Transpl Int (2003) 16: 806–813
DOI 10.1007/s00147-003-0634-y

ORIGINAL ARTICLE

Moritz von Frankenberg
Markus Golling
Arianeb Mehrabi
Hagen Nentwich
Ernst Klar
Thomas W. Kraus

Donor pretreatment with gadolinium chloride improves early graft function and survival after porcine liver transplantation

Received: 17 October 2002
Revised: 6 May 2003
Accepted: 14 May 2003
Published online: 15 July 2003

M. von Frankenberg (✉) · A. Mehrabi
H. Nentwich · E. Klar · T. W. Kraus
Department of Surgery,
University of Heidelberg,
Im Neuenheimer Feld 110,
69120 Heidelberg, Germany
E-mail: moritz_von_frankenberg@med.uni-heidelberg.de
Tel.: +49-6221-566110
Fax: +49-6221-565227

M. Golling
Department of Surgery,
Johann Wolfgang Goethe University
of Frankfurt, 60590 Frankfurt, Germany

Abstract Kupffer cell depletion by gadolinium chloride ($GdCl_3$) in rat livers has previously been proven to minimize hepatic ischemia/reperfusion injury after experimental liver transplantation (LTX). In the current study, we evaluated the effects of donor pretreatment with $GdCl_3$ on hepatic ischemia/reperfusion injury, macro- and microcirculation, and endotoxin clearance of the liver in a porcine model of experimental LTX. Two groups of 12 pigs were treated either with intravenous NaCl (0.9%; control) or $GdCl_3$ (20 mg/kg). Twenty-four hours after pretreatment, hepatic macrocirculation was quantified by Doppler flowmetry and liver parenchymous microcirculation by implanted thermodiffusion electrodes. The liver grafts were transplanted after 4–6 h of cold ischemia in University of Wisconsin (UW) solution. At 1 and 24 h after LTX, the perfusion values were re-evaluated and histology, biochemical (aspartate aminotransferase, AST) and functional parameters (partial thromboplastin time, prothrombin time, and bilirubin) were analyzed. Furthermore, endotoxin clearance of the liver was evaluated at all time points. In $GdCl_3$-treated animals 80% of the Kupffer cells were destroyed, and 24 h after LTX ischemia/reperfusion injury in treated grafts was significantly lower in comparison to controls, as shown by histology, AST levels (741 ± 490 U/l in controls vs 379 ± 159 U/l in treated grafts, $P < 0.05$), survival (67% vs 92%), and enhanced macro- (total transhepatic blood flow [THBF] = 112 ± 22 ml/min per 100 g in controls vs 157 ± 45 ml/min per 100 g in treated grafts, $P \leq 0.05$) and microcirculation (thermodiffusion [TD] = 73 ± 9 ml/min per 100 g in controls vs 90 ± 16 ml/min per 100 g in treated grafts, $P \leq 0.05$). Despite destruction of the macrophage system in the liver, the transhepatic endotoxin gradient of treated livers was enhanced before and 1 h after transplantation (58% in controls vs 85% in treated grafts, $P < 0.05$). Destruction of Kupffer cells of donors by pretreatment with $GdCl_3$ in pigs is effective in preventing liver graft dys- and nonfunction after LTX. Pretreatment with $GdCl_3$ does not diminish but increase hepatic endotoxin clearance.

Keywords Porcine liver transplantation · Kupffer cells · Endotoxin · Primary graft nonfunction · Hepatic microcirculation

Introduction

The existing critical shortage of grafts is still a major problem in clinical liver transplantation (LTX). A feasible way to expand the existing donor organ pool could be aggressive use of marginal grafts. As the rate of primary nonfunction after LTX can reach 15%, selective pretreatment of grafts appears to be a promising concept in order to optimize organ function after transplantation [1, 2, 3]. The function of nonparenchymal liver cells appears to be crucial to the success of LTX [4, 5, 6]. Kupffer cells play a key role in the pathophysiology of hepatic ischemia/reperfusion injury. One of their main functions is the elimination of toxins such as endotoxin (lipopolysaccharide, LPS) and bacteria from splanchnic circulation. Kupffer cells are also of relevance with regard to antigen presentation and specific immune response [7]. Activation of Kupffer cells during graft reperfusion by endotoxin leads to the production of reactive oxygen species, stimulates lipid peroxygenation [7, 8, 9], and augments the release of mediators such as thromboxane, prostaglandins, leukotrienes, tumor necrosis factor (TNF)-α, interleukins, and proteases [10, 11].

In a rat model it was documented that treatment with gadolinium chloride ($GdCl_3$) effectively destroys 80% of Kupffer cells within 24 h [12]. $GdCl_3$ particles are incorporated by macrophages via phagocytosis and are then ingested into lysosomes where acidic pH leads to free Gd^{3+} ions, which causes the destruction of Kupffer cells. Kupffer cell destruction by $GdCl_3$ in rats has proved successful in the prevention of alcoholic liver injury, minimizes ischemia/reperfusion injury particularly in pre-injured organs following both warm and cold ischemia, and prevents primary graft dysfunction after experimental LTX [13, 14, 15, 16]. $GdCl_3$ has never been used before on either humans or animals aside from rats.

