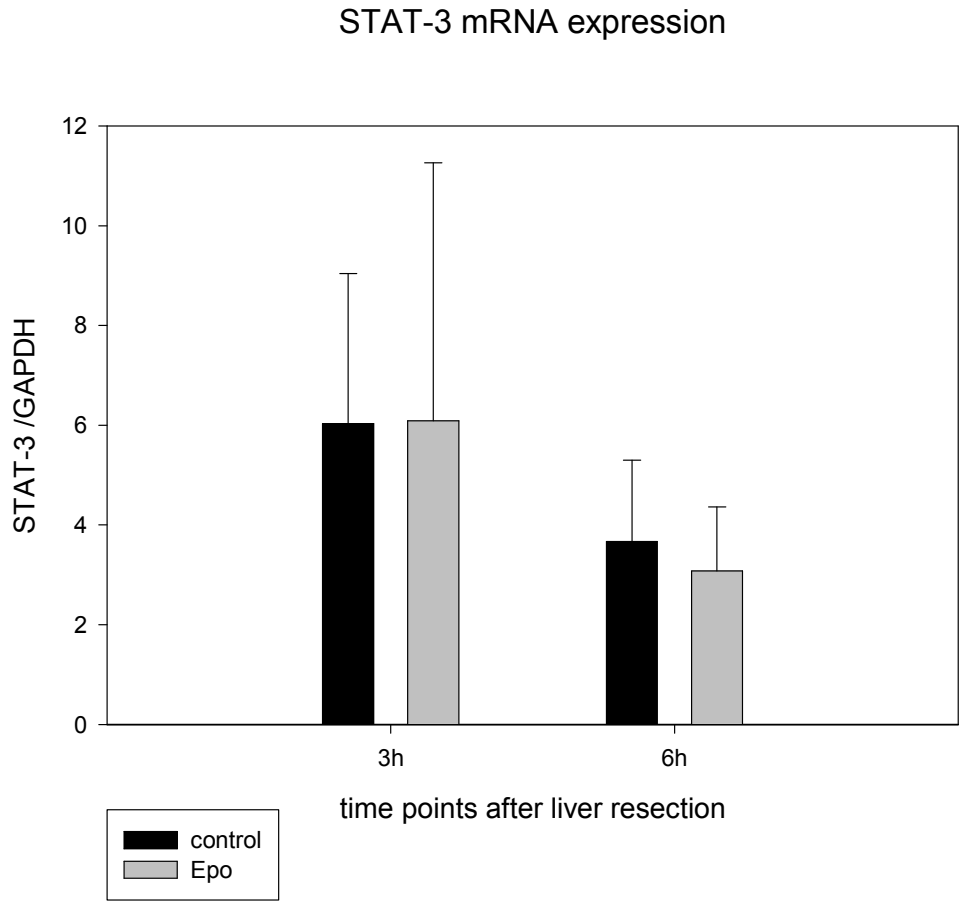

Figure 15.

STAT-3 mRNA expression 3 and 6 hours after partial liver resection in hepatic tissue (RT-PCR); (Epo = intraportalvenous application; 4,000 IU /kg)

5.5 Vascular endothelial growth factor

VEGF mRNA was measured 24 hours after liver surgery displaying considerable activation compared to controls, yet there were no detectable difference between animals treated with intraportalvenous Epo injection compared to subcutaneous application (figure 16).

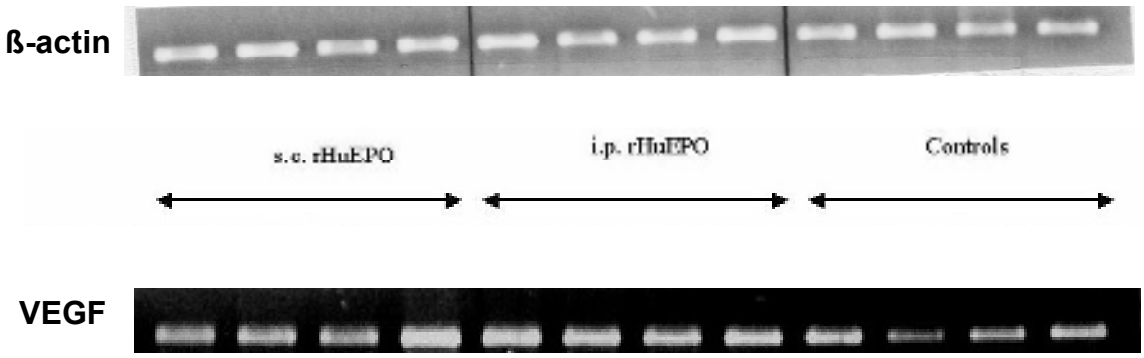

Figure 16.

a) RT-PCR showed increased expression of VEGF 24h after liver regeneration in both treatment groups. (Intraportalvenous = i.p.; Subcutaneous = s.c.) ß-actin mRNA expression served as internal control.

5.6 Survival data

After 90% hepatectomy, none of the control animals survived more than 10 days, however, four of the ten rHuEPO intraportal treated rats survived the full observation time of 10 days ($p<0.05$) (Figure 17).

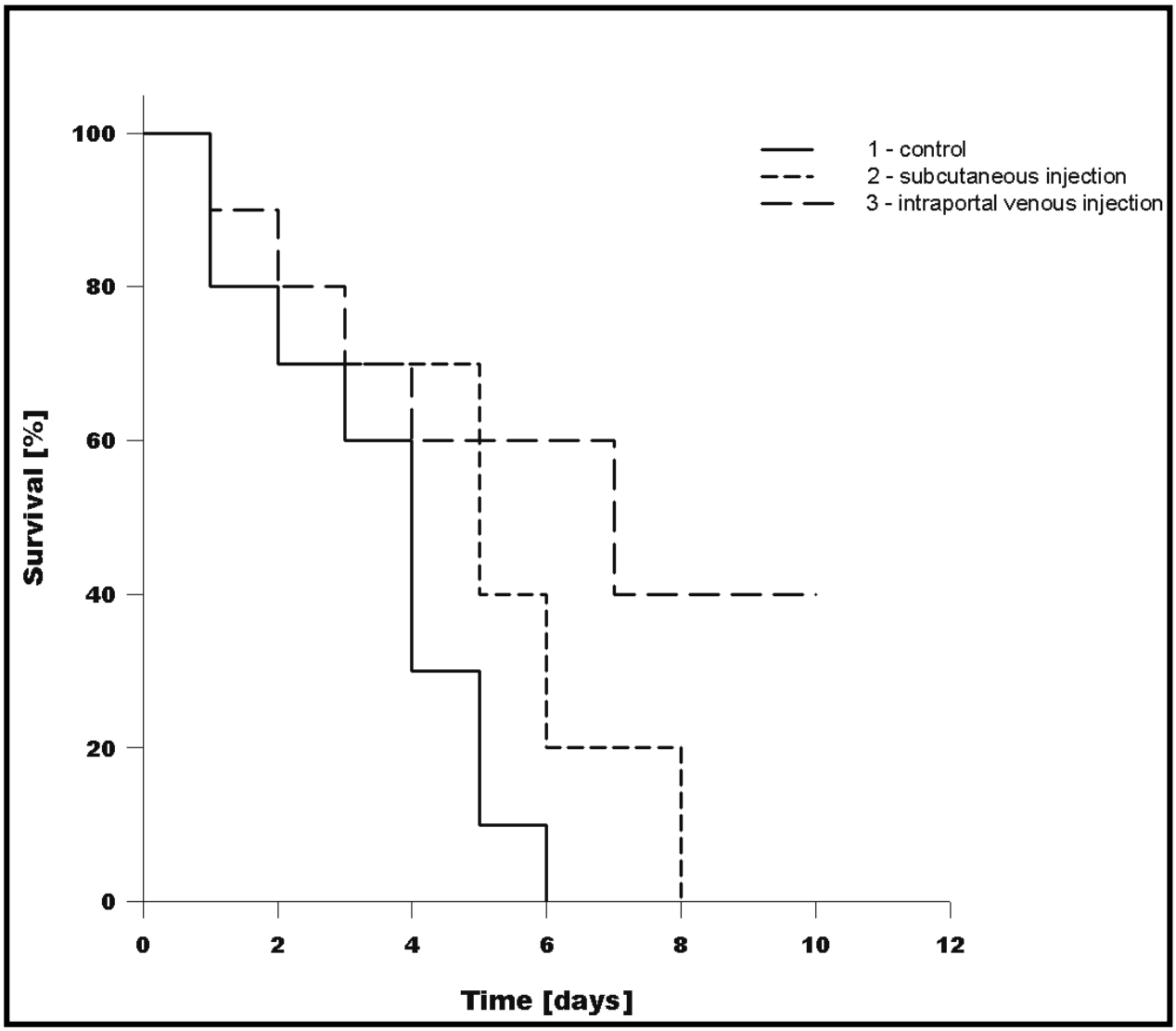

Figure 17.

