rHuEPo Reduces Ischemia-Reperfusion Injury and Improves Survival After Transplantation of Fatty Livers in Rats

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Background. The scarcity of appropriate donor organs remains to be a major problem in transplantation surgery today. This has led to increased acceptance of so-called marginal grafts, incorporating the increased risk of poor posttransplant function. Erythropoietin has been shown to reduce ischemia-reperfusion injury in transplanted rat livers. We investigated whether these capacities may contribute to improve marginal organ function.
Methods. One hundred and forty Lewis rats were used. Fatty liver (≥50% steatosis) was induced by a special diet in 70 donor animals. Seventy recipients received liver transplantation after donor organ treatment with 1000 IU rhuEpo or saline injection (controls) into portal veins (cold ischemia 6 hr, University of Wisconsin solution). Recipients were allocated to two groups which received 1000 IU rHuEpo at reperfusion or an equal amount of saline (control). Analysis of liver enzymes, histology (hematoxylin-eosin and periodic acid Schiff stain), immunostaining (terminal deoxynucleotide tranferase-mediated dUTP nick-end labeling, hypoxyprobe, and tumor necrosis factor-α), and reverse transcriptase-polymerase chain reaction of cytokine messenger RNA (interleukin-1, interleukin-6, hypoxia induced factor-1α, vascular endothelial growth factor, and hepatocyte growth factor) were performed at defined time points (2, 4.5, 24, 48 hr, and 7 days postoperatively).
Results. Alanine aminotransferase values were significantly reduced for epo-treated rats 48 hr after reperfusion; however, at all other time points enzyme levels were without significant differences. Terminal deoxynucleotide tranferase-mediated dUTP nick-end labeling and hypoxyprobe analysis and necrotic index evaluation displayed significant reduction of apoptosis and hypoxic cells in rHuEpo-treated graft livers. Overall survival was significantly improved among epo-treated rats.
Conclusion. Erythropoietin improves marginal graft function and recipient survival after transplantation of fatty livers in rats.

Keywords: Epo, Erythropoietin, Fatty liver, Liver transplantation, Marginal organs, Ischemia-reperfusion injury.

(*Transplantation* 2010;89: 161–168)

The growing demand for adequate donor organs in transplantation surgery has led to the increased acceptance and transplantation of so-called marginal grafts.

However, the limited quality of the graft in addition to cold storage and ischemia-reperfusion (I/R) injury may result in poor posttransplant function (*1–3*).

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Received 17 July 2009. Revision requested 17 August 2009.
Accepted 16 September 2009.

ISSN 0041-1337/10/8902-161
DOI: 10.1097/TP.0b013e3181c425fd

Therefore, graft function may substantially be improved not only by limiting ischemic time but also through protection of the organ by reducing the impact of I/R injury and improving regenerative capacities of the transplant.

As steatosis of the liver graft accounts for a major risk factor increasing the risk of posttransplant liver insufficiency, we chose to establish a small animal model of fatty liver transplantation to resemble the clinical situation of a "marginal graft" (*4–9*).

Erythropoietin (rHuEpo) has been shown to protect various tissue types from ischemic damage, improving posttraumatic organ function (*10–14*). Our group and others (*15–17*) have detected protective capacities for rHuEpo in warm hepatic ischemia in a rat model. These findings have been confirmed in a liver transplantation model demonstrating significantly reduced I/R injury in rats treated with epo (*18*). Furthermore, we were able to display enhanced regeneration and survival for rHuEpo-treated rats after large-volume hepatectomy (*19*).

rHuEPo is an endogenous hormone, which has been routinely applied to patients for many years. Current indications include tumor anemia and substitution for patients on dialysis. Large doses of rHuEpo can significantly increase the

risk of thrombosis; however, the overall spectrum of side effects is rather limited (*20*, *21*).

Various studies have elucidated the mechanisms of rHuEpo-mediated cell protection and reduction of apoptosis (*22*, *23*). The tissue-protective capacities of rHuEpo are mediated by the Jak-2 pathway and an Akt-dependent intracellular cascade downregulating the expression of proapoptotic mediators and molecules (*24*, *25*). The synthesis of cell-protective substances and signaling proteins such as STAT-3 is increased facilitating intracellular homeostasis by blocking apoptotic mechanisms. The exact molecular cascade by which rHuEpo eventually mediates cellular injury remains to be elaborated at this point.

The aim of this study was to evaluate the potential effect of rHuEpo preconditioning and treatment on posttransplant graft function in a rat model of marginal graft liver transplantation.

MATERIALS AND METHODS

Animals

Male Lewis rats (body weight 250–300 g; Charles River Laboratories, Sulzfeld, Germany) were used for the experiments. Animals were housed in standard animal laboratories with a 12-hr light-dark cycle and had free access to water and standard laboratory chow ad libitum. The experimental design was reviewed and approved by the local government (Senator fuer Gesundheit und Soziales, Berlin) and the ethics committee of the Charité University Hospital, and carried out according to the European Union regulations for animal experiments and the "*Guide for the Care and Use of Laboratory Animals*" (DHEW Publication No. (NIH) 85-23, Revised 1985, Office of Science and Health Reports, DRR/NIH, Bethesda, MD). A veterinarian responsible for the treatment and handling of laboratory animals in our institution was present at all times.