Theoretically, pretreatment of liver donors with $GdCl_3$ could completely diminish hepatic endotoxin clearance and could thereby significantly increase the risk of infection or sepsis. Although there have been no reports with regard to infections in rats treated with $GdCl_3$, only little data exist on the function of the reticulo-endothelial system and endotoxin clearance after $GdCl_3$ treatment in large animal models.

The aim of the present study was thus to evaluate the effects of $GdCl_3$ in a pig model. We intended to answer the following questions:

1. Does pretreatment with $GdCl_3$ effectively lead to the destruction of Kupffer cells in pigs?
2. Does the destruction of Kupffer cells modify hepatic macro- and microperfusion?
3. Does $GdCl_3$ diminish ischemia/reperfusion injury?
4. Does $GdCl_3$ improve graft function and survival after LTX?
5. Does the destruction of Kupffer cells influence hepatic endotoxin clearance?

Materials and methods

Groups of animals

Orthotopic LTX was performed on 24 healthy young pigs (German domestic pigs) in ITN. Two experimental groups of animals were formed:

(A) The control group ($n = 12$)
(B) The $GdCl_3$ group ($n = 12$)

In the control group A, LTX was performed without $GdCl_3$ donor pretreatment. In the therapy group B, donors were pretreated with $GdCl_3$ in a standardized dosage of 20 mg/kg. $GdCl_3$ was dissolved in acidic 0.9% NaCl solution (pH = 3.0) and over 5 min slowly infused into the central caval vein 24 h before surgical donor hepatectomy. Animals of group A received an infusion of acidic 0.9% NaCl solution in a comparable volume.

Experimental liver transplantation

All experiments were performed under general anesthesia and during continuous cardiopulmonary monitoring (systemic arterial and central venous blood pressure, oxygen saturation, pH, blood gases, and hemoglobin concentration). During donor hepatectomy, arterial and portal graft perfusion was performed with University of Wisconsin (UW) solution at 4°C. Orthotopic LTX was performed after 4–6 h of cold ischemia. A porto-jugular silicone shunt was inserted to bridge the anhepatic phase of 50 min at maximum. The suprahepatic inferior caval vein was sutured, while cuff anastomoses were performed in the portal and infrahepatic inferior caval vein. The graft was rinsed with 2 l Ringer's solution before reperfusion. For anastomosis of the hepatic artery VCS clips were used. The anastomoses of the common bile duct was stented with a tube.

Measurement protocol

Hepatic micro- and macroperfusion, liver histology, and blood samples were evaluated in donors before hepatectomy and in recipients 1 and 24 h after transplantation. Sterile blood samples for endotoxin level were taken from both the portal and jugular veins in recipients (1) directly before transplantation, (2) 1 h afterwards, and (3) 24 h after LTX. Recipients that survived 24 h (the technical failure rate was 10%) after LTX were re-operated, graft perfusion was re-evaluated, and blood samples and biopsies were taken. After the experiments, all animals were killed by infusion of potassium in deep anesthesia.

Laboratory parameters

Central venous blood samples were taken before explantation and in recipients before and during explantation, and then at 1 and 24 h after portal venous reperfusion. Blood counts, liver enzymes (aspartate aminotransferase [AST], alanine aminotransferase [ALT]), bilirubin, prothrombin time (PT), and partial thromboplastin time (pTT) were determined.

Liver perfusion

Blood flow in the hepatic artery and portal vein was measured by implanted Doppler flow probes (Transonic Systems, Ithaca, N.Y.). Total transhepatic blood flow (THBF) was calculated by the addition of both parameters. Thermodiffusion electrodes (Thermal Technologies, Cambridge, Mass.) were implanted in the liver parenchyma to evaluate parenchymous liver microperfusion [17]. This technique of thermodiffusion (TD) has been described previously in detail [18].

Histology

Liver wedge biopsy was performed immediately before graft explantation in the donor and at time points 60 min and 24 h after portal reperfusion in recipients. The biopsy specimens were cut into blocks of less than 1 mm in diameter and immediately stored in formalin. Paraffin sections were stained with hematoxylin and eosin. All histologic examinations were performed in a blinded fashion with the use of a computer-assisted histology analyzer and a video microscope (Leitz, Germany). The number of macrophages, necrotic hepatocytes, and intracellular vacuoles was analyzed in five random high power fields (magnification ×400), and average numbers were calculated.

Endotoxin assay/endotoxin neutralizing capacity

Sterile central and portal venous blood samples were taken immediately after laparotomy, before portal reperfusion at the end of the hepatic period (47 ± 6 min), and 60 min after portal reperfusion. The samples were centrifuged at 2000 rpm. Serum was stored at −80°C until definite analysis. Serum endotoxin and endotoxin neutralizing capacity in the plasma were measured by means of the automated kinetic turbimetric limulus amebocyte lysate microtiter test (a biological test for the toxic activity of endotoxin) with intraindividual internal standardization (Pyroquant-Associates of Cape Cod, Woods Hole, Mass.) [5].