Overall survival data after 90% liver resection (log-rank). Intraportalvenous rHuEPO 4000 U/kg showed improved overall survival 10 days after 90% liver resection ($p< 0.05$). In the subcutaneous rHuEPO group there was no significant improved survival 10 days after 90% hepatectomy.

6. DISCUSSION.

Higgins and Anderson (6) published the earliest studies on regeneration in 1931, when they noted the remarkable capacity of the rat liver to regenerate after partial hepatectomy. This model of 70% hepatic resection in rats was used extensively in the investigation of hepatic regeneration. Much work has subsequently been performed to investigate the factors that trigger the initiation of regeneration. In the case of surgical resection of rat liver, the process of regeneration begins within hours and is completed after 7-10 days. The liver adapts almost instantly after partial hepatectomy to the resulting increased functional load per cell. Both negative and positive growth factors regulate hepatocyte proliferation, and the ratio between these factors may be more important than their absolute levels (7)

6.1 Stage of liver regeneration

Understanding the molecular mechanism that regulates liver regeneration is of enormous scientific and clinical importance. The analysis of these mechanisms is intimately linked to efforts to understand cell cycle regulation in complex mammalian organs and regulation defects that may cause neoplasia. There is an urgent need for clinical therapies that would enhance regulated hepatocyte replication in acute liver failure, in liver transplantation (i.e.' small-for-size transplants', split transplants, living donor partial hepatectomy and transplants) as well as in cell and gene therapy strategies that require cell proliferation. Most of our knowledge about the molecular mechanisms that regulate hepatic growth derives from experimental studies of liver regeneration induced by either partial hepatectomy (removal of 68% of the liver in rat and slightly less in mice) or acute chemical injury (carbon tetra chloride being the most common agent). There is every reason to believe that the conclusions reached in

studies involving these experimental models are applicable to humans with only small variations. Liver regeneration after partial hepatectomy is a growth response that culminates in hepatocyte replication. This process has several important biological characteristics:

(a) It is a process of compensatory hyperplasia rather than true regeneration in that removed parts do not grow back, instead the liver remnant increases in mass;

(b) The process depends on the replication of differentiated hepatocytes (diploid, tetraploid, even octoploid cells) and does not involve precursor ('stem cell') cells or oval cells;

(c) Replication of hepatocytes proceeds in a synchronous wave and is followed (approximately 1 day later) by replication of non-parenchymal cells;

(d) Growth terminates when liver mass reaches normal values, within about 10% of the original liver mass.

Hepatocytes rarely divide under normal conditions in adult humans or animals. In the adult liver only one in 1000 cells is in mitosis at any given time (70). There are three main phases of liver regeneration. Cells are normally in a resting G0 state. After partial hepatectomy, all hepatic cells simultaneously undergo transition into G1 phase almost immediately. Hepatocytes enter S phase (DNA synthesis) roughly 12-15 h after partial hepatectomy. The G2 phase and mitosis (M phase) follow 6-8 h after DNA synthesis. Non-hepatocyte replication is usually delayed by approximately 24 h as a result of prolongation of their G1 phase (71). During this initial phase of regeneration most hepatocytes will replicate at least once. When the original mass-volume ratio of the liver has been achieved, the hepatocytes revert to their quiescent G0 phase. The rat liver can almost double its size within 48 h of partial hepatectomy. Once the original liver mass is restored, liver regeneration stops abruptly (71).

The majority of hepatocytes are involved in this process, with each dividing at least once. However, this number decreases with increasing age, making regeneration slower and less complete in older animals. Hepatocytes are heterogenous with respect to their gene expression and metabolic function. This heterogeneity is maintained after liver regeneration and may, in part, be attributable to differences in blood flow and nutrient availability within normal hepatic architecture (7). *In vivo,* the periportal hepatocytes are the first to begin replication, initially without any corresponding sinusoids or matrix. Cell clusters develop and replication spreads to the pericentral regions. Hepatocyte replication decreases by day 4 after partial hepatectomy, and thereafter non-parenchymal cells begin to recreate normal lobular hepatic architecture by dividing cell clusters into cell plates surrounded by vascular spaces, sinusoids and spaces of Disse. The restoration of this complex microarchitecture may well exert some influence on the eventual arrest of the regenerative process (7). During cell replication, hepatocytes do not appear to dedifferentiate, and are capable of continuing their metabolic and synthetic function (72). The regenerating liver has little reserve capacity and does not efficiently remove excess amino acids or ammonia from the blood. Therefore there is a minimum volume of residual hepatocytes necessary if homoeostasis is to be maintained during replication.

The remaining functional liver volume limits liver regeneration, because the liver must be capable of maintaining its normal metabolic functions at the same time as undergoing cell division. The minimal volume liver remnant compatible with functional viability after liver resection has been the subject of much debate and several studies. Patients with greater than 80% liver cell mass damage due to drugs or a virus undergo complete liver regeneration if they survive the insult. Operations in the rat model involving a reduction in liver mass greater than 80% are associated with a reduced rate

of liver regeneration compared with the 70% resection. In fulminant hepatic failure a hepatocyte volume fraction of less than 12% is associated with a high mortality rate. Studies in rats have predicted a minimal hepatic energy charge; below this level regeneration does not proceed, because the metabolic demands of the liver take precedence (73). Similar methods for predicting the energy balance of liver remnant have been calculated for humans. However, survivable 90% liver resections have been performed successfully in rats (74), and have also been reported in humans (75).

6.2 Basic mechanisms of liver regeneration after partial hepatectomy.

The process of regeneration is considered to be divided into several stages, all of which are dependent on the presence or absence of a number of factors acting in a cascade-like fashion. After partial hepatectomy or toxic liver injury, the regenerative response is orchestrated by the release of cytokines and growth factors, with subsequent increases in activity of growth response genes and telomerase (76).

Efficient regeneration depends on the activation of more than 100 genes and the participation of growth factors and cytokines (Table 2). It is unclear at this time how many of these genes and factors are absolutely required for liver regeneration after PH. Because of multiple interactions among genes and overlapping effects of growth factors, it is unlikely that a single agent by itself would determine the outcome of hepatic growth after PH. Animal experiments have shown that hepatocytes in an intact resting liver are not very sensitive to the mitogenic effect of growth factors such as hepatocyte growth factor (HGF), epidermal growth factor (EGF) and transforming growth factor-α (TGF-α), which strongly stimulate DNA replication in cultured hepatocytes (77, 78). Only HGF and TGF-α are able to stimulate hepatocytes to replicate in culture. Each of these

complete mitogens can stimulate DNA synthesis in cultured hepatocytes. If hepatocytes are exposed to complete mitogens in the absence of liver injury, there is little increase in the expression of immediate early genes (79). However, Liver hyperplasia can be induced by these agents, and this regresses on mitogen withdrawal. This suggests that, without liver injury, replication can occur via a different pathway (79).