One hundred and forty Lewis rats were used for the experiments. Seventy rats served as liver donors. All donor animals had been fed by a special diet for 8 days before organ harvest. This choline-free diet induces fatty involution of the liver with a degree of at least 50% steatosis after 8 days. (This had been clarified in previous experiments, see Fig. 1).

Thirty-five of these donor animals received a dose of 4000 IU rHuEpo/kg body weight (0.1 mL NeoRecormon [Epoetin beta, Roche Pharma, Welwyn Garden City, Great Britain] diluted in saline solution) into the portal vein 30 min before harvest. The other 35 donor rats were treated with an intraportalvenous saline injection of identical volume 30 min before organ removal.

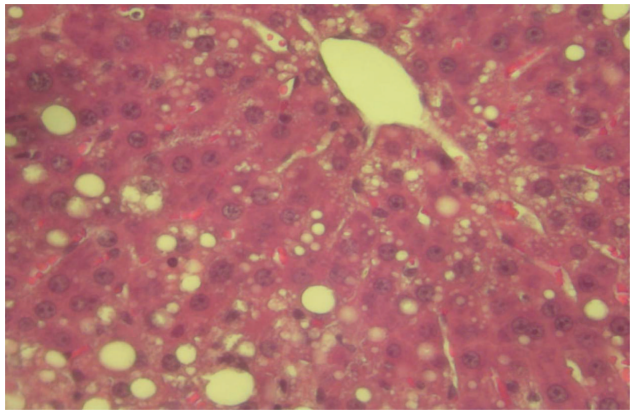

FIGURE 1. Steatotic liver graft (steatosis ≥50%).

Seventy rats served as recipients. Because of the extremely strenuous procedure, we calculated a potential dropout rate of approximately 25%, therefore a minimum of five animals alive at each scheduled sacrifice time point was intended. For later time points (48 hr, 7 days), where immunohistologic evaluation was the top priority, a minimum group size of four animals was aimed at.

Animals were allocated to two groups. Group 1 (n=30; 26 survived/4 died before scheduled sacrifice, all within the first 4 hr after liver transplantation [LT]) received a bolus injection of 4000 IU rHuEpo/kg body weight into the portal vein immediately at reperfusion. Donor livers for these recipients had been pretreated with an equal dose 30 min before harvesting. Epo doses were chosen in accordance to previously published protocols (*13*, *16*, *19*).

Group 2 (n=40; 26 survived/14 died before scheduled sacrifice, all within the first 4 hr after LT) received 0.1 mL saline as a bolus injection (0.1 mL) into the portal vein at the time of reperfusion. Liver graft for these recipients had not been pretreated with rhuEpo but with saline injection.

Animals were sacrificed 2, 4.5, 24, 48 hr, and 7 days after transplantation (n=6 each, n=4 for 2 hr and 7 days) for collection of serum (not for animals sacrificed after 2 hr and after 7 days) and liver tissue.

Surgical Procedure

Donor organ harvest: Anesthesia was performed using isoflurane/air inhalation with 40% oxygen. Ten minutes before incision of the abdominal wall animals received an intraperitoneal injection of 5 mg/kg tramadol for analgesia (identical procedure for allotransplantation).

The abdomen was opened by a midline incision, the liver was mobilized from all ligamentous attachments, and the hepatic artery and the portal vein were isolated. The distal aorta was cannulated with a 14 G catheter; the supratruncal aorta and both renal arteries were ligated. Aortic perfusion was performed by gravity with a pressure of 120 cm H_2O, portal perfusion was executed with a pressure of 20 cm H_2O. Taking into account the gradual rewarming after cold storage perfusion in the clinical setting of multiorgan procurement, all livers remained within the body for an additional 30 min before removal and back-table perfusion.

Allotransplantation: Donor livers underwent 6 hr of cold ischemic storage in University of Wisconsin solution. To resemble the warm ischemia time during human liver transplantation procedures, the livers were kept at 21°C (room temperature) for 15 min before surgical implantation started. All livers were reflushed with 10 mL saline solution through the portal vein and weighed. Orthotopic rat liver transplantation was performed with hepatic artery revascularization and bile duct reconstruction by stent implantation. Except for hepatic artery and bile duct, all anastomoses were conducted by hand-sewn sutures. Portal clamping time in all transplantation procedures included in this study was less than 18 min.

After reperfusion and control of potential bleeding sources the abdominal cavity was closed, and the animals were placed in their cages for postoperative monitoring. All animals received

analgesic treatment with 10 mg/kg of Tramadol by intraperitoneal injection immediately before closure of the abdomen.