In order to evaluate the endotoxin clearance function of the liver during the early phase of reperfusion, we assessed the transhepatic LPS gradient defined as:

Hepatic LPS gradient = (portal LPS concentration−systemic LPS concentration)/(portal LPS concentration × 100)

Ethics and animal rights

Approval for the experimental procedures was obtained by the German Committee for Animal Care, Regierungspräsidium Karlsruhe, Germany. During the experiments all animals received human care in compliance with the United States National Research Council's criteria for human care, as outlined in the *Guide for the Care and Use of Laboratory Animals*, prepared by the National Institutes of Health (NIH publication no. 86-23, revised 1985). All experiments were performed under general anesthesia and during continuous cardiopulmonary monitoring.

Statistics

Data are given as mean values ± standard deviation (SD). Fisher's exact test or Student's *t*-test were used to test the statistical significance of differences where appropriate (SigmaStat 2.03 for Windows). *P*-values of less than 0.05 were considered to define statistical significance.

Table 1 Liver histology 24 h after reperfusion of control or $GdCl_3$-treated grafts. Biopsy specimens were fixed in formalin, embedded in paraffin, and stained with hematoxylin and eosin. All histologic examinations were performed using a computer-assisted histology analyzer and a video microscope (Leitz, Germany). In every biopsy, five randomized high power fields (magnification 400×) were examined by two different analyzers

	Necroses (*n*)	Polymorph nucleated leukocytes per field (400×)	Vacuoles
Controls	57 ± 9	96 ± 33	27 ± 10
$GdCl_3$ group	21 ± 2	38 ± 5	5 ± 2
P	< 0.005	< 0.05	< 0.05

Results

Kupffer cell depletion

$GdCl_3$ treatment in pigs was effective in the destruction of Kupffer cells. In donor liver biopsies of animals of group B, the number of macrophages was reduced by 80% compared to the control group. An average number of 20 ± 8 macrophages per high power field was seen in the control livers, while $GdCl_3$-treated animals only had 4 ± 5 macrophages per field in the tissue ($P < 0.05$, Table 1).

Graft function and animal survival after liver transplantation

The survival rate at 24 h after LTX in Kupffer cell-depleted animals was considerably improved compared to recipients of untreated grafts (11 vs 8, Fig. 1). Hepatocellular injury, reflected by serum AST at 1 h after reperfusion, was significantly lower in recipients of $GdCl_3$-treated livers (203 ± 94 U/l vs 127 ± 63 U/l, $P < 0.05$). On the first postoperative day, AST was lower in recipients of treated livers (741 ± 490 U/l vs 379 ± 159 U/l, $P < 0.05$, Fig. 2). No differences in transaminases were found in donors between treated and untreated animals.

Biopsy specimens of $GdCl_3$-treated grafts taken 24 h after transplantation had significantly less severe signs of ischemia/reperfusion injury compared to untreated grafts, as shown by the number of necrotic cells, infiltrating leukocytes, and vacuoles (Table 1, Fig. 3). With regard to excretory liver function, no differences were seen in donors 1 h after reperfusion, while at 24 h after transplantation bilirubin levels in $GdCl_3$-treated livers were markedly lower than in controls (1.03 ± 1.05 mg/dl in controls vs 0.36 ± 0.28 mg/dl bilirubin i.s. in the treatment group). Partial thromboplastin time was lower in Kupffer cell-depleted organs compared to controls 1 day after LTX (30 ± 34 s in controls vs 17 ± 2 s in the treatment group). No differences between groups were seen for prothrombin time.

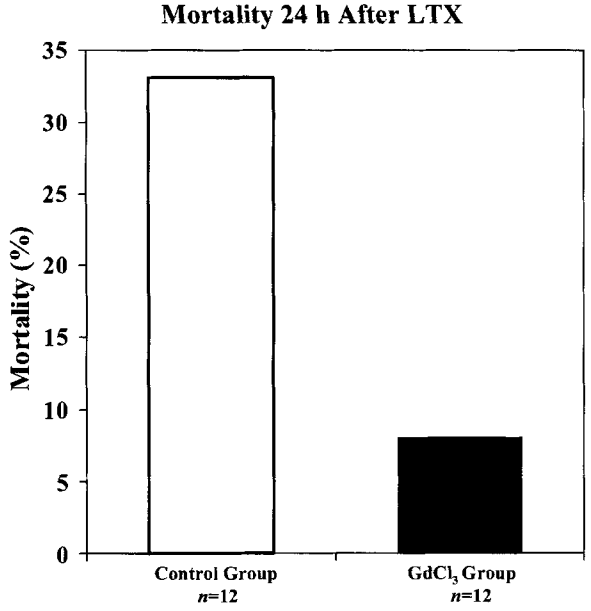

Fig. 1 Mortality 24 h after liver transplantation (*LTX*) in control or Kupffer cell-depleted animals ($n = 12$)

