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ERYTHROPOIETIN STIMULATES HEPATOCYTE REGENERATION AFTER LIVER RESECTION

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ABSTRACT

Aim The increased relevance of liver surgery and transplantation as a therapeutic modality over the last two decades mandates the development of novel strategies to improve liver regeneration. Here we studied whether erythropoietin (EPO) improves liver regeneration after hepatectomy in pigs.
Methods Eighteen female pigs underwent laparoscopic left lateral liver resection and were allocated randomly into three groups. No EPO was administered to the control group (group 1, n=6). Group 2 (n=6) received EPO topically to the liver resection surface in a fibrin sealant. Group 3 (n=6) received EPO topically and systemically. Pigs were sacrificed 14 days after hepatectomy. The fraction of proliferating hepatocytes was determined by ki-67 immunostaining. Liver volume was determined by the principle of Archimedes,
Results Liver weight and volume were significantly increased in group 3 (1249 ± 223 g, 1073 ± 190 ml) compared to group 2 (1027 ± 167 g, 894 ± 105 ml) and group 1 (923 ± 186 g, 813 ± 165 ml). Ki-67 immunostaining of liver tissue close to the resection surface demonstrated a significantly increased percentage of proliferating hepatocytes in group 3 (4.3 ± 1.96 %) and in group 2 (3.5% ± 0.98 %) as compared to group 1 (1.15 ± 1.2 %) 14 days after hepatectomy.
Conclusions Our results indicate for the first time that EPO supports liver regeneration after hepatectomy.

Keywords: erythropoietin, hepatocytes, laparoscopic hepatectomy, regeneration

Abbreviations: EPO: erythropoietin, i.v.: intravenous, i.m.: intramuscular, PH: partial hepatectomy, Hb: haemoglobin, SD: standard deviation, PCNA: Proliferating Nuclear Antigen, d: day, OP: operation

INTRODUCTION

The remarkable proliferative capacity of the liver is vividly captured in the Greek myth of Prometheus(Lee, 2001; Bucher, 1991). Nevertheless, the exquisite regenerative capacity of the liver is finite and often insufficient to combat many liver diseases (Cataldegirmen et al., 2005). For example, hepatic failure occurring after liver resection, a complication dreaded by surgeons, is associated with a poor prognosis (Mullin et al., 2005). The maximum extent of hepatic resection compatible with a safe postoperative outcome is difficult to determine and depends on many factors including residual liver function (Yigitler et al., 2003). Despite widespread interest in the molecular mechanisms underlying liver regeneration (Michalopoulos and DeFrances, 1997, 2005), the clinically relevant limits of the ability of the liver to regenerate are not well understood. Presently, no efficient therapy is available to enhance regeneration and optimize survival when the residual liver is excessively small. On the other hand, the growth of liver surgery and transplantation as a therapeutic modality over the last two decades mandates the development of novel strategies to improve liver regeneration and optimize outcomes (Cataldegirmen et al., 2005).

Experimental evidence supports a role for erythropoietin (EPO) in the repair and regeneration also of non-hematpoietic organs (Sasaki, 2003). Recent studies have identified multiple paracrine and autocrine functions of EPO. These functions coordinate local responses to injury by maintaining vascular autoregulation and attenuating both primary (apoptotic) and secondary (inflammatory) causes of cell death. EPO has been shown to prevent apoptosis and to stimulate mitosis and signalling in astrocytes (Sugawa et al., 2002), endothelial cells (Jaquet et al., 2002), and cardiomyoblasts (Ogilvie et al., 2000) maintained in vitro. Although it is well known that EPO plays an important role in myocardial and cerebral regeneration, the role of EPO in liver regeneration is unknown. Shinozuka et al. (2000) conclude in their trial that the simultaneously preoperative administration of autologous blood and EPO, markedly reduced the requirement for homologous blood transfusion during the surgery and reduced postoperative complications.

In the present study we studied the influence of EPO on liver regeneration using a laparoscopic porcine liver resection model. We report for the first time that EPO improves liver regeneration after topical administration to the resection surface in a fibrin sealant and even the more after combined topical and systemic administration.

MATERIALS AND METHODS

Experimental design and operative procedure

Eighteen commercially available, female German Landrace pigs (Lehr- und Versuchsgut, Oberholz, Leipzig, Germany) were allocated randomly into three groups and studied with the approval of the local Institutional Animal Use Committee (Regierungspräsidium, Leipzig, Germany). Animals were fed twice daily at 7 a. m. and 3 p. m. with standard pig diet (ATR Starter Extra 13.0, ATR Landhandel, Ratzeburg, Germany). The animals were housed in an animal resource facility at the Center for Experimental Medicine (Medizinisch Experimentelles Zentrum, University of Leipzig, Germany). Pigs underwent laparoscopic left hemihepatectomy and were observed postoperatively for two weeks. The operation was performed using CUSA (CUSA; Valleylab Boulder, CO, USA) and Ultracision (UltraCision, Ethicon). The pigs were placed in the supine position and six 12 mm laparoscopic port sheets were inserted through the anterior abdominal wall. Surgical procedures were performed without the Pringle manoeuvre (Pringle, 1908). The weight of the resectate was similar in all groups. No EPO was administered to the control group (n=6). In group 2 (n=6) 10,000 units of EPO (ERYPO, Epoetin alfa, ORTHO BIOTECH, Division of Janssen-Cilag GmbH, Neuss, Germany) were mixed with 2 ml of fibrin sealant (Quixil, Omrix,