Animal Sacrifice and Organ Harvest

All animals received complete anesthesia at 2, 4.5, 24, 48 hr, or 7 days after transplantation. The abdomen was opened again and a clamp was placed on the infrarenal aorta. Blood was drawn from the aorta until circulation stopped. The heart was incised to secure animal death; liver tissue was harvested and forwarded to histologic and polymerase chain reaction (PCR) evaluation.

Hepatocellular Damage

The extent of hepatocellular damage was assessed at 4.5, 24, and 48 hr after ischemia by spectrophotometric determination of aspartate aminotransferase and alanine aminotransferase (ALT) using a commercially available reaction kit (Roche Diagnostics, Mannheim, Germany). (Evaluation of liver enzymes 2 hr after LT was not performed due to preliminary experiments showing a transaminase peak at approximately 6 hr postoperatively. After 7 days, liver enzymes had almost normalized in all previous experiments in all animals. We therefore abstained from evaluation of 2 hr and 7 day enzyme values.)

Histology

Remnant liver tissue was fixed in 4% phosphate-buffered formalin for 2 to 3 days and then embedded in paraffin. From the paraffin-embedded tissue blocks, 5 μm sections were cut and stained with hematoxylin-eosin (H&E) and periodic acid Schiff stain (PAS). For each animal and time point, nine high power fields were analyzed, and necrotic, apoptotic, and mitotic cells were counted.

To evaluate hepatocyte replication, mitotic figures were counted in 1000 hepatocytes (200-fold magnification) and analyzed as mitotic index (number of mitotic figures per 1000 hepatocytes). All slides were judged by the same investigator who had been blinded to the corresponding study group.

Terminal Deoxynucleotide Tranferase-Mediated dUTP Nick-End Labeling/Apoptosis Detection

To identify apoptosis in hepatocytes, the TACS TdT in situ apoptosis detection kit was used in sequential order according to the procedures of the manufacturer (R&D Systems). Briefly, after cryopreservation and cutting of tissue slices, the cryosection was first fixed in cold acetone and permeabilized in Cytonin solution for 60 min. Thereafter, chymase was stained by adding the primary anti-chymase mAb (1 mg/mL) for 60 min at 37°C, the alkaline phosphatase-conjugated secondary Ab (1:100) (Vector) for 60 min at room temperature, and finally Red Label Substrate (R&D Systems) for 30 min. Next, endogenous peroxidase activity was quenched in 3% H_2O_2 for 5 min followed by incubation in the TdT labeling buffer for 5 min. The labeling reaction was initiated by adding 50 mL per slide of the TdT labeling buffer containing TdT-dNTPs, Mn2, and TdT enzyme.

After 60 min, reaction period at 37°C, the labeling was stopped in TdT Stop buffer for 5 min. The slides were washed twice in H_2O, incubated in streptavidin-HRP for 10 min, washed again twice in H_2O, and finally incubated in TACS Blue Label for 2 to 5 min. DNase-free deionized water was used throughout the procedure. The slides were processed through increasing concentrations of ethanol, then xylene and finally covered with Depex.

TACS-nuclease-treated sample was used as a control for labeling reaction and a slide stained without TdT enzyme was used as a negative control. Positive tissue control slides for apoptosis were provided by R&D Systems. The hepatocyte was judged to be apoptotic when the red color for cytoplasmic chymase and bluish color for nuclear apoptosis were seen in close contact, and the morphologic criteria for apoptosis were fulfilled. The cells were counted as explained earlier, and the apoptosis index (%) was defined as the number of apoptotic hepatocytes in relation to all chymase-positive cells. Examination and grading of all tissue samples was performed by the same investigator who had been blinded to the corresponding study group.

Hypoxyprobe

We used the HypoxyprobeTM-1 Kit for detection of tissue hypoxia (Chemicon, USA).

The marker Pimonidazole possesses a heterocyclic nitro-group (nitroimidazole). This is a substance which is reduced to a nitro-radical anion by intracellular nitro-reductases. This anion binds to intracellular makro-molecules, usually RNA. The reduced anion is stabilized for this binding procedure only in the absence of oxygen, therefore stable binding can only occur in hypoxic or anoxic cells.

Animals were treated with 60 mg/kg body weight of hypoxyprobe 30 min before they were sacrificed. Five-micrometer slices of paraffinized liver tissue were used for staining according to the manufacturer's recommendations in the Dako Cytomation Autostainer (Dako Cytomation, Hamburg, Germany).

Tumor necrosis factor (TNF)-α was measured by immunohistological staining using a polyclonal antibody in a 1:250 dilution (Biomol Inc., Hamburg, Germany) according to the manufacturers' recommendations.