Table 2. Main cytokines, growth factors, and transcription factors involved in liver regeneration

Cytokines
Tumor necrosis factor (TNF)
Tumor necrosis factor receptor 1 (TNFR-1)
Interleukin-6 (IL-6)
Growth factors
Transforming growth factor-α (TGF- α)
Hepatocyte growth factor (HGF)
Epidermal growth factor (EGF)
Heparin-binding epidermal growth factor (HB-EGF)
Keratinocyte growth factor (KGF)
Transcription factors
Nuclear factor-κB (NF-κB)
Signal transducer and activator of transcription 3 (STAT-3)
Activating protein-1 (AP-1)
CAAT enhancer binding protein β(C/EBP β)

Gene Expression, Cytokines, and Growth Factors in the Regenerating Liver

The liver is a recognized target organ for proinflammatory cytokines such as tumor necrotic factor (TNF) α, interleukin (IL) 1 and IL-6. These cytokines are released within minutes of partial hepatectomy from non-parenchymal liver cells, and induce hepatocytes to synthesize further acute-phase proteins, mainly protease inhibitors, via activation of hepatocyte DNA-binding proteins. TNF-α binds to receptors on non-parenchymal liver cells and induces the synthesis of other cytokines, including IL-6. IL-6 is a common, multifunctional cytokine, which is produced in local tissue as part of the

acute-phase response after a variety of systemic insults, such as trauma or acute infection. It acts on various cells and is involved in the differentiation of B cells, T cells and osteoclasts as well as hepatocytes. It has also been found to have a role in the growth and development of some tumors, such as renal cell carcinoma and Kaposi's sarcoma. In liver regeneration, IL-6 is thought to result in enhanced transcription, triggering hepatocytes to leave their quiescent state (G0) and enter a prereplicative phase (G1), as well as being involved in further stages of replication.

The priming phase of liver regeneration corresponds to the time at which quiescent hepatocytes enter the cell cycle (G0 to G1 transition) and lasts for 4 to 6 hours after partial hepatectomy (Figure 18). During this phase, there are increases in TNF and interleukin-6 (IL-6) levels in blood and liver, as well as protease activation in the hepatic remnant. Once the hepatocytes have been initiated, exposure to further mitogens allows DNA synthesis to occur. Expression of IL-6 appears to be essential for the priming of hepatocytes. Serum levels of both TNF-α and IL-6 are raised within the first few hours after partial hepatectomy, despite DNA synthesis being delayed up to 24 hrs. In mice with IL-6 knockout gene, there is impaired DNA synthesis and an abnormal G1 phase of partial hepatectomy leading to liver failure. This suggests that IL-6 has a leading role in the induction of hepatocyte proliferation. The administration of IL-6 to TNF-α knockout mice has been shown to induce regeneration; however, the reverse effect in IL-6 knockout mice is not seen. This suggests that the role of TNF-α starts much earlier in the regenerative process than that of IL-6 (80). IL-6 has also been found to protect hepatocytes against transforming growth factor (TGF) β–induced apoptosis (81).

Once the cell has been primed, the progression to G1 phase is dependent on the presence of continued stimulation by mitogens, such as epidermal growth factor (EGF),

TGF-α, insulin and glucagon. The effects of growth factors are thought to be short term and their mode of action may be dependent on the metabolic state of the hepatocytes and presence or absence of other effectors. When progression occurs, the cell proceeds to DNA synthesis, since once it has entered the S phase it is committed to undergo replication. In animal studies, peak rates of mitosis after 70% hepatectomy have been recorded at 24 - 48 h in rats, and at 4 days in dogs.

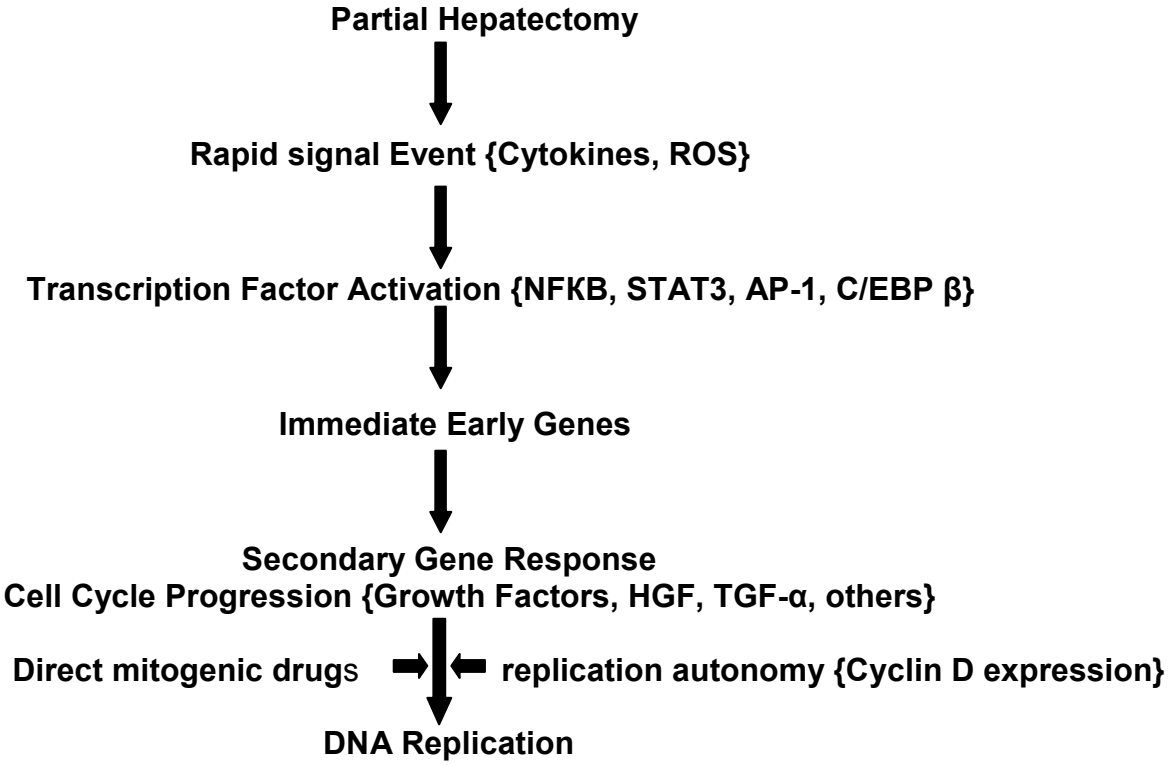

Figure 18. Major steps in liver regeneration after PH. A broad outline of important events in liver regeneration, which suggests that rapid events which initiate regeneration are associated with cytokine networks and reactive oxygen species (ROS) generation. Cell-cycle progression is regulated by growth factors, principally HGF and TGF-α. Replication autonomy is reached at a stage in cell-cycle progression in which cyclin D1 is activated. Drugs that have a mitogenic effect on the liver may act at this stage or at later stages (cyclin E) of the cell cycle.