Rhode-St-Genèse, Belgium). The mixture was applied topically to the liver resection surface (Carless et al., 2002). The same procedure was conducted with group 3 (n=6). In addition to the topical EPO treatment all pigs in group 3 received 10,000 units of EPO systemically (i. v.) on days 0, 3, 7, and 11. The pigs were sacrificed on day 14.

Medication

Pre- and postoperative medication, exposure to anesthesia, and postoperative care were identical for all animals. After premedication with azaperone (5 $mg \cdot kg^{-1}$, i. m.) and atropine (0.05 $mg \cdot kg^{-1}$, i. m.), anaesthesia was performed using thiopental (10 $mg \cdot kg^{-1}$, i. v.) and fentanyl (5 $\mu g \cdot kg^{-1}$, i. v.). Muscle relaxation was induced with pancuronium bromide. Postoperative analgesia was provided by metamizole and piritramide. Prior to taking blood samples the animals were sedated by i. m. administration of a mixture of azaperone (15 $mg \cdot kg^{-1}$ body weight, Stresnil, Janssen–Cilag GmbH, Germany), ketamine hydrochloride (10 $mg \cdot kg^{-1}$ body weight, ketamine, Sanofi-Cefa GmbH, Germany) and atropine sulfate (0.05 $mg \cdot kg^{-1}$body weight, Atropin, B. Braun Melsungen, Germany).

Sample collection

Liver biopsies were taken during the first operation (day 0). The pigs underwent a second laparoscopic intervention 24 hrs later (day one). During this procedure, approximately 5 ml of liver tissue were taken from 1 cm beneath the resection surface in order to avoid necrotic tissue from the operation. On day 14 another specimen was taken from beneath the resection surface. Additionally bone marrow from the femur was examined on day 14 (Fig. 1). The volume of the resectate and the volume of the residual liver on day 1 and day 14, respectively, were measured according to the principle of Archimedes ($F(A) = p * V * g$). Blood samples were taken immediately before surgery, after wound closure, three hours after the operation, after 24 hours, on day 3, on day 7 and on day 14.

Immunohistochemical analysis

Along with conventional hematoxylin-eosin (HE) stains for all tissues examined, immunohistochemistry was performed as described previously (Tannapfel et al., 2003). The bone marrow was stained with CD117 antibody (polyclonal rabbit, c-kit, DAKO, Copenhagen, Denmark, 1:250) and counterstained with hematoxylin. The positive cells were counted and the relation be-