Interleukin-1b, Interleukin-6, TNF-α, Hypoxia Induced Factor-1α, Hepatocyte Growth Factor, and Vascular Endothelial Growth Factor Messenger RNA Expressions Were Analyzed Using Noncompetitive Semiquantitative Reverse Transcriptase-PCR

Expression of messenger RNA (mRNA) in liver and tissue samples was analyzed by reverse transcriptase-PCR (RT-PCR). For RNA isolation by phenol extraction we used a TRIZOL reaction kit. Every sample was incubated with Desoxypolymerase I (DNAse) for 15 min to exclude DNA contamination thereafter. After photometric determination of RNA purity and concentration, it was stored in aliquots of 10 μL at –80°C. RNA was amplified using a RT-PCR kit (Boehringer, Mannheim, Germany) that combines reverse transcriptase and taq polymerase activity in a final reaction volume of 50 μL. Controls using no RNA or only taq polymerase without reverse transcriptase activity were performed. Amplification products were analyzed for size and quantity by gel electrophoresis (agarosis 2%). In addition to cytokines, ß-actin was also determined for each sample to assess differences in RNA concentration. Primers for ß-actin were designed to anneal in two different exons to further control possible DNA contamination that would result in a bigger size PCR product including the intron in-between. Finally, the amplification products of all cytokines were analyzed for correct sequence by restriction

analysis. (Primers were all commercially obtained from Quiagen Corp., Hilden, Germany.)

Total absolute values were normalized with respect to GAPDH; the quotient of the measured parameter/GAPDH was then multiplied by ×1000 to receive whole numbers.

Statistical Analysis

Results of liver enzyme measurement were expressed as mean values with standard deviations. After proving the assumption of normality and equal variance across respective groups, differences between groups were assessed using analysis of variance (overall differences) followed by Fisher's exact test. In all instances, *P* values less than 0.05 were considered to be statistically significant. Longitudinal laboratory data were analyzed using a linear regression model.

SPSS software was used for statistical analysis (SPSS version 16.0, SPSS Inc., USA); graphical design was performed using SPSS and Sigma-Plot (Sigma-Plot version 8.0, SPSS Inc., USA).

RESULTS

Liver Enzymes

All liver enzymes measured in this experimental setting increased extensively after LT, which can be identified in Figure 2.

Mean total enzyme values were partially increased for epo-treated animals compared with controls; however, this was not statistically significant. 48 hr after reperfusion ALT values were significantly reduced for epo-treated rats ($P<0.05$), at all other time points differences between epo-treated and control animals were without statistical significance. Standard deviations were rather large (n=7 for each time point except n=5 for 7 days).

Histology

H&E and PAS staining revealed the amount of necrotic cell damage after LT and the associated I/R injury: While the epo-treated group displayed increased necrosis rates 2 and 4.5 hr after reperfusion compared with controls (0.44 vs. 0.11 and 0.37 vs. 0.15), 24 hr after LT epo-treated animals presented with less necrotic liver cells (0.67 vs. 0.75), continuously decreasing until 7 days after LT (0.0014 vs. 0.132; Fig. 2).

Immunohistologic Staining: Terminal Deoxynucleotide Tranferase-Mediated dUTP Nick-End Labeling/Hypoxyprobe

The degree of apoptosis in graft tissue was determined by terminal deoxynucleotide tranferase-mediated dUTP nick-end labeling (TUNEL) staining. Two hours after reperfusion, the degree of apoptosis was 29% vs. 38%, after 4.5 hr this difference was significant with 13.2% vs. 26.4% ($P=0.048$). This trend continues until 48 hr after I/R with 29.5% for the epo-group vs. 37.5% for controls.

Hypoxyprobe staining revealed significantly less hypoxic cells 4.5 hr after reperfusion (13.6 vs. 27.4; $P=0.014$). At all other time points, hypoxyprobe staining displayed less hypoxia for epo-treated animals; however, these differences were without statistical significance (Figs. 3 and 4).

Interleukin-1ß mRNA Expression

Interleukin (IL)-1β mRNA expression was measured at 2, 4.5, 24, and 48 hr after I/R. Although IL-1ß mRNA expression was increased for epo-treated rats at all time points, these differences were without statistical significance.

IL-6 mRNA Expression

IL-6 mRNA expression was increased for epo-treated animals 2, 4.5, and 24 hr after reperfusion (152.9±216.5 vs. 124.5±63.3 [2 hr]; 118.4±199.2 vs. 48.7±57.9 [4.5 hr]; 23.4±34.1 vs. 4.7±2.7 [24 hr]). However, none of these differences reached statistical significance.