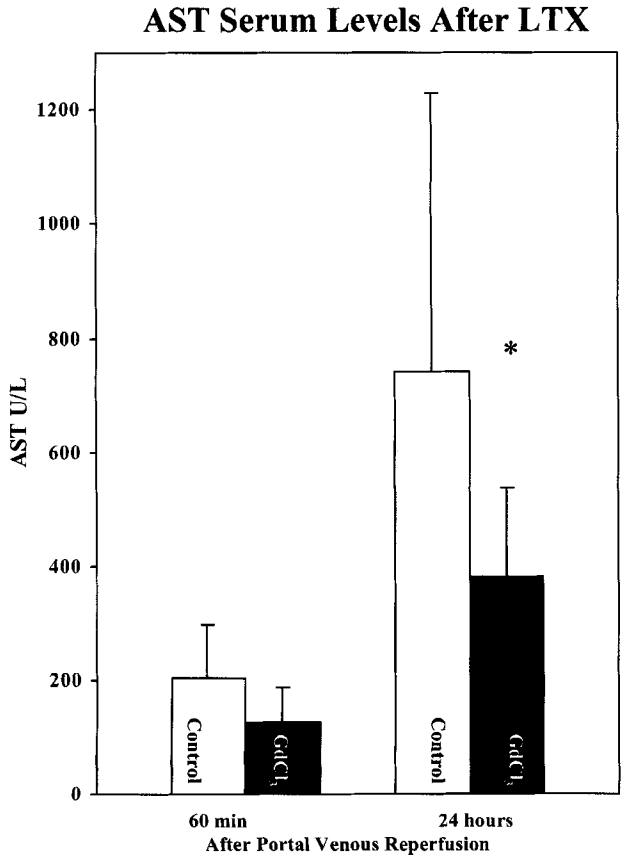

Fig. 2 Serum transaminases 60 min and 24 h after liver transplantation (*LTX*) in recipient pigs with and without $GdCl_3$ treatment (*AST* aspartate aminotransferase). *$P < 0.05$, Student's *t*-test

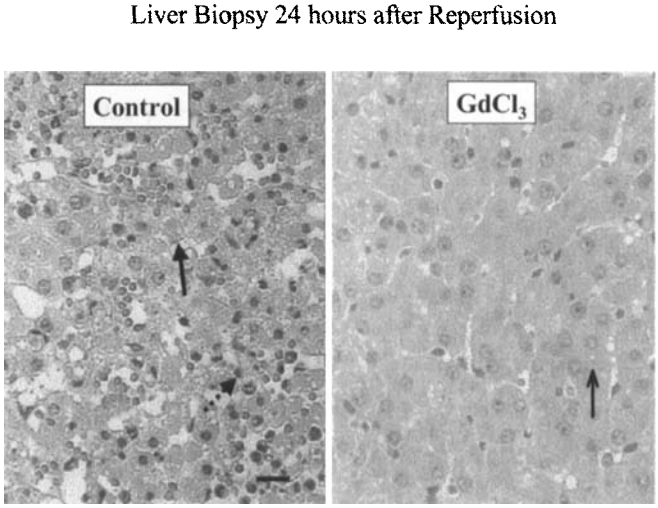

Fig. 3 Hematoxylin and eosin stain (magnification 400×) of liver histology 24 h after liver transplantation (*LTX*). Biopsy samples were taken from control or Kupffer cell-depleted grafts 24 h after LTX. Note the marked ischemia/reperfusion injury in the control with necrotic cells (*arrow*), leukocytes (*dotted arrow*), and vacuoles compared to $GdCl_3$ grafts. The *bar* indicates 25 μm

Macro- and microcirculation

Twenty-four hours after injection of $GdCl_3$, hepatic arterial and portal blood flow (THBF) and hepatic microcirculation (TD) were significantly enhanced. In untreated donors, THBF was 112 ± 24 ml/min per 100 g compared to 171 ± 40 ml/min per 100 g in $GdCl_3$-treated pigs ($P < 0.001$, Fig. 4). Hepatic arterial blood flow had almost doubled (21 ± 9 ml/min per 100 g vs 39 ± 17 ml/min per 100 g, $P < 0.01$), while portal venous flow was increased by more than 30% in treated livers (118 ± 17 ml/min per 100 g vs 170 ± 54 ml/min per 100 g, $P < 0.01$). Microcirculation was substantially increased in $GdCl_3$-treated livers compared to controls. In untreated animals TD was 65 ± 4 ml/min per 100 g, after Kupffer cell depletion TD increased to 90 ± 14 ml/min per 100 g ($P < 0.001$, Fig. 4). No significant differences in THBF or TD mean values between both groups were seen at 60 min after reperfusion. In therapy group B however, a significant rise in THBF and TD was found 24 h after LTX compared to controls. THBF was increased by 40% (112 ± 22 ml/min per 100 g vs 157 ± 45 ml/min per 100 g, $P \leq 0.05$). TD showed a 20% elevation compared to controls (73 ± 9 ml/min per 100 g vs 90 ± 16 ml/min per 100 g, $P \leq 0.05$, Fig. 5).