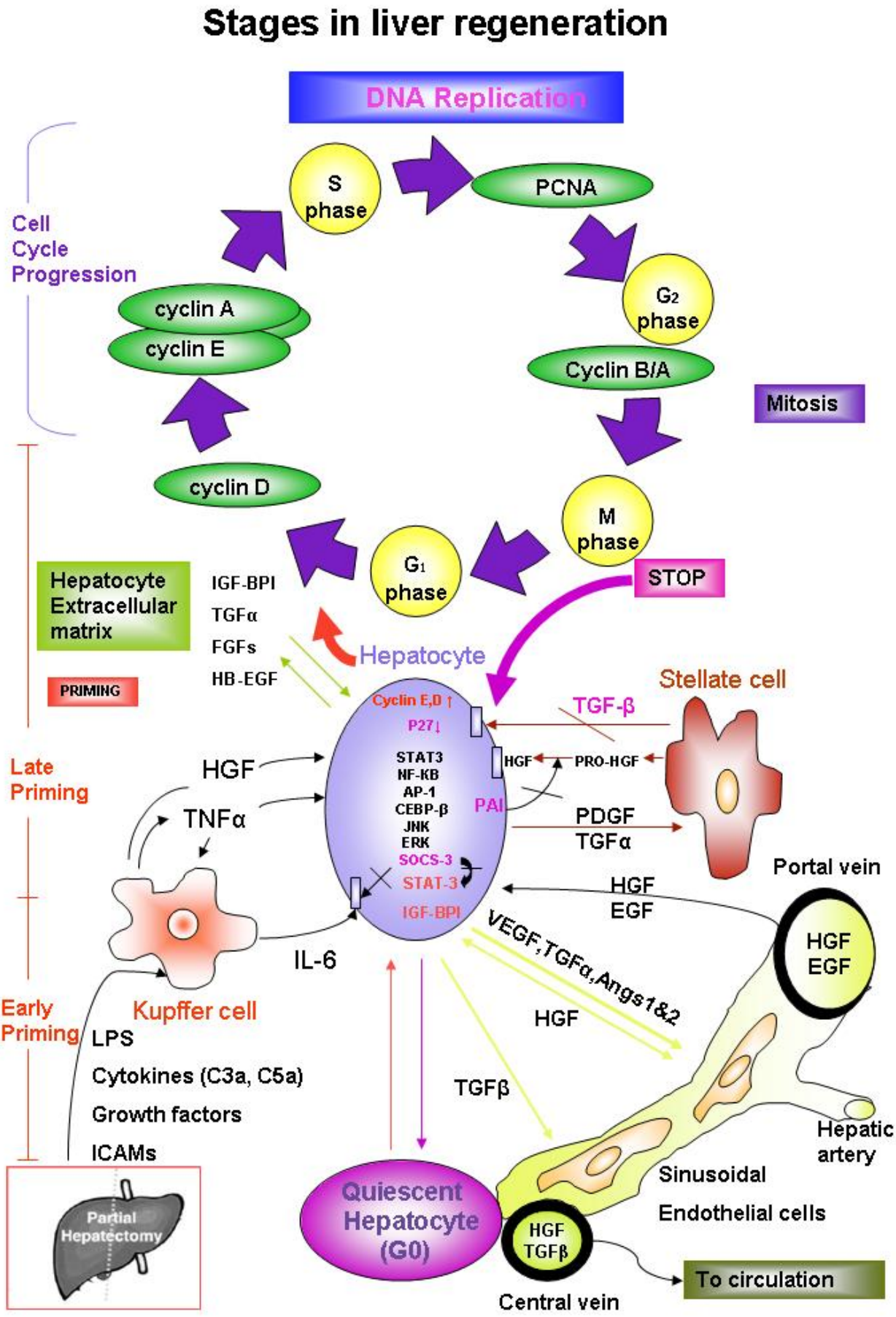
Stages in liver regeneration
DNA Replication
S phase
PCNA
G2 phase
Cyclin B/A
Mitosis
M phase
STOP
G1 phase
cyclin D
cyclin A
cyclin E
Cell Cycle Progression
Hepatocyte Extracellular matrix
PRIMING
IGF-BPI
TGFα
FGFs
HB-EGF
Hepatocyte
Stellate cell
Cyclin E,D ↑
P27↓
STAT3
NF-KB
AP-1
CEBP-β
JNK
ERK
SOCS-3
STAT-3
IGF-BPI
HGF
PRO-HGF
PAI
TGF-β
PDGF
TGFα
Late Priming
HGF
TNFα
IL-6
Kupffer cell
Early Priming
LPS
Cytokines (C3a, C5a)
Growth factors
ICAMs
Portal vein
HGF
EGF
VEGF,TGFα,Angs1&2
HGF
TGFβ
Hepatic artery
Sinusoidal
Endothelial cells
Quiescent Hepatocyte (G0)
HGF
TGFβ
Central vein
To circulation
Partial Hepatectomy

Figure19.

Figure 19. A modified picture of stages in liver regeneration contained references number 83-86 Major steps in hepatocyte replication during liver regeneration. In the normal liver, hepatocytes are in a quiescent state, outside of the cell cycle (G0 phase). To enter the cell cycle at the G1 phase, quiescent hepatocytes are primed in a reversible process associated with cytokine activity. Priming of hepatocytes for replication is not sufficient to induce cell-cycle progression (G1 through S phases) and DNA replication (S phase). During liver regeneration hepatocytes undergo one or two cycles of DNA replication and mitosis (M). The newly divided cells may continue through G1 and around the cell cycle again, or may exit from the cell under the regulation of a 'stop' signal, and return to the G0 quiescent state (84, 85). The growth-factor-mediated pathway. Vascular endothelial growth factor (VEGF) binds to endothelial cells, which triggers the release of the hepatocyte growth factor (HGF) precursor, pro-HGF, from stellate cells. HGF **signalling releases transforming growth factor (TGF) α and other downstream signals that are shared with the cytokine-mediated pathway, such as AP1, Jun amino-terminal kinase (JNK), phosphorylated extracellular signal-regulated kinases (pERKs) (83), CCAAT-enhancer-binding protein (C/EBP) β and insulin-like-growth-factor binding-protein (IGFBP)1. These factors are proposed to activate target of rapamycin (TOR), although this remains to be established, and this leads to cell-cycle transition by increasing the expression of cyclins D and E and reducing p27 levels. TNFα and IL-6 activate neighbouring hepatocytes, which cause signal transducer and activator of transcription (STAT) 3 activation and the expression of several proteins that are shared with the growth-factor-mediated pathway. Various inhibitory proteins that are important for terminating liver regeneration are also activated (shown in pink), including TGFβ (which is produced by stellate cells), plasminogen-activator inhibitor (PAI), suppressor of cytokine signalling-3 (SOCS3), which downregulates STAT3, and inhibits the IL-6 signalling pathway (83, 86), and p27 and other cyclin-dependent kinase inhibitors, and their effects on the two pathways are shown.**

Most hepatocytes are in a state of quiescence and require priming before undergoing replication. A number of factors involved in replication are present in the circulation even when replication is not stimulated, suggesting that hepatocytes do not react to these factors unless they have been primed. Priming, or initiation, is controlled by a series of genes, which act within minutes of partial hepatectomy or on exposure to complete mitogens (figure 19). This stage may be divided into early (A) and late stages (B).

A. The early stage is stimulated by proto-oncogenes, such as c-fos, c-myc and c-jun; which are transcriptional regulators. Expression of genes occurs without protein synthesis. Thus, the cell is primed for proliferation, however, this stage can easily be reversed and its progression to cell division is dependent on the presence of other signalling factors. If these factors are absent, the cell simply returns to its quiescent phase (70). Transcription factors, nuclear factor-κB (NF-κB), signal transducer and activator of transcription 3 (STAT3), activating protein-1, and CAAT enhancer binding protein β(C/EBPβ) are also activated during the initial stages of liver regeneration. NF-

κB activation is particularly rapid, occurring within 30 minutes of PH. NF-κB activation involves a post-translation mechanism that consists of the phosphorylation and degradation of a cellular inhibitor (IκB) bound to the NF-κB protein subunits. Free from the inhibitor, heterodimeric NF-κB consisting of p65 and p50 subunits migrates into the nucleus and activates multiple target genes that have an NF-κB recognition sequence. Among these is the cytokine IL-6, which itself causes activation of STAT3, establishing a signal transduction pathway with the sequence TNF →TNF receptor 1 (TNFR-1) → NF-κB→IL-6→STAT3. NF-κB and STAT3 can transactivate many different genes, and IL-6 has many gene targets besides STAT3 and may serve as a proliferative or antiapoptotic agent. In this manner, stimuli received after PH by a specific receptor in the plasma membrane can be greatly amplified and lead to the transcription of a large number of genes.