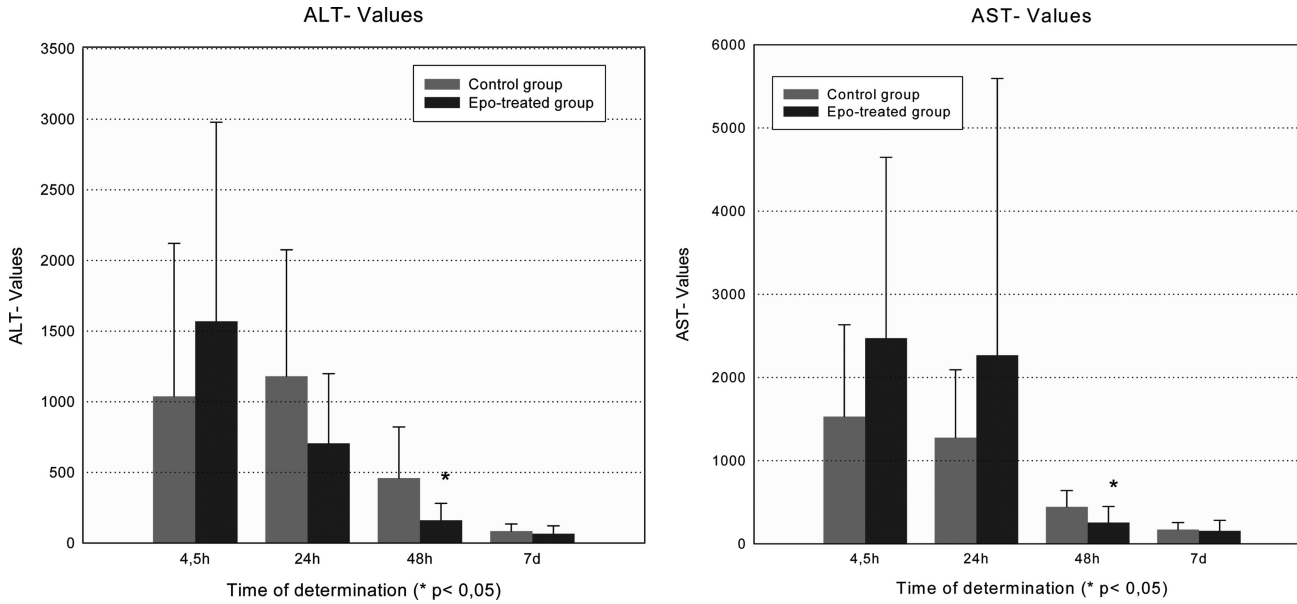

FIGURE 2. Alanine aminotransferase (ALT) and aspartate aminotransferase values at corresponding time points after reperfusion.

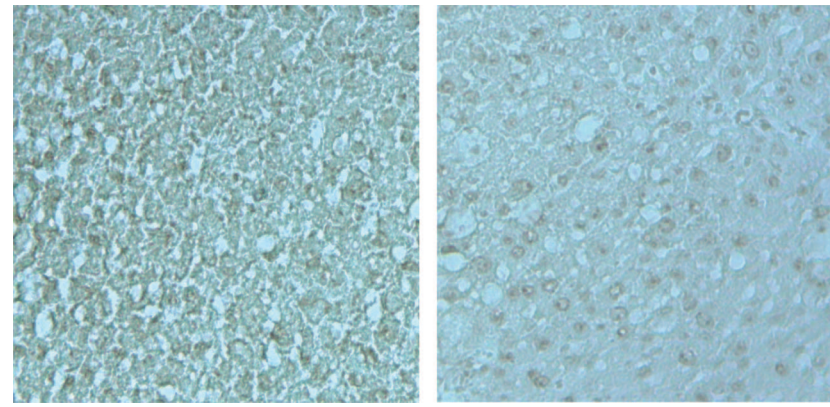

FIGURE 3. Terminal deoxynucleotide tranferase-mediated dUTP nick-end labeling (TUNEL) staining displays apoptotic cells 4.5 hr after reperfusion.