Endotoxin levels

While portal endotoxin levels were not different before, during, and after LTX, systemic endotoxin levels were significantly lower 1 h after reperfusion in recipients of

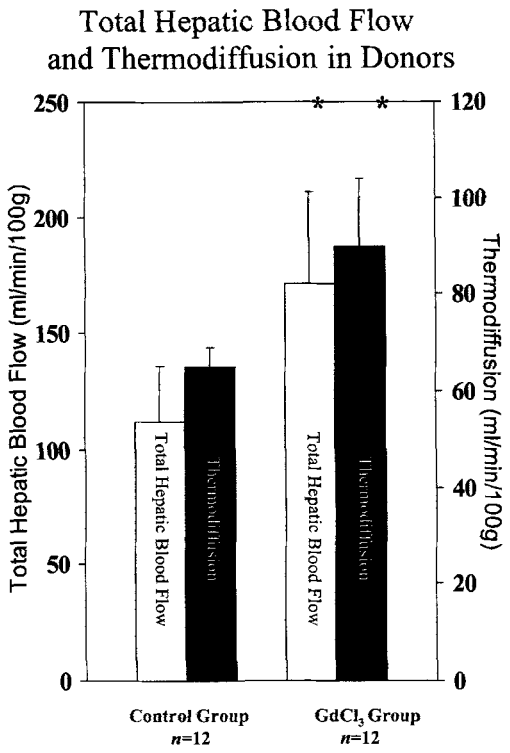

Fig. 4 Transhepatic blood flow (*THBF*) and parenchymous liver microcirculation (*TD*) in Kupffer cell-depleted and untreated donors before liver graft hepatectomy. $^*P < 0.05$, Student's *t*-test

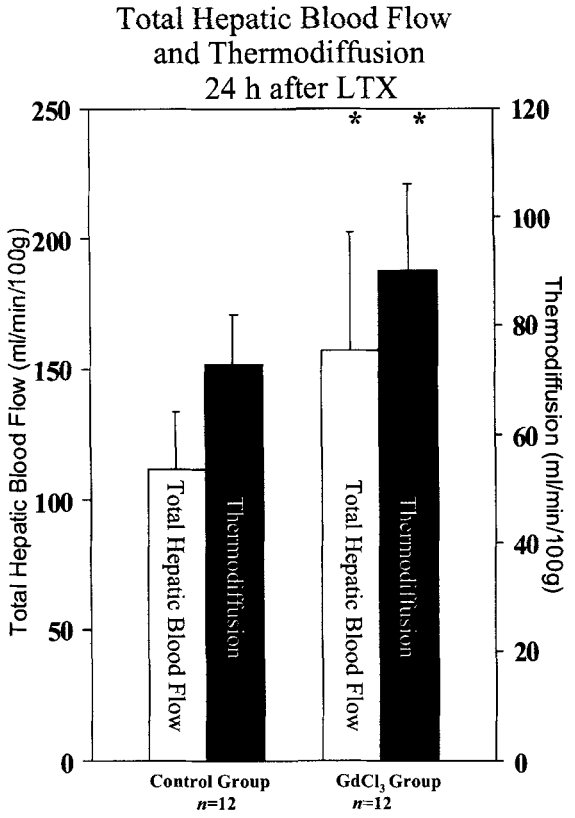

Fig. 5 Transhepatic blood flow (*THBF*) and parenchymous liver microcirculation (*TD*) 24 h after liver transplantation (*LTX*) in control or Kupffer cell-depleted grafts. $^*P < 0.05$, Student's *t*-test

$GdCl_3$-treated grafts than in recipients of untreated control grafts. One hour after reperfusion, the systemic endotoxin level in recipients of control livers was 39 ± 23 pg/ml, while recipients of $GdCl_3$ livers had a value of 14 ± 7 pg/ml ($P < 0.001$, Fig. 6). While systemic and portal endotoxin concentrations rose more than thirty-fold in both groups during the anhepatic phase, the hepatic endotoxin clearance only recovered in Kupffer cell-depleted livers after reperfusion. The transhepatic endotoxin gradient at 1 h after reperfusion was significantly higher in Kupffer cell-depleted grafts compared to controls (58% vs 85%, $P < 0.05$).

Discussion

$GdCl_3$ destroys Kupffer cells in pigs

Knowledge of the mechanisms of Kupffer cell destruction is scarce and originates exclusively from experiments with rats or mice. The preparation of $GdCl_3$ solution and the correct mode of infusion are crucial to the effectiveness of treatment. $GdCl_3$ dissolves in acidic pH only. Furthermore, only by cautious systemic infusion is the crystallization process slow enough to form small particles, which can be ingested by macrophages through phagocytosis. If performed in the correct way, the destruction of Kupffer cells with $GdCl_3$ is easily achievable. As shown by histology, more than 80% of macrophages in porcine livers were destroyed in the present study. The data are comparable to results in small animal studies, where Kupffer cell depletion by $GdCl_3$ has been shown to be effective while no other toxic or pharmacological effects of $GdCl_3$ treatment were observed [12, 19].