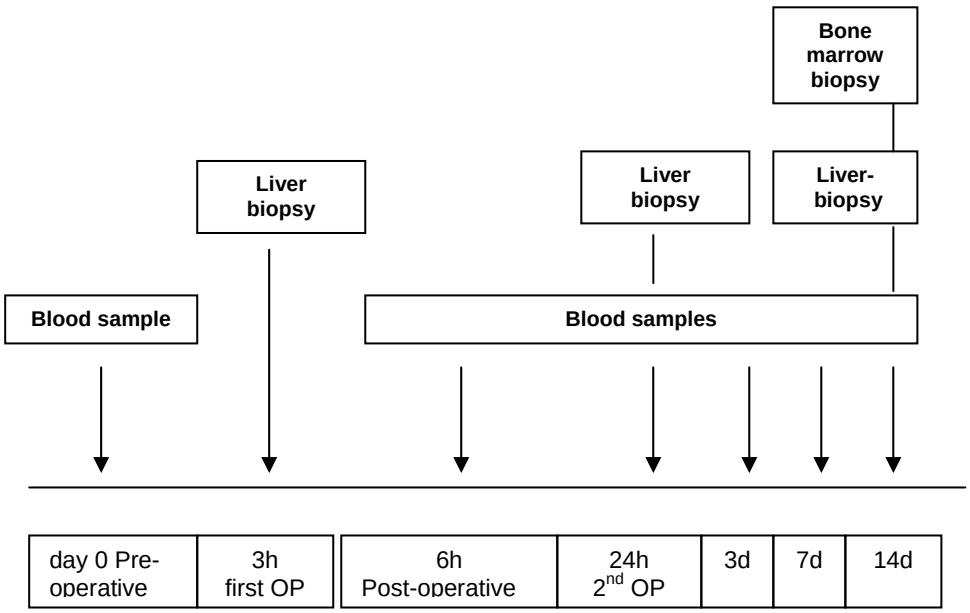


Figure 1: Schedule for operations (OP) and biopsies

tween erythropoiesis and myelopoiesis was examined. Immunohistochemical staining for Ki-67 [MIB-1] and PCNA [Proliferating Cell Nuclear Antigen] was performed to assess the proliferative activity of the liver specimens at various time points. The tissues were excised at designated time points after partial hepatectomy, fixed in 4 % buffered formaline solution and embedded in paraffin wax. For immunohistochemistry 3 µm thick sections of formaline fixed paraffin embedded tumor tissue were mounted on poly-L-lysine capillary slides, dried for 20 minutes at 70 °C, dehydrated through graded alcohol and cleared in xylene. For detection, we used the streptavidin-biotin immunoperoxidase technique using a LAB kit (DAKO, Copenhagen, Denmark). A counterstaining with hematoxylin was used to visualize the nuclei in the tissue sections. The number of Ki-67 and PCNA positive hepatocyte nuclei was determined and expressed as a percentage of all hepatocytes. Only cells with normal hepatocyte morphology were counted. Each tissue sample was examined and counted by two pathologists blinded for any experimental information (AT, EH).

Statistical analysis

The results were expressed as the mean ± SD. The statistical significance of differences between sample means was assessed with Kruskal-Wallis One Way Analysis of Variance on Rank test and Mann-Whitney Rank Sum Test (SigmaStat). Differences were considered significant at $p \leq 0.05$. Statistics were compiled in cooperation with the Institute for Medical Informatics, Statistics and Epidemiology, University of Leipzig.

RESULTS

EPO accelerates liver weight gain after resection

Eighteen pigs underwent successful laparoscopic left liver resection. Mean body weight (± standard deviation) of the pigs prior to operation was 48 ± 4.42, 53 ± 6.76 and 51 ± 9.80 kg in groups 1, 2 and 3, respectively. The mean weight of the resected tissue in groups 1-3 was 183 ± 69, 205 ± 49 and 206 ± 88 g, respectively.

Interestingly, combined systemic and topical administration of EPO (group 3) significantly accelerated liver weight gain by 26 % compared to the controls (group 1) (p=0.039). Fourteen days after surgery mean liver weight was 1249 ± 222.5 g in group 3 compared to only 923 ± 186 g in the controls (Fig. 2).

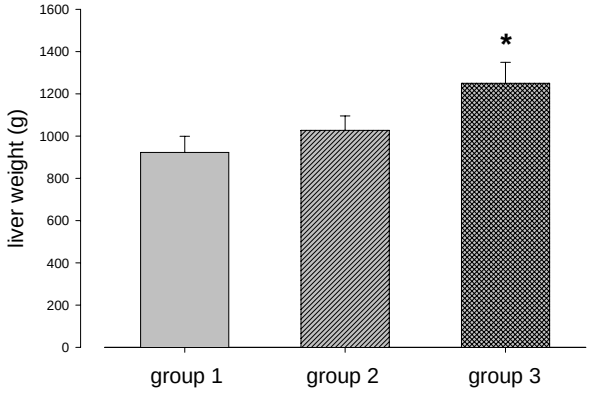


Figure 2: Liver weight 14 days after partial left sided hepatectomy. Systemic administration of EPO produced a 26 % increase of liver weight compared to the control group (p=0.039*).

Topical administration of EPO alone (group 2) did not cause a significant increase in liver weight. Nevertheless, mean liver weight in group 2 was 1027 ± 167 g that was 10.1 % more compared to the controls. A similar result as for liver weight was obtained for liver volume. Fourteen days after surgery mean liver volumes were 813.4 ± 164.6, 894.0 ± 104.7 and 1073 ± 190.1 ml for groups 1-3, respectively. Thus, combined systemic and topical administration of EPO (group 3) led to a 31.9 % increase in liver volume over the control group (p=0.043) (Fig. 3).

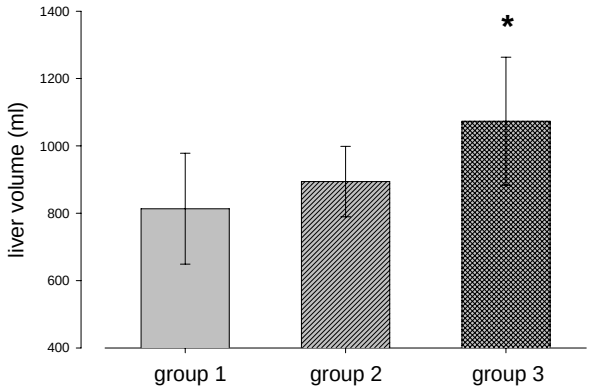


Figure 3: Liver volume 14 days after partial left sided hepatectomy. Systemic administration of EPO produced a 24.2 % increase in liver volume compared to the control group (p=0.043*).