B. Delayed or late initiation occurs several hours later, and is controlled by the induction of genes requiring protein synthesis (70). Other factors, such as an increase in ornithine decarboxylase activity, which is involved in the stabilization of messenger RNA (mRNA), influence the late stages of initiation, beginning at 1 h after partial hepatectomy and reaching a peak at 4 h. The cell cycle-related genes include both inducers and inhibitors of the cycle, such as cyclin D1 and mdm2 (inducers), and p53, p21 and p107 (inhibitors). Progression through the cycle is regulated by growth factors. Expression of cyclins D1 and E corresponds to a stage in the cell cycle (G1 restriction point) after which hepatocytes no longer depend on growth factor activity to progress to replication. The exact signal that induces the activation of these processes remains to be determined. It is thought that loss of functional liver mass after partial hepatectomy

stimulates the release of cytokines, which in turn initiate DNA synthesis and regeneration by induction of appropriate genes.

Blood HGF levels increase shortly after PH, and HGF, and TGF-α messenger RNAs (mRNAs) are expressed within the liver before DNA replication (86). These two factors are considered the most important for liver regeneration (87). Studies by Tomiya et al. (88) showed that the level of TGF-α in blood may be a good indicator of liver growth and correlates better than HGF levels with the extent of the regenerative response. However, it is not known whether growth factor production in LDLT is adequate for optimal growth of the donor and recipient livers. Hepatocytes in both these livers are likely to be primed (competent) for replication as a consequence of cytokine release after the surgical procedures. It remains to be explored whether the infusion of growth factors into the donor and recipient livers shortly after surgery would accelerate hepatocyte replication and enhance growth. Based on experimental data, infusion of HGF with either TGF-α or EGF may prove to be particularly effective.

Nevertheless, it is not known whether hepatocytes may preferentially respond to one of these factors, or if HGF and TGF-α act in parallel or sequentially during liver regeneration. In addition, other growth factors may have a role in the regenerating liver, such as heparin binding–EGF (HB-EGF) and keratinocyte growth factor. In particular, HB-EGF mRNA increases earlier in the regeneration process of the liver than TGF-α and HGF mRNAs (89). Except for TGF-α, which is produced by hepatocytes (autocrine effect), all other growth factors are synthesized by hepatic nonparenchymal cells and have a paracrine effect on hepatocytes. (HGF also may act as an endocrine factor.) Thus, the regenerative response that culminates with hepatocyte replication requires the precise interaction between parenchymal and nonparenchymal cells, and can be greatly inhibited in vivo by nonparenchymal blockage (89).

Hepatocyte growth factor (HGF) appears to be the most potent mitogen of all. Levels are greatly increased in blood and liver in cases of extensive liver damage. It is synthesized and secreted by a number of tissues as well as by non-parenchymal liver cells, particularly Kupffer cells, however, it is not found in hepatocytes, suggesting that it acts in a paracrine fashion. Its action is augmented in the presence of insulin, glucagon and EGF.

Transgenic mice that overexpress HGF have correspondingly high levels of c-*myc* and c-*jun* mRNA, suggesting that they may have some role in the induction of HGF action. These mice recover in half the normal time after partial hepatectomy. This tissue response can be suppressed by increasing doses of anti-HGF antibody *in vitro* (71). Transgenic HGF knockout mice have been found to die *in utero* with atrophic livers.

Transforming growth factor α (TGF-α) is also produced by non-parenchymal liver cells, mainly Kupffer cells. It is capable of increasing DNA synthesis *in vitro*. It is similar to EGF in both its structure and its receptor. An increase in TGF mRNA occurs at 24 h after partial hepatectomy. Transgenic mice that overexpress TGF-α have significantly enlarged livers during infancy. However, this effect is lost in older mice, suggesting this may simply be a postnatal effect (71). Of those transgenic mice that express TGF-α, 50-70% develop hepatocellular tumours at 18 months. Overexpressions of HGF have not increased the incidence of tumours; HGF has actually been found to suppress the growth of many hepatocellular carcinomas *in vitro*.

Epidermal growth factor (EGF) induces culture hepatocytes to synthesize DNA. This effect is augmented by insulin. Administration of EGF and insulin increases DNA synthesis after partial hepatectomy in cirrhotic rat (90).

Hepatic stimulatory substance is able to stimulate DNA synthesis only in those cells that have previously been primed. However, it is a potent mitogen for malignant hepatocytes. It appears to be liver specific in that it has no effect on other organs either *in vivo* or *in vitro*, and is secreted by liver tissue itself. *In vitro* studies have detected a synergistic effect with EGF (71).

Co-mitogens have no direct stimulatory effect on hepatocyte proliferation on their own, but are capable of enhancing the effect of complete mitogens and reducing the effect of inhibitory substances. The latter include hormones and neurotransmitters, such as insulin, glucagon, noradrenaline, adrenaline, and thyroid and parathyroid hormones, as well as calcium and vitamin D. Insulin-like growth factors, or somatomedins, are produced in the liver in response to growth hormone and are thought to be involved in the progression of regeneration rather than its initiation. In 1975 Bucher and Swaffield (91) found that eviscerated rats had much lower rates of DNA synthesis after partial hepatectomy than normal rats, and this effect could be reversed by the administration of insulin and glucagon. This was confirmed by subsequent studies in which somatostatin (an inhibitor of insulin and glucagon secrete) was administered preoperatively, resulting in the inhibition of DNA synthesis; DNA synthesis was restored by the subsequent administration of both hormones (92).

Noradrenaline can enhance the effect of other growth factors on regenerating hepatocytes, as can several other hormones. Thyroid hormone does not specifically affect proliferation, but it does affect the overall metabolic state. Parathyroid hormone has been found to have an effect on cells in the later stages of regeneration.

Liver resection is the only curative treatment for several primary and metastatic malignant liver diseases. However, the number of patients qualifying for R0 resections

is limited due to the remaining liver tissue and altered postoperative liver function, which is the main cause of mortality after major liver resection. This has prompted various strategies of increasing the remnant liver volume prior to resection or performing pre-conditioning of the liver parenchyma (65, 66).

A number of growth factors are potential candidates for the improvement of postoperative liver regeneration. Liver regeneration is a multi-step process in which HGF, VEGF and TNF are important initiators, priming remnant hepatocytes for cell division. EPO is an epidermal growth factor which is in clinical use for patients with anemia. Several recent investigations indicated protective mechanisms for tissues other than the hematopoietic system (93-96). However, information on its value for inducing regeneration in the liver has not been reported yet.

In this study we demonstrate for the first time that rHuEPO has the ability to increase liver regeneration after major liver resections in the rat. Our data indicates that rHuEPO doubles the mitotic index early within 24 hours after liver resection. This finding has been confirmed by increased Ki-67 and PCNA expression. The beneficial effect of rHuEPO was confirmed by the experiment involving 90% liver resection in which rHuEPO significantly enhanced postoperative survival.

Consequently, treatment of patients undergoing major liver resection with rHuEPO may have a positive impact on postoperative liver regeneration and decrease peri- and postoperative morbidity and mortality.

6.3 The pharmacokinetics and pharmacodynamics of rHuEPO after intravenous and subcutaneous administration and the effect of proliferative cells on liver regeneration in rat

Despite the fact that the subcutaneous administration of rHuEPO produced lower drug concentrations due to incomplete bioavailability because of prolonged exposure and slow absorption, it produced equal or greater efficacy in rHuEPO treatment compared with intravenous administration at the same dose. The slow absorption process producing T_{max} (time that maximal concentration occurs) at ~8 to 12 h and more prolonged terminal phases were observed in rats after subcutaneous administration. The prolonged plasma concentrations after subcutaneous dosing and slow absorption indicate flip-flop kinetics.

Following intravenous injection in humans, rHuEPO occupies an apparent volume of distribution (Vd) equivalent to the plasma volume, implying that rHuEPO is distributed mainly intravascularly (97). Both in normal subjects and in patients with renal disease, the estimated t1/2b (elimination half-life) ranges from 2 to 13 h following intravenous administration, and tends to increase dose-dependently. The apparent Vd has also been found to increase dose-dependently, but for doses of 50–1000 U/kg it has usually been found within the range of 30–100 ml/kg (98). After subcutaneous administration of 100 U/kg, the Cmax (peak concentration) of rHuEPO was 124 mU/ml compared with 3136 mU/ml after intravenous administration (99). The subcutaneous route is associated with only 18–49% of the bioavailability of the intravenous route. Despite their differing bioavailability and pharmacological profiles, both intravenous and subcutaneous routes are effective for the administration of rHuEPO. The metabolic fate of EPO has not been accurately determined. It is likely that haemopoietic cells, the liver

and the kidney are involved in its elimination. Renal excretion usually accounts for less than 10% of the daily endogenous production (100).