Kupffer cell destruction prevents ischemia/reperfusion injury in pigs

The role of ischemia/reperfusion injury in nonparenchymal cells is crucial. The prevention of injury in these cells is a major goal that can be achieved by organ preservation. Kupffer cells play a sizable role in the mediation of reperfusion injury by production of toxic substances like reactive oxygen species, proteases, and the release of mediators such as interleukins, adhesion molecules, and TNF-α [7, 9, 10, 11, 20]. In rats, destruction of Kupffer cells minimizes a) ischemia/reperfusion injury in the liver after warm and cold ischemia, b) toxic injury by LPS, and c) acute and chronic

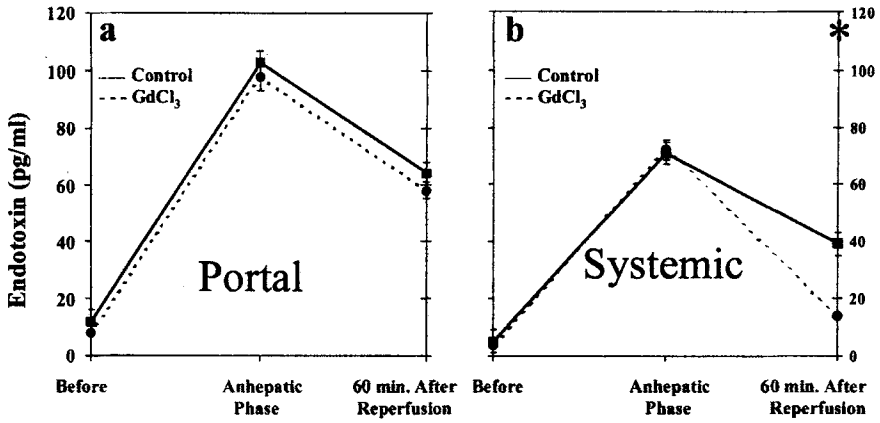

Fig. 6 Endotoxin serum concentrations in the portal (**a**) or suprahepatic caval vein (**b**) during different stages of liver transplantation (*LTX*) of grafts from pigs with and without $GdCl_3$ preconditioning. $^*P < 0.05$, $GdCl_3$-treated grafts against controls, Student's *t*-test

ethanol intoxication [9, 14, 15, 19, 21, 22, 23]. Furthermore, pretreatment of alcohol-fed donors with $GdCl_3$ prevents failure of fatty marginal grafts after experimental LTX [19, 21, 24]. Treatment of porcine donors with $GdCl_3$ also impressively diminished ischemia/reperfusion injury in our current investigation, as shown by both functional and histological parameters. Mortality, hepatocellular injury, and histologic reperfusion injury after LTX was significantly reduced by prior destruction of Kupffer cells (Figs. 1, 5 and 3, and Table 1). This is the first study to prove that the destruction of Kupffer cells for donor preconditioning is feasible in a large animal model.

Kupffer cell depletion increases macro- and microperfusion in the pig liver

Disturbance of liver graft perfusion is one of the earliest signs of ischemia/reperfusion injury and may reflect early graft function. Several methods have been used to evaluate liver microcirculation. In vivo microscopy with and without fluorescence markers has been used in experimental transplantation of the liver, gut, and pancreas [25]. Thermodiffusion can be used clinically for continuous surveillance of tissue perfusion in the liver as well as in other organs [17]. $GdCl_3$ pretreatment increased both macro- as well as microperfusion in the donor. The greatest enhancement of more than 80% was detected in the hepatic artery. This effect cannot be attributed just to larger intrahepatic arterio-venous shunt flow volumes since microperfusion in the tissue was also improved (Fig. 4).

The pathophysiological mechanisms of the effects of gadolinium are not yet fully clarified. As Kupffer cells play an important role in intercellular hepatic communication, the lack of mediators like TNF-α, PAF, and leukotrienes could lead to a decreased aggregation of platelets or a diminished release of TNF-α and endothelin-1 by endothelial cells [7, 9, 10, 14, 15, 20, 26]. This could be responsible for a decreased contraction of the intrahepatic vascular system or of stellate cells [27]. Another mechanism might be the reduced production of reactive oxygen species. These block the enzyme guanylate cyclase, which leads to lower cGMP concentrations and further decreased NO production. This mechanism is enhanced by mediators from activated Kupffer cells. By eliminating Kupffer cells, this pathway might be reduced and more NO would then be produced and released, which would lead to vascular relaxation [3, 19, 26].