EPO mediated liver weight gain is associated with increased hepatocyte proliferation

Hepatocyte proliferation was quantified by determination of the percentage of Ki-67 positive hepatocyte nuclei before as well as 1 and 14 days after liver resection. The expression of Ki-67 was not significantly different between day 0 and day 1 (Fig. 4A). Additionally, no relevant difference was seen between pigs treated with EPO (groups 2 and 3) and the controls (group 1). However, a clear influence of EPO was observed on day 14 after surgery. The mean percentage of Ki-67 positive nuclei was 1.52 ± 1.17, 3.50 ± 0.98 and 4.30 ± 1.96 % in groups 1, 2 and 3, respectively (Fig. 4A and 4B). Thus, topical administration of EPO alone (group 2) caused a 2.3-fold increase in Ki-67 positive nuclei (p=0.0176), whereas combined topical and systemic administration of EPO (group 3) led to a 2.8-fold increase (p=0.0173). In conclusion, both, topical as well as combined systemic and topical administration of EPO, caused a clear increase in proliferation of hepatocytes close to the resection surface 14 days after surgery. The percentage of PCNA positive nuclei to total nuclei was increased in groups 2 and 3 as compared to group 1, but the difference did not reach statistical significance. In groups 1-3 the percentage of PCNA positive nuclei was 25.8 ± 8.1, 29.9 ± 7.2 and 37.2 ± 14.3, respectively.

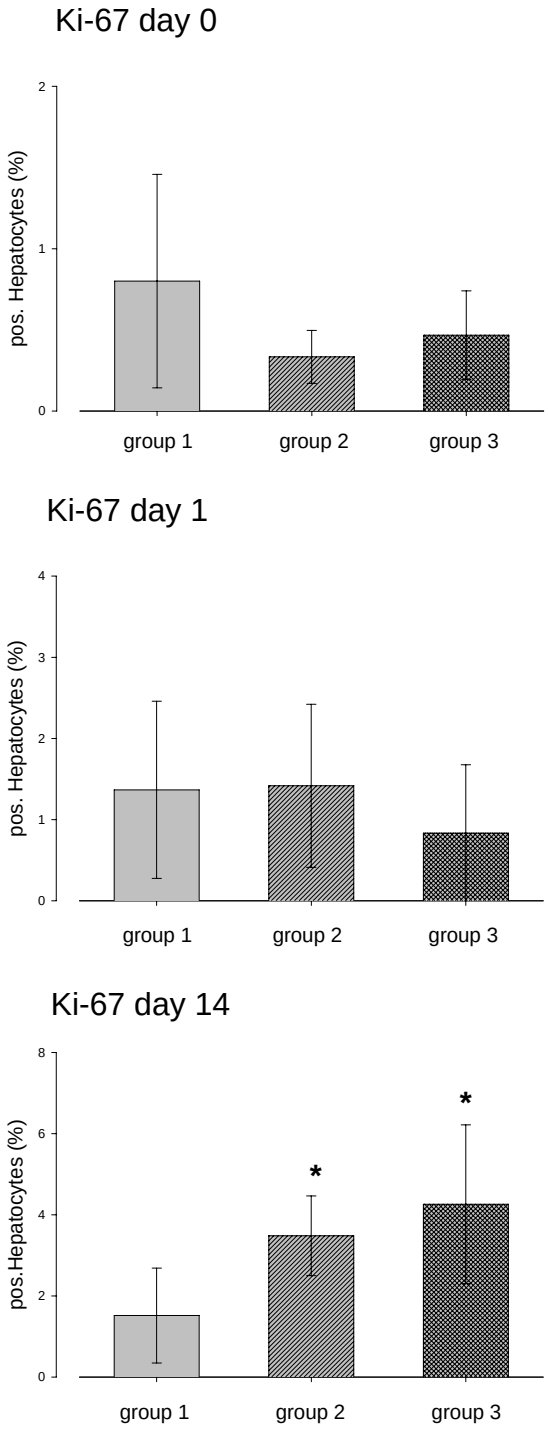
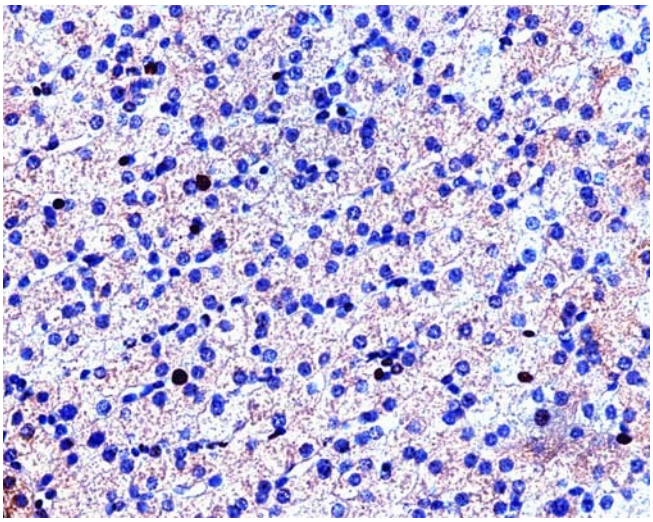