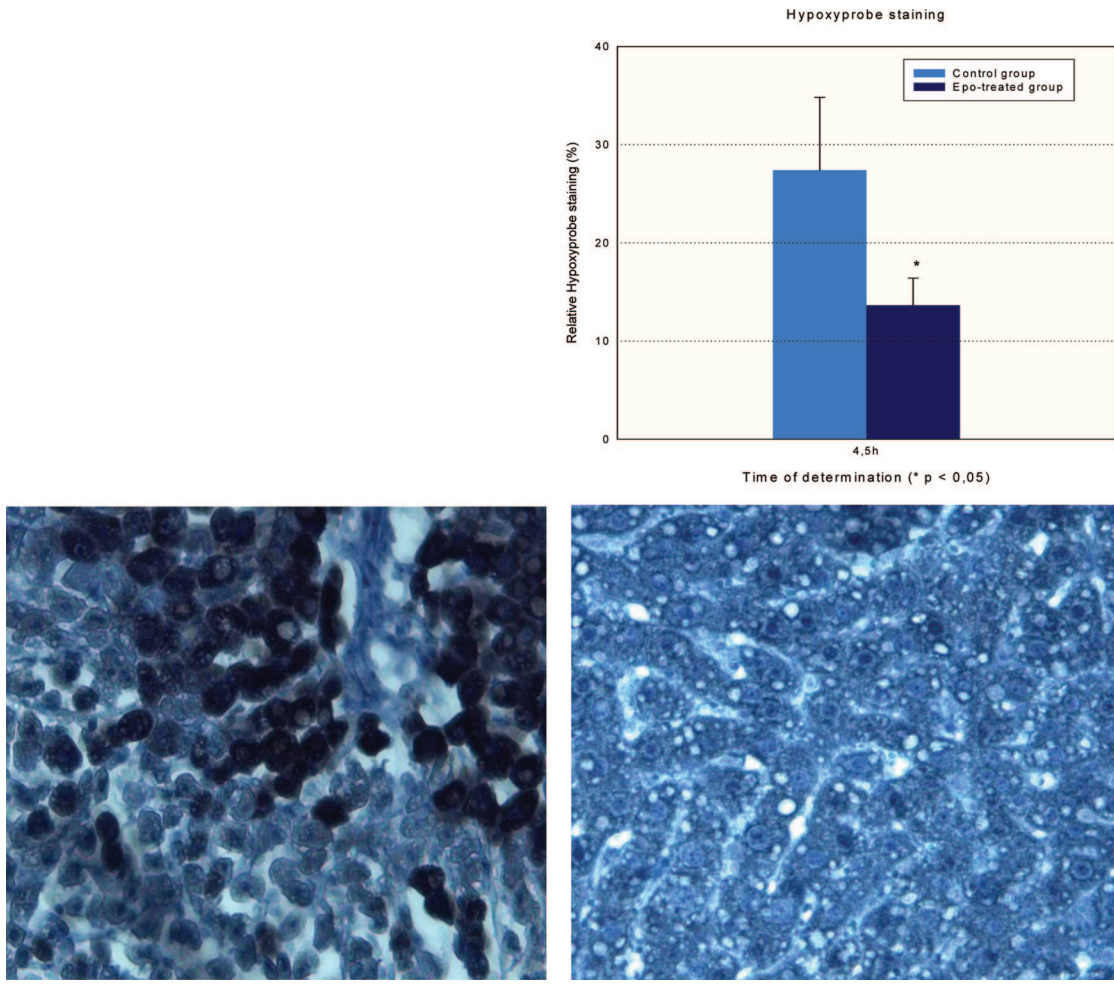

FIGURE 4. Hypoxyprobe immunostaining displays hypoxic cell damage 4.5 hr after reperfusion.

TNF-α mRNA Expression

TNF-α mRNA was measured 24 and 48 hr after reperfusion by immunohistological staining. At 24 hr after I/R values were lower for epo-treated animals (0.75 vs. 1.0), after 48 hr this difference reached statistical significance with 0.14 vs. 0.86 (P=0.017; Fig. 5).

Hypoxia Induced Factor-1α-mRNA Expression

Hypoxia induced factor (HIF)-1α-mRNA was measured at 2, 4.5, 24, and 48 hr after reperfusion. mRNA expression was increased for epo-treated animals at all time points; however, statistical significance was not reached (17.3 vs. 8.3 at 2 hr, 15.7 vs. 7.4 after 4.5 hr, 220.2 vs. 118.9 after 24 hr, 220.9 vs. 191.3 after 48 hr).

Cytokines Associated With Regeneration

Vascular Endothelial Growth Factor-mRNA Expression

Vascular endothelial growth factor (VEGF) mRNA expression was measured at 2, 4.5, 24, 48 hr, and 7 days after I/R.