Kupffer cell depletion improves reperfusion after transplantation

Diminished hepatic blood flow and microcirculatory disturbances can lead to primary nonfunction of the graft. Transplantation of Kupffer cell-depleted livers is clearly associated with a better hepatic macro- and microperfusion 1 day after LTX. The best improvement was seen in the arterial blood flow of Kupffer cell-depleted grafts (60%), which reflects diminished tissue injury, but also leads to faster washout of toxic substances and mediators and, thus, an improved supply of oxygen and protective agents (Fig. 5) [9, 19].

Activated Kupffer cells release reactive oxygen species, proteases, TNF-α, adhesion molecules, and other mediators (s.a.), which lead to microcirculatory disturbances and injury, destruction, or apoptosis of other nonparenchymal cells and hepatocytes [7, 8, 9, 10, 14, 20, 28, 29]. This mechanism is boosted by the release of chemotactic mediators and infiltrating leukocytes. Both pathways are reduced in Kupffer cell-depleted livers, even though the release of toxic mediators, reactive oxygen species, and chemotactic factors also originates from endothelial cells [8, 28]. Consequently, after LTX, hepatic perfusion is lower than that in the donor situation. This might also be the cause of the equally reduced perfusion volumes in both groups soon after LTX.

Kupffer cell depletion improves endotoxin clearance in the liver

Kupffer cells represent 80% of the reticulo-endothelial system (RES) in the liver, and their phagocytic function is an important part of the immunologic defense line. By destruction of Kupffer cells, possible danger could arise from bacteria or toxic bacterial products like endotoxin [7, 30]. Even though there are no reports on toxic or sepsis-promoting effects of $GdCl_3$ treatment to date, the destruction of Kupffer cells in the liver must prove to be a safe procedure before it can be used for donor pretreatment in the human clinical setting. Surprisingly, even though a dramatic rise in LPS concentration during the anhepatic phase was seen in the portal vein in both groups, the extraction of LPS in Kupffer cell-depleted livers was significantly higher 1 h after reperfusion. This effect cannot be explained by improved function of Kupffer cells since the endotoxin clearance of hepatocytes probably was the major cause here [31, 32]. Two reasons can possibly be defined for this effect. Firstly, improved organ perfusion leads to enhanced endotoxin distribution in the liver and, thus, more hepatocytes are perfused. Secondly, the protective effect of Kupffer cell depletion in ischemia/reperfusion injury leads to diminished hepatocellular injury, thus causing improved function of hepatocytes.

Summary and conclusion

Treatment of donors with $GdCl_3$ causes destruction of more than 80% of the Kupffer cells in the liver. Kupffer cell-depleted livers have a significantly improved perfusion compared to untreated livers. After ischemia/reperfusion, further enhancement of macro- and microperfusion is found in recipients of $GdCl_3$-treated grafts. $GdCl_3$ treatment also has a marked and significant protective effect on ischemia/reperfusion injury in pigs, as reflected by graft function, hepatocellular injury, and histology. This protective effect leads to improved endotoxin extraction in transplanted grafts. Treatment of liver donors by Kupffer cell destruction appears to be effective and can potentially be transferred to the clinical setting. Toxic side effects, especially to other transplantable organs of the donor, have to be evaluated before using $GdCl_3$ in humans.

Acknowledgements This paper was initiated and partially revised by Dr. Ron G. Thurman, Director of the Laboratory of Hepatobiology and Toxicology, University of North Carolina, USA, who died suddenly in July 2001. I had the good fortune to work with him in his laboratory from December 1994 to December 1996. He was a wonderful man and teacher and a brilliant scientist, and I take great pleasure in dedicating this work to him. We wish to thank Prof. R. Urbaschek for the analysis of serum endotoxin concentration. This study was supported by Forschungsförderungsprogramm Universität Heidelberg Nr. 110/99.