Figure 4.A: Effect of EPO on proliferation of hepatocytes as evidenced by expression of Ki-67. Biopsies were taken 1 cm beneath the resection surface on the day of liver resection, 24 h later and on day 14. Topical and systemic administration of EPO led to a significant increase in hepatocytes with Ki-67 positive nuclei 14 days after liver resection compared to group 1 (p= 0.0176*, p= 0.0173*).

A.



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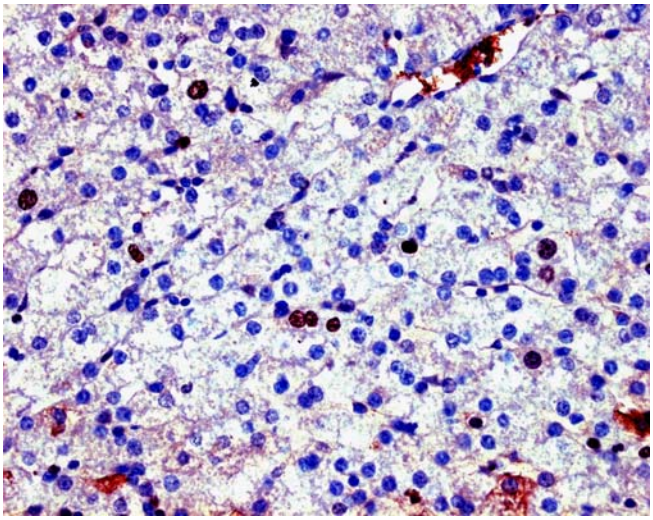

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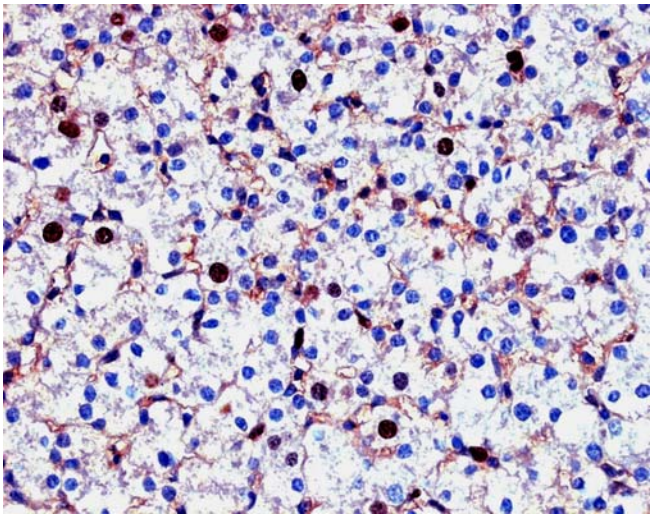


Figure 4.B: Ki-67 immunostaining of liver slices 14 days after liver resection. Ki-67 positive nuclei are stained reddish-brown. Photos A, B and C show representative results from groups 1, 2 and 3, respectively. Magnification: x 400

Systemic but not topic EPO administration stimulates erythropoiesis

In the present study we administered human EPO to pigs using concentrations usually applied for treatment of anaemic patients. In order to analyze whether the human EPO regimen is also functional in pigs we analyzed CD117 expression in bone marrow cells and determined the ratios between erythropoiesis and myelopoiesis at day 14 after surgery. Mean ratios of erythropoiesis to myelopoiesis were 0.42 ± 0.15, 0.47 ± 0.18 and 1.64 ± 0.14 in groups 1, 2, and 3, respectively (Fig. 5). The differences between group 1 and group 3 ($p=0.0043$) as well as between group 2 and group 3 ($p=0.0043$) were significant. Thus, systemic administration of human EPO leads to a stimulation of erythropoiesis also in pigs. As expected the topical administration of EPO in the fibrin sealant did not induce erythropoiesis in the bone marrow.