Our data shows that both routes of rHuEPO administration are able to promote liver regeneration after partial hepatectomy. Pretreatment intraportalvenous significantly increased hepatocyte proliferation during the first 24 hrs predominantly with Ki-67, however, after 4 days the percentage of hepatocyte proliferation declined below the level of controls and subcutaneous administration groups. The animals receiving postoperatively subcutaneous rHuEPO showed a remarkably higher percentage of mitotic index and Ki-67 positive cells after 2 days, and also the percentage of PCNA expressed was significantly higher on day 7. This phenomenon corresponds directly to the variability in pharmacokinetics and pharmacodynamics of different administration routes. A high dosage of intraportal venous rHuEPO shows benefits of early stimulated regenerative effects, but a lower subcutaneous postoperative dosage on three consecutive days displays a positive effect on regeneration after 2 days.

We have analysed two different modalities of rHuEPO application (subcutaneous and intraportalvenous) and our overall results displayed almost identical effects for both study groups, indicating that subcutaneous (considering in Cmax in properly time for absorption) pre-treatment with lower consecutive doses prior to the scheduled surgical procedure can be as beneficial as a large intraoperative volume prior to resection, which is comparable to the widely established application of steroids before parenchymal transsection (65, 66). With regard to pharmacokinetics and pharmacodynamics previously described, the alternative option is to use combined intraportal venous and subcutaneous administration. However, this conclusion should be verified in detail in future studies. The doses of rHuEPO used in this study were chosen in accordance with the current literature, however further analyses need to be conducted to see

whether altered dosages may grant even greater effects or generate undesired events such as thrombosis (43-44, 101-104). Additionally, if transferred directly from the animal model used in this study, a dose of 1000 IU / kg would add significant costs to the treatment of liver surgery patients and potentially increase the risk of thrombosis. Therefore studies using smaller amounts of rHuEPO should be undertaken.

6.4 Effect of rHuEPO on hematopoietic system during liver regeneration

Although the effects of rHuEPO on the hematocrit (erythrocyte mass) seem to be of minor concern in these acute experiences, this would not be true if rHuEPO is used clinically in a chronic dosage regimen. Nevertheless, carbamylated EPO and various nonhematopoietic mutants were cytoprotective in vitro and conferred neuroprotection against stroke, spinal cord compression, diabetic neuropathy and experimental autoimmune encephalomyelitis at a potency and efficacy comparable to EPO (61). The development of nonerythropoietic analogs of EPO might make it possible to avoid such undesirable effects in the clinical settings stated before. Although tissue protective and regenerative cytokines signaling needs to be further clarified, the availability of compounds such as Carbamylated EPO (EPO where all the lysines were transformed to homocitrulline by carbamilation) that do not trigger EPOR2 also opens up possibilities of distinguishing experimentally between EPO's tissue regenerative effects and its potentially detrimental effects (e.g. pro-coagulant and prothrombotic effects within the microvasculature) and excessive erythropoiesis upon chronic dosing (61). This might be able to trigger EPO-mediated tissue regerative pathways without cross-talk with the hematopoietic system.

There are a number of different possible mechanisms which may contribute towards promoting the outcome following rHuEPO treatment after major liver resections.

Hematopoietic cells located in the liver may be one source of increased regeneration potential of the liver after rHuEPO pretreatment (105-107). Studies of hematopoietic cells and bone marrow mesenchymal stem cells revealed that they are capable of generating various types of tissue cells. It has been shown recently that hepatocytes can be produced by cultures of multi-potent adult progenitor cells. These properties of stem cells have been introduced to accelerate liver regeneration at the time of portal venous embolization prior to trisegmentectomy in humans (65, 66). Previously it has been shown that a single high dose of EPO does not increase hemoglobin levels during the first 2 to 3 days (108, 109). The peak RET response occurred at ~4 to 5 days after the treatments. The dose-dependent increases in RET followed by an immediate decline below the baseline were observed at all dose levels, reflecting the tolerance and rebound phenomena. The RET then slowly returned to the baseline by days 21 to 24. The red blood cell count and hemoglobin level started rising steadily until peaks were achieved at approximately days 5 to 6, at which point reticulocytes started decreasing because of their conversion into mature red blood cells. Erythropoietic production of rHuEPO is expressed more slowly than the regenerative effect of rHuEPO on the liver. A previous study showed that subcutaneous and intravenous administrations of rHuEPO increased hemoglobin and red blood cell count at peaks approximately at the 5-6th day (110). The elevation of hemoglobin level does not promote liver regeneration, but may be of help later in supporting oxygenation and promoting survival after 90% liver resection and high dose intravenous portal injection. However, this hypothesis remains to be substantiated in further studies.

6.5 Effect of rHuEPO on TGF-β during liver regeneration

TGF-β is a multifunctional cytokine that can have either inhibitory or stimulatory effects, depending on cell type and conditions. It cancels the effects of TGF-α when administrated at the same time, but has no effect on any of the other complete or incomplete mitogens, such as HGF or insulin. TGF-β is also able to stimulate fibrogenesis in the liver (71). It is produced by both hepatocytes and non-parenchymal cells, but requires cleavage from its binding protein by plasmin for activation (70). Normal hepatocytes cannot activate TGF-β, only those undergoing regeneration. Administration of TGF-β to rats after partial hepatectomy inhibits replication for up to 22 hours, after which the hepatocytes begin to replicate again (111). In obstructive jaundiced rats after liver resection, TGF- β 1 blockage with anti-TGF- β 1 monoclonal antibody improved liver regeneration both morphologically and functionally (112). Transgenic mice that overexpress TGF-β have delayed regeneration. TGF- β levels are increased at 4 h after partial hepatectomy, with a peak at 72 h, suggesting that its action may be during the later stages of the regenerative process, perhaps by inducing apoptosis (4). TGF-β family cytokines signal cells through the Smad pathway, leading to a cascade of events that eventually result in the induction of apoptosis.

The amount of damage to the surgically altered parenchyma may be quantified by the activation of certain immunologic mediators such as TGF-ß (113, 114). In our study, TGF-ß mRNA was expressed to a considerably higher extent in controls than in EPO-treated animals 3 hours after surgery. Although this effect did not reach statistical significance, this may be regarded as a potential protective effect attributed to Erythropoietin.

6.6 Effect of rHuEPO on HIF-1 α during liver regeneration

Hypoxia is well-known as one of the strongest stimulants for angiogenesis, and hypoxia inducible factor-1α (HIF-1α) has been well recognized to play an essential role in hypoxic adaptation during mammalian development, wound healing and tumor growth (115, 116). HIF-1 is a heterodimer protein, which is composed of an alpha and a beta subunit, both of which are members of the basic-helix–loop-helix PAS family. HIF- 1β is an aryl hydrocarbon receptor nuclear translocator and is constitutively expressed in most of the tissues. HIF-1 α is unable to bind hypoxia response elements without forming a complex with HIF-1β. The exact stimulus responsible for induction of the HIF-1α gene in ischemic tissue is yet to be determined. Several factors including the phosphatidylinositol 3-kinase/Akt pathway, reactive oxygen species, HER2 (neu) signalling pathway, tissue oxygen concentration and chemical factors like cobalt chloride may modulate the level of HIF-1α (117, 118). Maeno et al. (119) have shown that nuclear HIF-1α expression peaked at 24 h and remained elevated through 120 h following hepatectomy. HIF-1a expression preceded the VEGF and flt-1 expression in regenerating rat liver and may indicate that HIF-1 serves as a trigger for downstream VEGF expression. Recent studies highlighted the association of hepatic angiogenesis with inflammation and fibrogenesis during chronic liver injury. Furthermore, it has become evident that hypoxia-induced increase of VEGF expression is a common response to liver damage and might play a major role in the angiogenesis of the cirrhotic liver (120, 121). It is now known that hypoxia initiates an intracellular signalling pathway leading to the activation of the transcription factor HIF-1α. HIF-1α binds to conserved regulatory sequences known as hypoxia-responsive elements (HRE) found in the promoter of several target genes, including VEGF, and controls their expression in response to hypoxia, leading to the adaptation of cells to decreased oxygen levels. In

experimental liver fibrogenesis, HIF-1a expression gradually increases according to the severity of fibrosis and strongly correlates with VEGF expression and angiogenesis. Sevgi B. et al. (122) suggest that HIF-1a might influence angiogenesis via regulation of VEGF contribute towards the wound healing response of liver injury, and could be a potential target in the manipulation of angiogenesis in chronic inflammatory liver diseases ending with cirrhosis.