References

1. Alexander JW, Vaughn WK. The use of "marginal" donors for organ transplantation. Transplantation 1991; 51:135.
2. Mirza FM, Gunson GK, DaSilva RF, Mayer D, Buckels JA, McMaster P. Policies in Europe on "marginal quality" donor livers. Lancet 1994; 344:1480.
3. Serracino-Inglott F, Habib NA, Mathie RT. Hepatic ischemia-reperfusion injury. Am J Surg 2001; 181:160.
4. Marzi I, Zhong Z, Lemasters JJ, Thurman RG. Evidence that graft survival is not related to parenchymal cell viability in rat liver transplantation. The importance of nonparenchymal cells. Transplantation 1989; 48:463.
5. Caldwell-Kenkel JC, Thurman RG, Lemasters JJ. Selective loss of nonparenchymal cell viability after cold ischemic storage of rat livers. Transplantation 1988; 45:834.
6. Wang L, Florman S, Roayaie S, et al. Differential in vivo recovery of sinusoidal endothelial cells, hepatocytes, and Kupffer cells after cold preservation and liver transplantation in rats. Transplantation 1998; 66:573.
7. Wardle EN. Kupffer cells and their function. Liver 1987; 7:63.
8. Zhong Z, Connor H, Stachlewitz RF, et al. Role of free radicals in primary nonfunction of marginal fatty grafts from rats treated acutely with ethanol. Mol Pharmacol 1997; 52:912.
9. Urata K, Brault A, Rocheleau B, Huet PM. Role of Kupffer cells in the survival after rat liver transplantation with long portal vein clamping times. Transpl Int 2000; 13:420.
10. Nathan CF. Secretory products of macrophages. J Clin Invest 1987; 79:319.
11. Decker K. Biologically active products of stimulated liver macrophages (Kupffer cells). Eur J Biochem 1990; 192:245.
12. Hardonk MJ, Dijkhuis FW, Hulstaert CE, Koudstaal J. Heterogeneity of rat liver and spleen macrophages in gadolinium chloride-induced elimination and repopulation. J Leukoc Biol 1992; 52:296.
13. Sarphie TG, D'Souza NB, Deaciuc IV. Kupffer cell inactivation prevents lipopolysaccharide-induced structural changes in the rat liver sinusoid: an electron–microscopic study. Hepatology 1996; 23:788.
14. Bremer C, Bradford BU, Hunt KJ, et al. Role of Kupffer cells in the pathogenesis of hepatic reperfusion injury. Am J Physiol 1994; 267: G630.
15. Iimuro Y, Yamamoto M, Kohno H, Itakura J, Fujii H, Matsumoto Y. Blockade of liver macrophages by gadolinium chloride reduces lethality in endotoxemic rats – analysis of mechanisms of lethality in endotoxemia. J Leukoc Biol 1994; 55:723.

16. Niwano M, Arii S, Monden K, et al. Amelioration of sinusoidal endothelial cell damage by Kupffer cell blockade during cold preservation of rat liver. J Surg Res 1997; 72:36.
17. Klar E, Kraus T, Bleyl J, et al. Thermodiffusion as a novel method for continuous monitoring of the hepatic microcirculation after liver transplantation. Transplant Proc 1995; 28:2610.
18. Kraus T, Mehrabi A, Angelescu M, Golling M, Allenberg JR, Klar E. Characterization of renal parenchymal perfusion during experimental infrarenal aortic clamping and declamping with enhanced thermodiffusion electrodes. Ann Vasc Surg 2001; 15:447.
19. Frankenberg M von, Stachlewitz RF, Forman DT, et al. Amino acids in rinse effluents as a predictor of graft function after transplantation of fatty livers in rats. Transpl Int 1999; 12:168.
20. Schauer RJ, Bilzer M, Kalmuk S, et al. Microcirculatory failure after rat liver transplantation is related to Kupffer cell-derived oxidant stress but not involved in early graft dysfunction. Transplantation 2001; 72:1692.
21. Frankenberg M von, Forman DT, Frey W, Stachlewitz RF, Bunzendahl H, Thurman RG. Release of amino acids from fatty livers during organ harvest for transplantation. Ann Clin Lab Sci 1997; 26:287.
22. Zhong Z, Connor H, Mason RP, et al. Destruction of Kupffer cells increases survival and reduces graft injury after transplantation of fatty livers from ethanol-treated rats. Liver Transpl Surg 1996; 2:383.
23. Iimuro Y, Frankenberg M von, Arteel GE, Bradford BU, Wall CA, Thurman RG. Female rats exhibit greater susceptibility to early alcohol-induced liver injury than males. Am J Physiol 1997; 272: G1186.
24. Frankenberg M von, Forman DT, Frey W, Bunzendahl H, Lemasters JJ, Thurman RG. Amino acids in storage solution predict primary nonfunction in fatty liver grafts. Transplant Proc 1997; 63:1661.
25. Rentsch M, Post S, Palma P, Gonzalez AP, Menger MD, Messmer K. Intravital studies on beneficial effects of warm Ringer's lactate rinse in liver transplantation. Transpl Int 1996; 9:461.
26. Kraus T, Mehrabi A, Klar E, et al. Peri- and postoperative plasma kinetics of big endothelin and endothelin 1/2 after liver transplantation. Transplant Proc 1992; 24:2569.
27. Sakamoto M, Ueno T, Kin M, et al. Ito cell contraction in response to endothelin-1 and substance P. Hepatology 1993; 18:978.
28. McCord JM. Oxygen-derived free radicals in postischemic tissue injury. N Engl J Med 1985; 312:159.
29. Stachlewitz RF, Gao W, Zhong Z, Connor HD, Mason RP, Thurman RG. Generation of lipid free radicals by adherent leukocytes from transplanted rat liver. Transpl Int 1998; 11:353.
30. Zipfel A, Schenk M, You MS, Lauchart W, Bode C, Viebahn R. Endotoxemia in organ donors: graft function following liver transplantation. Transpl Int 2000; 13 [Suppl 1]: S286.
31. Mimura Y, Sakisaka S, Harada M, Sata M, Tanikawa K. Role of hepatocytes in direct clearance of lipopolysaccharide in rats. Gastroenterology 1995; 109:1969.
32. Parent JB. Membrane receptors on rat hepatocytes for the inner core region of bacterial lipopolysaccharides. J Biol Chem 1990; 265:3455.