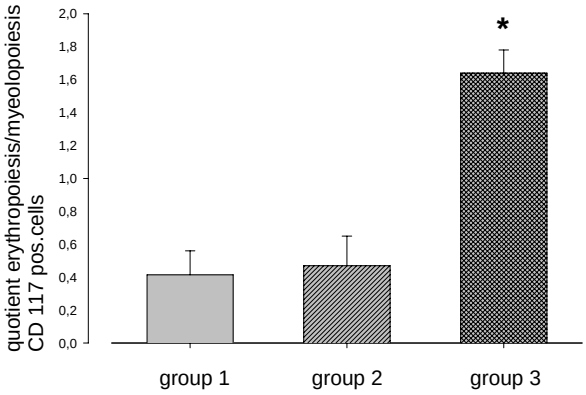
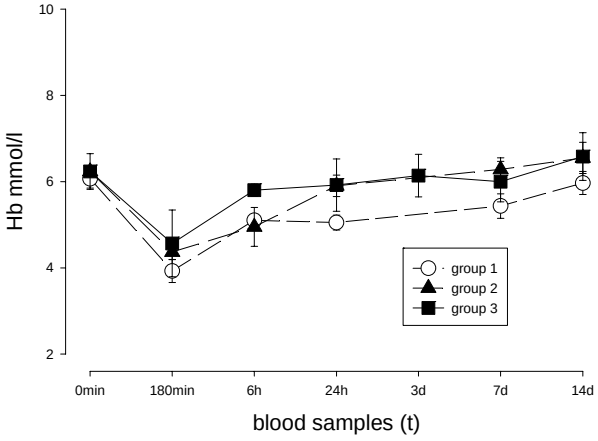


Figure 5: CD 117 staining of bone marrow on day 14 after liver resection. Systemic EPO administration (group 3) leads to a significant increase in the ratio of erythropoiesis/myelopoiesis ($p=0.0043$*).

The mean blood hemoglobin (Hb) level was 6.37 ± 0.44 mmol/l before surgery. Six hours after hepatectomy Hb decreased because of blood loss, resulting in haemoglobin levels of 5.10 ± 0.14, 4.95 ± 0.64 and 5.80 ± 0.14 mmol/l in groups 1-3, respectively. One day after hepatectomy Hb levels were 5.05 ± 0.34 (group 1), 5.9 ± 0.56 (group 2) and 5.92 ± 0.60 (group 3) mmol/l and increased to 6.01 ± 0.66 (group 1), 6.55

± 0.89 (group 2) and 6.6 ± 0.55 (group 3) mmol/l 14 days after surgery. Surgery and EPO administration did not significantly influence hematocrit levels. Prior to liver resection, the mean blood hematocrit level was 0.32 ± 0.05. Six hours after surgery the mean hematocrit levels were 0.30 ± 0.03 (group 1), 0.32 ± 0.05 (group 2) and 0.36 ± 0.04 (group 3). At day 14 after surgery the respective levels were 0.36 ± 1.10 (group 1), 0.39 ± 0.06 (group 2) and 0.40 ± 0.02 (group 3) (Fig. 6).

A **Hemoglobin**



B **Hematocrit**

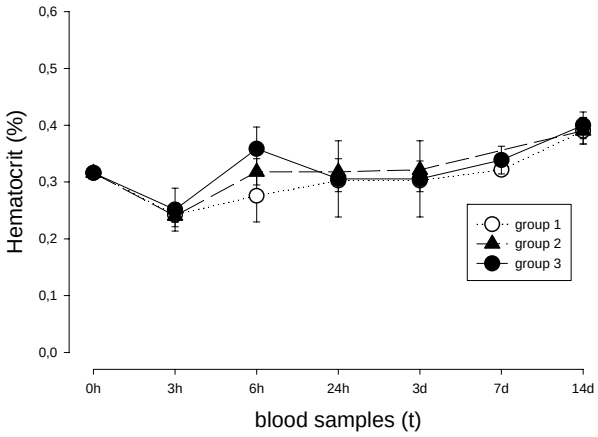


Figure 6: Effect of EPO on hemoglobin levels and on the hematocrit after left lateral sided liver resection. The slight increase in hemoglobin and hematocrit in group 3 did not reach statistical significance.

DISCUSSION

Liver regeneration after hepatectomy: a need for supportive therapy

Despite of the progress in understanding the molecular mechanisms underlying liver regeneration (Michalopoulos and DeFrances, 1997, 2005; Gebhardt et al., 2003), the clinically relevant limits of the ability of the liver to regenerate are not well understood, and there has been little progress in the development of therapy to enhance regeneration and optimize survival when the residual liver after hepatectomy is excessively small (Cataldegirmen et al., 2005). Human liver regeneration is of particular importance in patients who have undergone partial hepatectomy, to patients suffering from fulminant hepatic failure, and to liver transplant recipients. Although the liver has a remarkable regenerative capacity (Kountouras et al., 2001) it nevertheless is often insufficient (Cataldegirmen et al., 2005). In particular, hepatic failure can occur following therapeutic liver resection. Postoperative hepatic insufficiency in a large study by Jarnagin et al. occurred in approximately 5 % (Jarnagin et al., 2002). Therefore, agents improving liver regeneration after hepatectomy would be of considerable benefit for patients. Promising candidates include interleukin 6 (IL-6) and hepatocyte growth factor (HGR) (Borowiak et al. 2004). IL-6 administration in male Wistar rats increases liver weight exemplified by positive immunostaining for PCNA (Ohira et al., 1996). The designer cytokine, Hyper-IL-6, consisting of the soluble IL-6 receptor covalently linked to IL-6 which directly stimulates gp130 and activates intracellular signalling, has been shown to improve liver regeneration after partial hepatectomy in mice (Peters et al., 2000). Previous studies of HGF supplementation in liver resection models in rats have led to a higher proliferation rate (Ishii et al., 1995). However, despite promising data in the field of conceptual research, neither Il-6 or HGF can be applied in clinical studies in the near future, due to their high cost and low availability (Giordano et al., 1993).