Significant reduction of HIF mRNA expression in EPO-treated animals 3 hours after partial hepatectomy suggests a potential influence of hypoxia-related cell damage on liver regeneration. It may be speculated that the positive effect of EPO treatment on regenerative capacities of hepatocytes is transmitted via protective mechanisms directed against ischemic cell damage (43-44, 104).

6.7 Effect of rHuEPO on STAT-3 Expression during liver regeneration

STAT3 belongs to the STAT family of transcription factors which consists of at least seven members in mammals (123). STAT3, initially named acute-phase response factor, was shown to be activated by all members of the IL-6 family, including IL-6, IL-11, oncostatin M (OSM), leukemia inhibitory factor, cardiotropin 1 and ciliary neutrophic factor. The targeted disruption of Stat3 leads to early embryonic lethality, indicating an essential function of Stat3 in embryonic cell growth (124). In the liver, activation of STAT3 by IL-6, its related cytokines, and IL-22, plays an important role in acute-phase response and hepatoprotection (125-127). Akira Moh et al. (128) have demonstrated that STAT3 is required for survival in the acute stage after 70% hepatectomy. After the acute stage, STAT3 plays a limited role in DNA synthesis and liver mass recovery in lean mice. In addition, exaggerated inflammatory reaction exists after hepatocyte necrosis in STAT3-deficiency mice. Intracellular expression of STAT-3, an important

component of regeneration and repair mechanisms, has been shown to increase dramatically following ischemia and reperfusion injury. Stephanou et al (129) demonstrated that STAT-3 is significantly upregulated following tissue ischemia. The protective capacities of STAT-3 for hepatic tissue were published by Haga et al (130). An additional possible mechanism is the regulation of STAT binding elements, which are involved in tissue protection by ischemia, liver regeneration and activation of HGF (131, 132). A recent study demonstrated that ex vivo adenoviral gene transfer of activated STAT-3 protects the liver from ischemic damage and promotes liver regeneration. These changes in STAT-3 regulation during liver regeneration were underlined by previous investigations, showing enhanced STAT-3 mRNA expression six hours after rHuEPO treatment in rats with intra-portal vein administration of rHuEPO (48) and improvement of liver function after warm ischemia using rHuEPO administration. However, despite this effect, we could not identify any differences in STAT3 mRNA expression within the three groups after liver resection.

This may be attributed to the time points investigated in our study. STAT-3 mRNA was extensively expressed in all animals 12 hours after liver resection. A possible explanation for the rHuEPO effects could be the STAT-3 mRNA expression in the very early phase after hepatic resection. Additionally, since STAT-3 has anti-apoptotic capacities effected by Bcl-2 mediated mechanisms, it may have protective effects during cold and warm ischemia of the liver, which contributes towards the reduction of ischemia-induced cell damage when hilar occlusion is applied (43).

6.8 Effect of rHuEPO on VEGF mRNA Expression during liver regeneration

Furthermore, liver parenchyma regeneration may be induced by VEGF-dependent and independent up-regulation of vascular growth, as shown previously. In liver regeneration

after PH, both hepatocytes and non-parenchymal cells express the vascular endothelial growth factor (VEGF) mRNA, suggesting that VEGF plays a significant role in this process. Hepatocellular production of VEGF shows the maximal levels between 48 and 72 h after PH (133, 134).The endogenous and exogenous VEGF promoted the proliferation of sinusoidal endothelial cells during liver regeneration after PH, and the reconstruction of hepatic sinusoids appeared to promote the proliferation of hepatocytes. Maximilian et al. (135) have shown that VEGF is an important factor in the early phase of liver regeneration after PH. The exogenous administration of VEGF leads to an increase in vessel density and vessel diameter. VEGF administration leads to an increase of liver body weight ratio and Ki-67 immunostaining compared with controls, and a significant increase of IL-6 concentrations. To further elucidate the role of endogenous VEGF, animals were treated with anti-VEGF after PH. These animals showed suppressed angiogenesis as demonstrated by a decrease in vessel density and diameter. Furthermore, the liver body weight ratio was significantly suppressed (1% versus 1.5%), and Ki-67 immunostaining showed a markedly suppressed proliferation index of only 3% versus 62% at 24 h after resection. LeCouter et al. (136) have already demonstrated the importance of VEGF during liver regeneration mediated through its receptor VEGFR1 (Flt-1). Injury, such as resection, leads to the secretion of VEGF A, which binds to its receptors VEGFR1 and VEGFR2. Endothelial cells then proliferate and release growth factors such as IL-6 and hepatocyte growth factor (HGF) triggering angiogenesis and liver regeneration.

Nevertheless, exogenous VEGF stimulates liver regeneration following 70% hepatectomy as indicated by Ki-67 immunostaining. Blocking of endogenous VEGF could delay regeneration almost completely for about 24 h, and suggests an important role for this factor in the first phase of liver regeneration after PH, but is not essential in

the second phase of liver regeneration (135). Subsequent studies of Maximilian et al. have shown that VEGF administration does not improve liver regeneration and survival after 90% subtotal liver resection. This indicates that the relevant mechanisms that stimulate liver regeneration after hepatectomy at least partially depend upon the extent of liver resection (137). Tanigushi et al. (134) have shown that the percentage of Ki-67 expressing sinusoid endothelial cells was progressively increased after 24 hrs and reached significantly higher maximal levels after 72 hrs in the periportal area after partial hepatectomy. However, proliferation of sinusoidal endothelial cells has been demonstrated only in periportal areas, but not in perivenular and midzonal areas. The results show that proliferation of sinusoidal endothelial cells was most active in periportal areas, moderately active in perivenular areas, and less active in midzonal areas, suggesting that VEGF produced by periportal and perivenular hepatocytes promote proliferative activity of sinusoidal endothelial cells, probably in a paracrine fashion. Furthermore, the proliferative activity of periportal sinusoidal endothelial cells was significantly higher 48 and 72 hrs after PH, corresponding to increased proliferative activity of periportal hepatocytes.

In our study, VEGF mRNA expression significantly increased in the treatment group compared to controls at only 24 hours after 70% partial hepatectomy. This may show that VEGF was stimulated by an indirect effect (paracrine) of EPO in early sinusoidal cell production and promoted in the early phase of liver regeneration after partial hepatectomy. Nevertheless, VEGF administration alone seems to be insignificant in improving survival and regeneration in 90% liver resection in contrast to EPO administration. However, EPO is a far less potent stimulator of angiogenesis than VEGF, which may, however, be reduced in its importance by its short half-life.

7. SUMMARY

Liver regeneration is a tightly controlled response to loss of liver mass, a complex chain of interrelated molecular and cellular events usually resulting in rapid and remarkable growth. As hepatobiliary and transplant surgeons continue to expand the magnitude and complexity of liver resection and explore beyond the boundaries of transplantation, the role of liver regeneration in the clinical realm becomes increasingly relevant. Investigators need to continue to explore with new technologies not only the process of replacement of lost liver mass, but also the repair of injured cells and the maintenance of metabolic homeostasis. Understanding how regeneration processes are initiated, progress and cease and appreciating how pre-existing liver disease, patient status, and exogenous factors can influence these processes can improve outcomes after liver resection and transplantation.