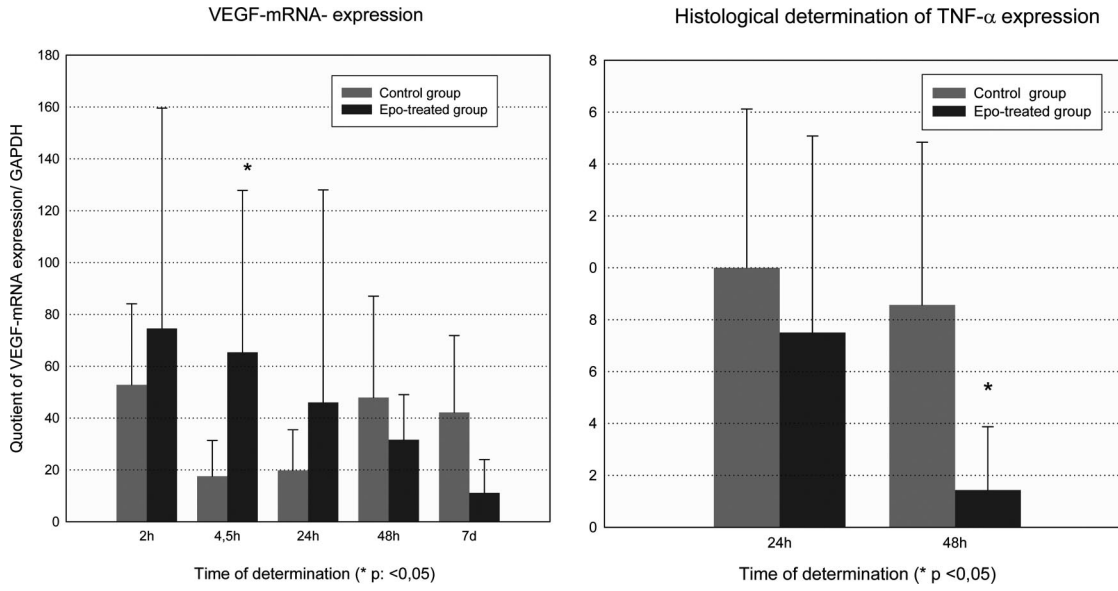

FIGURE 5. Tumor necrosis factor (TNF)-α concentration determined by immunohistological staining 24 and 48 hr after reperfusion. After 48 hr, I/R epo-treated animals display significantly less TNF-α activation. Vascular endothelial growth factor (VEGF) messenger RNA (mRNA) expression determined by reverse transcriptase-polymerase chain reaction. 4.5 hr after reperfusion VEGF mRNA expression is significantly increased for epo-treated animals.

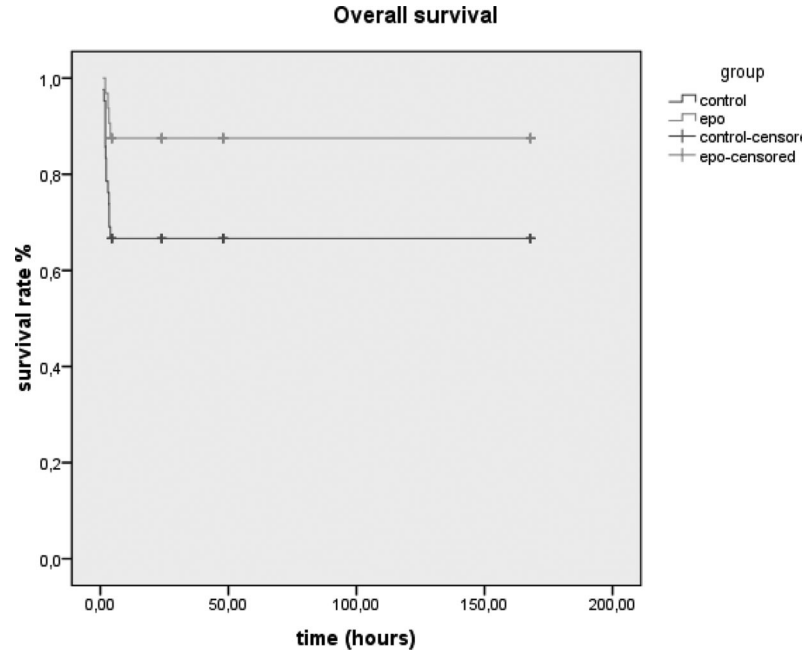

FIGURE 6. Kaplan-Meier analysis displays significantly improved survival for animals treated with rHuEpo (84% vs. 68%).

At all early time points, VEGF-mRNA was more pronounced among epo-treated animals (74.5 vs. 52.8 after 2 hr, 65.3 vs. 17.6 after 4.5 hr, and 46.0 vs. 19.8 after 24 hr) with statistical significance after 4.5 hr.

This trend turned around at later time points with stronger VEGF-mRNA expression for controls after 48 hr and 7 days (47.9 vs. 31.6 and 42.1 vs. 11.1; Fig. 6).

Hepatocyte Growth Factor-mRNA Expression

Hepatocyte growth factor (HGF)-mRNA was determined 2, 4.5, 24, 48 hr, and 7 days after liver transplantation.

During the first 24 hr, HGF-mRNA expression was elevated for epo-treated rats compared with controls; however, differences were without statistical significance (504 vs. 482 after 2 hr, 176 vs. 80 after 4.5 hr, 260 vs. 126 after 24 hr). In the later course, this trend turned around with slightly stronger HGF-mRNA expression among controls after 48 hr and 7 days (130 vs. 127 and 432 vs. 409, no statistical significance).

Survival

For all animals that were scheduled to survive longer than 4 hr after liver transplantation, cumulative survival was

determined. Epo-treated rats showed significantly improved survival compared with controls (84 vs. 68%, $P=0.031$; see Fig. 6).

DISCUSSION

The presented data demonstrate that rhuEpo application to donor livers and to recipients at reperfusion attenuates I/R injury and improves survival after transplantation of fatty livers in rats.

This is reflected not so much by a reduction in liver enzyme levels, in fact, only ALT values were significantly reduced 48 hr after I/R injury for epo-treated animals. This finding stands slightly in contrast to our previously presented results on liver transplantation of nonfatty livers under epo-treatment (*18*). The reason for almost identical liver enzyme activation with or without epo application might be found in the fatty liver issue itself which generates larger amounts of enzyme after I/R damage than normal liver tissue does. The effect of epo-treatment on the detectable enzyme level may be limited by the shear amount of enzyme present.