Many other factors have been shown to be relevant in liver regeneration (Michalopoulos and DeFrances, 2005) including HB-EGF, norepinephrine, amphiregulin and TGF-α, but these factors are also many years away from being clinically relevant. At a first glance erythropoietin (EPO) would not be considered to be a first line candidate in this field, especially since the adult liver has been reported to express only very low levels of the EPO receptor (Liu et al., 1997; Masuda et al. 1992). However, the observations discussed below suggest that EPO nevertheless represents a promising candidate for preclinical and clinical studies on liver regeneration.

EPO in tissue regeneration: systemic versus cell type specific mechanisms

The primary action of EPO is to prevent the programmed cell death of erythrocytic progenitors and to stimulate their growth and maturation to normoblasts. The action of EPO is induced through its binding to the homodimeric EPO receptor, which is a member of the cytokine class I receptor superfamily (Sawyer, 1989; Swameye et al., 2003). After EPO binding, the two receptor subunits undergo a conformational change resulting in the phosphorylation of tyrosine residues of two Janus kinase 2 (JAK2) molecules and the receptors themselves (Remy et al., 1999). EPO receptor signaling involves the expression of the anti-apoptotic protein bcl-x_L (Gregory et al., 1999), the activation of various protein kinases and the homodimerization of the signal transducer and activator of transcription 5 (STAT5) (Oda and Sawada, 2000; Yoshimura and Misawa, 1998). Recombinant human EPO is used therapeutically to stimulate production, differentiation and maturation of erythroid progenitors in patients with chronic renal failure or those on dialysis (Buemi et al., 2002). The administration of EPO to liver resection patients was first reported in 1994 (Kajikawa et al. 1994) and its purpose was to reduce the need for excessive donor blood since donated blood may have adverse effects on the immune system. This study demonstrated a swifter decrease in serum bilirubin levels (indicating a return to normal) following postoperative EPO treatment as well as a reduction in postoperative complications. A second trial (Shinozuka et al., 2000) in which EPO was administrated preoperatively to liver resection patients to stimulate erythropoiesis, similarly demonstrated that patients given EPO experienced a significant reduction in postoperative complications compared to the control group. Both studies concentrated on the systemic effects of EPO, especially to avoid the need for donor blood, and did not discuss a possible direct influence of EPO on liver regeneration.

Meanwhile, knowledge has improved about the physiological functions of EPO beyond the classical stimulating effect on erythropoiesis: a survey of rat organs has revealed hypoxia-inducible expression of EPO mRNA in testis, brain, liver and kidney (Tan et al., 1992). EPO enhances the maturation of oligodendrocytes and the proliferation of astrocytes (Sugawa et al., 2002). In cardiomyoblasts expression of EPO receptors and EPO binding stimulate cell proliferation and repress cell differentiation (Ogilvie et al., 2000). EPO receptors were identified on human hepatoma cells of the line Hep 3B (Ohigashi et al., 1996). Recently, expression and signaling through the EPO receptor has been demonstrated in a variety of non-hematopoietic organs, including the brain (neurons and glia), cardiovascular tissues (endothelium, vascular smooth muscle, cardiomyocytes), gastrointestinal tissues, pancreatic islands, the kidney, the testis and female reproductive organs (Juul, 2000; Moritz et al., 1997; Sasaki et al., 2000; Adamson and Ludwig, 1999). Clearly, EPO is a more pleiotropic survival and growth factor than earlier thought. In particular, the neurotrophic and neuroprotective effects of EPO have been documented in detail (Cerami et al., 2002; Chong et al., 2003; Marti et al., 2000; Masuda et al., 1999). Recently, recombinant human EPO has been administered successfully to patients with acute stroke for reduction of brain infarct size (Ehrenreich et al.,

2002). With respect to the effects of EPO in the vascular system, the growth factor induces a pro-angiogenic phenotype of endothelial cells and neo-vascularization (Jaquet et al., 2002; Carlini et al., 1995). Furthermore, EPO promotes blood vessel formation in the uterus of ovariectomized mice (Yasuda et al., 1998). The signaling pathways in neuronal cells (Chong et al., 2003) and in vascular cells (Fodinger et al., 2000; Haller et al., 1996) involve the activation of kinases and transcription factors as in erythrocytic cells.