Erythropoietin was shown to have dramatic hematopoietic, tissue-protective and regeneration promoting effect in multiple organs. To date no data is available on whether rHuEPO has the ability to stimulate liver regeneration after liver resection. Using a rat model of liver resection we aimed to determine the effect of the administration of human recombinant erythropoietin (rHuEPO) on liver regeneration. Rats were subjected to 70% or 90% liver resection and received an intraportal venous administration of rHuEPO (4000 U/kg) prior to resection or a subcutaneous injection for 3 consecutive days (total dose 4000 U/kg) postoperatively. Control rats were treated with saline intraportal injection and surgery. Groups with 70% hepatectomy were studied for regenerative capacity (Ki-67, PCNA, TGF-β, HIF, STAT-3 and VEGF), and groups with 90% hepatectomy were studied for 10 days for survival analysis.

Regenerative capacity is supported by increased mitotic function and intracellular proliferation activity as demonstrated by increased Ki-67 and PCNA. Mitotic index and

Ki-67 were significantly increased in the treated groups 24 hrs and 48 hrs after 70% hepatectomy. A surprising finding is that PCNA-positive cells increased significantly early in the intraportal group both at 12 and 24 hrs, and at 48 hr in the groups after liver resection. TGF-ß and HIF mRNA are both up-regulated in control animals 3 hr after surgery. VEGF mRNA expression is increased significantly earlier in both treatment groups compared to controls 24 hr after hepatectomy. 10-day survival in rats undergoing 90% hepatectomy displayed significant survival advantage when treated with rHuEPO.

In this study we are able to conclude that rHuEPO effectively increased liver regeneration in the rat after 70% liver resection and enhanced survival even after 90% liver resection. The possibility of using rHuEPO to induce ischemic tolerance and promote liver regeneration suggests that there are advantages in its clinical application in liver resection and transplantation. Firstly, rHuEPO is a safe drug in clinical practice, secondly, the induction of regeneration seems to be relatively rapid after a single injection of rHuEPO and thirdly, no additional or special equipment is required for the induction of regeneration in the patient. Fourthly, patient's treatment of rHuEPO may be effective in preventing anaemia after operation.

In living donor liver transplantation (LDLT), the unique regenerating capacity of the liver is important for the donor as well as for the recipient to guarantee a good outcome. Therefore, treatment with erythropoietin may represent a promising strategy to optimize liver regeneration in the setting of living donor liver transplantation or after massive resection of the liver, especially due to its excellent general practicability. Clinical studies will be necessary to evaluate the therapeutic properties of rHuEPO in preventing an ischemic reperfusion injury and promoting regeneration not only in major liver resection and transplantation, but also when considering the transplantation of other

solid organs. The exact activation mechanisms of these tissue regeneration processes, including intracellular signal transmission and survival ability, remain to be clarified in detail. However, the route of pretreatment administration of rHuEPO (intraportalvenous, intravenous, subcutaneous and combined), pre-reperfusion, dose dependent properties for promoting regeneration and side effects with respect to tumor growth and thrombosis must be studied in more detail to ensure safer clinical application in the future.

8. ZUSAMMENFASSUNG

Die Leberregeneration ist ein komplexer Mechanismus, der infolge des Verlustes einer signifikanten Menge von funktionell aktivem Leberparenchym in Gang gesetzt wird. Hierbei sind auf molekularer wie zellulärer Ebene vielschichtige Signalkaskaden involviert. Für den Leberchirurgen spielt die hepatische Regeneration vor allem auf den Gebieten der Tumorchirurgie und der Lebertransplantation eine entscheidende Rolle. Das Verständnis der mechanistischen Grundlagen der Leberregeneration könnte einen wichtigen Beitrag zur Verbesserung der Ergebnisse nach Leberresektion und Lebertransplantation leisten.

Erythropoietin (EPO) hat sich in verschiedensten Gewebetypen als potenter Organ-Protektor und Regenerations-Stimulator erwiesen. Bis heute sind jedoch keinerlei Daten bezüglich des Einflusses von EPO auf die Leberregeration verfügbar.

Wir haben daher in einem Rattenmodell den Einfluß von EPO auf die Leberregeneration nach 70%-iger Leberteilresektion untersucht. Hierbei wurden drei Studiengruppen gebildet:

Gruppe 1 erhielt eine intraportalvenöse EPO-Gabe 30 Minuten vor Resektion (4000 U/kg), Gruppe 2 erhielt drei konsekutive EPO-Gaben subcutan während der ersten 48 Stunden postoperativ (Kumulativ-Dosis 4000 U/kg), Gruppe 3 diente als Kontroll-Gruppe und erhielt eine intrapoartalvenöse Kochsalz-Injektion 30 Minuten vor Resektion.

Die regenerative Kapazität wurde mittels Bestimmung von Ki-67, PCNA, TGF-ß, HIF, STAT-3 und VEGF untersucht.

Eine weitere Teil-Studie untersuchte das Überleben nach 90%-iger Leberteilresektion mit bzw. ohne EPO-Behandlung.

PCNA und Ki-67 stellen sensitive Marker für die im Rahmen der Gewebs-Regeneration erhöhte intrazelluläre Proliferations-Aktivität und die Mitose-Rate dar; 24 und 48 Stunden nach Leberteilresektion zeigten sich beide Parameter deutlich erhöht. Hierbei fällt auf, dass PCNA in der intraportalvenös mit EPO behandelten Studiengruppe nach 12 Stunden vermehrt exprimiert wird, in der subcutan behandelten Gruppe nach 24 Stunden.

TGF-ß und HIF mRNA sind bereits 3 Stunden nach Leberteilresektion deutlich hoch-reguliert. VEGF mRNA wurde bei den EPO-behandelten Tieren deutlich früher (24h) vermehrt nachgewiesen als in der Kontroll-Gruppe.

Das 10-Tages Überleben nach 90%-iger Leberteilresektion stellte sich für EPO-behandelte Tiere deutlich verbessert dar.

In der vorliegenden Studie konnte gezeigt werden, dass EPO die Leberregenration nach 70%-iger Leberteilresektion signifikant verbessert und einen günstigen Einfluß auf das Überleben nach 90%-iger Resektion besitzt.

EPO besitzt die positive Eigenschaft, eine bereits im klinischen Einsatz befindliche Substanz zu sein, deren Nebenwirkungsprofil bestens bekannt und relativ überschaubar ist. Die Substanz ist rasch verfügbar, unkompliziert zu verabreichen und vermittelt eine rasche Induktion der regenerativen Kapazität.

Besonders im Falle der Leber-Lebendspende und der gross-volumigen Leber-Teilresektion bei ausgedehnten Lebertumoren mit dem Risiko des „small-for-size“ Syndroms könnte EPO-Konditionierung einen besonders günstigen Einfluss auf die Organfunktion ausüben.

Die exakten Mechanismen der Stimulation der hepatischen Regeneration sind Gegenstand der aktuellen Forschung und erfordern eine detaillierte Darstellung der molekularen und intrazellulären Signalkaskaden.

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10.1) ABBREVIATIONS

EGF	Epidermal growth factor
EPO / rHuEPO	Erythropoietin / Recombinant Human Erythropoietin
EPOR	Erythropoietin receptor
ESAs	Erythropoiesis stimulating agents
I.P.	Intraportalvenous
HEF	Hepatic erythropoietic factor
HGF	Hepatocyte growth factor
HIF	Hypoxia induced factor
PCNA	Proliferating cell nuclear antigen
PH	Partial hepatectomy
RET	Reticulocyte
RES	Reticuloendothelial system
RBC	Red blood cell
S.C.	Subcutaneous
SFSS	Small-for-size liver syndrome
STAT-3	Signal transducing activator 3
TGF-ß	Transforming growth factor ß
Vd	Volume of distribution
VEGF	Vascular endothelial growth factor

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