Hypoxyprobe-immunostaining displayed significantly less hypoxic alteration in epo-treated graft hepatocytes. The attenuation of hypoxic damage is followed by a reduced extent of liver tissue necrosis compared with untreated controls.

In our study, this is reflected by TUNEL staining and evaluation of H&E- and PAS-stained tissue to determine the amount of apoptosis and necrosis in liver graft tissue. Both mechanisms of cell death play important roles in I/R injury. Malhi et al. (*26*) published a thorough analysis on the importance of both apoptosis and necrosis in liver cell death after I/R injury.

Increased apoptosis and necrosis rates in control animal livers compared with epo-treated rats were detected as early as 2 hr after I/R injury. When analyzing the extent of apoptosis and necrosis in the presented study, it may be suggested that epo-treatment reduces the number of cells undergoing apoptosis by directly protecting them from fatal I/R injury. Apparently, necrotic cell tissue, although present to an even greater extent than in control animals early after I/R injury, can be eliminated more effectively than without epo-treatment. This may be reflected by the larger amounts of necrotic tissue in control animals at later time points. Thus, rHuEpo may not only protect cells directly from fatal I/R injury but may also improve cellular "remodeling" leading to faster and more sustained organ regeneration. Our group has been able to demonstrate this effect of improved regeneration in a rat model of large liver resection (*19*); similar findings have been demonstrated concerning regeneration of cardiomyocytes and neuronal cells (*11*, *27–29*).

mRNA analysis of various cytokines did not display a clear-cut picture on the extent of I/R injury with values partially varying extensively. However, more intense analysis of our cytokine data shows a rather global increase in regeneration-associated parameters like VEGF, HGF, and IL-6 among epo-treated rats compared with controls. IL-6 has been named to be an important factor involved in hepatic regeneration after I/R injury (*30*, *31*).

Concerning the impact of ischemic injury, I/R associated tissue damage is well reflected by the amount of TNF-α expression, a cytokine which was significantly reduced in our study after epo-treatment.

IL-1ß data does not present an easily interpretable picture in our study with elevation in both study groups to an almost identical extent. The reason for this phenomenon remains unclear at this point; however, we made almost identical experiences in previous experiments with transplantation of nonfatty livers. IL-6, a cytokine not only involved in acute phase response to I/R injury but also in tissue regeneration was upregulated among epo-treated rats in this study; however, statistical significance was not reached, potentially due to large standard variations.

Our study design was based on previous work from our group (*16*, *18*, *19*), and the results of multiple studies in neuronal and cardiac tissue demonstrating reduced cellular injury after rHuEpo pretreatment (*11–15*). Regarding the fact, that acceptance of so-called marginal grafts is increasing worldwide it was the aim of this study to evaluate the potential capacities of rHuEpo in optimizing postoperative function of these marginal grafts. Reduction of ischemic damage and improved tissue regeneration are potential pathways by which epo conveys its attributes. We have previously shown that rHuEpo treatment reduces I/R injury after transplantation of non-fatty livers in rats and after 45 min of warm liver ischemia (*16*, *18*). Furthermore, it could be demonstrated that rHuEpo improves hepatic tissue regeneration after large-volume liver resection (*19*).

Recent studies have demonstrated the effectiveness of rHuEpo treatment concerning the reduction of ischemic tissue damage in the clinical setting (*32*). However, the exact mechanisms of epo-mediated cell protection remain to be clarified. It has been demonstrated that both PI3-kinase/Akt-triggered procedures and the JAK/STAT pathway play a decisive role in the intracellular signaling cascade between extracellular epo-receptor binding and gene transcription (*33–35*). Proapoptotic gene expression is downregulated by epo whereas Akt activation induces HIF-gene transcription and consecutive upregulation of epo-gene transcription (*36*, *37*). A recent study from Sweden demonstrated additional epo-mediated effects in astrocytes which are not dependent on gene-activation but depend on direct interference with astroglial water channels (*28*).

To limit I/R injury, many different substances have been studied in recent years, partially with great success, in small or even large animal models. Most of the examined drugs, though, are not approved for medical use in humans which makes their clinical application even more complicated. rHuEpo has been used in clinical routines for many years and can be regarded as a fairly safe substance with few side effects (*38–44*).

The combination of I/R injury reduction and improved tissue regeneration puts rHuEpo into a interesting and promising position with regard to improving initial graft function of increasingly accepted "marginal transplants." Significantly improved survival rates among epo-treated rats in our experiment underline this aspect.

Therefore, the application of rHuEpo in conditioning the "marginal" donor organ before and at/after transplantation may serve as a valuable tool in improving organ function after liver transplantation of "marginal liver grafts."

CONCLUSION

In conclusion, donor and recipient treatment with rHuEpo significantly increases survival after orthotopic

transplantation of fatty livers in rats. This effect is provided by the reduction of I/R injury and improved hepatic tissue regeneration.

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