Topical versus systemic administration of EPO to pigs after hepatectomy

Liver regeneration has been investigated mostly in rats or in vitro with hepatocyte cultures. Because of the anatomical and physiological similarities between human and porcine livers, pig liver resection and transplantation models have been the backbone of experimental surgical research in this field (Swindle, 1984; Swindle et al., 1988; Court et al., 2003). Unfortunately, porcine EPO was not available for our studies. Therefore, human EPO was used because of its ample availability. Previous studies have already described positive effects of human EPO in pigs (Romsi et al., 2002; Vogel et al., 1997), and Wen et al. (1993) have demonstrated that human and pig EPO share 82 % amino acid identity. For the EPO dosage, we used a concentration close to the upper limit recommended for the treatment of tumor-related anemia in human patients (Adamson and Ludwig, 1999). Analysis of CD 117 antibody, a marker for hematopoetic stem cells found in pig bone marrow, demonstrated a significant increase following systemic administration of EPO in our study. Erythropoiesis was stimulated in the bone marrow after systemic administration of EPO (group 3), suggesting that human EPO is functional in pigs under our study conditions.

Combined topical and systemic administration of EPO led to a 26 % increase in liver weight 14 days after hepatectomy. The accelerated weight gain was accompanied by a 2.8-fold increase in proliferating hepatocytes. In contrast, topical EPO application alone had no significant effect on liver weight, but also increased hepatocyte proliferation close to the resection surface of the left liver lobe, where EPO had been topically administered in the fibrin sealant. The latter result suggests a direct influence of EPO on the liver, since under our study conditions topical EPO administration was not associated with systemic effects such as erythropoiesis in the bone marrow. Clearly combined systemic and topical administration of EPO was much more efficient and should be used in further clinical studies. The EPO induced increase in hepatocyte proliferation was plainly detectable using an antibody against Ki-67. In contrast, immunostaining for PCNA did not result in a significant difference between EPO exposed pigs and controls. This discrepancy was not unexpected. Ki-67 immunostaining is a well accepted marker specific for cell proliferation that has been demonstrated to correlate well with BrdU incorporation (Muskhelishvili et al., 2003). In contrast, a correlation between PCNA expression and proliferation, as evidenced by BrdU incorporation, has not consistently been observed, since PCNA is also involved in other processes than proliferation, such as stress response and DNA-repair (Muskhelishvili et al., 2003; Zou et al., 2003).

Activation of anti-apoptotic or proliferative signal transduction pathways by EPO seems to be improbable since adult hepatocytes express no or very low levels of the EPO receptor (Masuda et al., 1992). Alternatively EPO may be inducing angiogenesis in the regenerating liver. EPO has been shown to have a role in promoting angiogenesis, acting as a survival factor for endothelial cells as well as increasing the amount of circulating progenitor endothelial cells (Heeschen et al., 2003). More recent publications related to the angiogenic potential of EPO further describe the indirect role for EPO in liver regeneration. Among these publications are two which use animal models to illustrate the role of angiogenic factors and endothelial cells in

the regeneration of liver. A 2003 Science publication (LeCouter et al., 2003) reported that following liver damage, hepatocytes were shown to secrete VEGF-A which stimulated liver endothelium to proliferate and in turn release IL-6 and HGF. A second publication (Greene et al., 2003) confirms that the stimulation of endothelial proliferation and migration through fibroblast growth factor significantly increases the rate of regeneration of resected liver while an inhibitor of endothelial cells likewise stunts liver regeneration. These data indicate an indirect effect of EPO through angiogenic mechanisms, whereas our study suggests also a direct effect on hepatocytes. In our experiments we compared EPO mediated phosphorylation of Akt/PKB in hepatocytes co-cultured with non-parenchymal liver cells to hepatocyte mono-cultures and did not observe a relevant difference. Therefore, phosphorylation of Akt/PKB seems to be independent from the non-parenchymal liver cells. Of course this does not exclude that other EPO mediated effects on hepatocytes could be mediated by endothelial or other types of non-parenchymal liver cells. Both indirect and direct effects may synergize to result in the observed accelerated regeneration.

Erythropoietin: a first line candidate for clinical studies in liver regeneration

Combined systemic and topical administration of EPO caused a 26 % increase in liver weight 14 days after hepatectomy, which corresponds to more than 300 g of additional liver tissue in the EPO treated compared to the control group. Should EPO induce a similar improvement of liver regeneration also in humans a reduction of frequency as well as severity of complications after liver resection can be expected. Particularly, the risk of hepatic failure occurring after liver resection will be reduced if regeneration of the residual liver is improved. Therefore, we have initiated a double-blind, randomized, placebo-controlled, monocentre clinical trial in patients treated post-operatively with erythropoietin, monitoring regeneration rates in patients undergoing liver resection.

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