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ORIGINAL ARTICLE

Gadolinium chloride-induced improvement of postischemic hepatic perfusion after warm ischemia is associated with reduced hepatic endothelin secretion

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Summary

Selective Kupffer cell blockade by gadolinium chloride ($GdCl_3$) pretreatment of liver donors previously proved to be effective in reducing ischemia/reperfusion injury in rat liver transplants. Physiological mechanisms of this effect have not been specified so far. Vasoactive peptides are involved in liver blood flow regulation. We tested the hypothesis, that hepatic hemodynamic effects of $GdCl_3$ pretreatment are mediated by intrahepatic endothelin-1 (ET) secretion in a standardized porcine model of warm liver ischemia and reperfusion. Standardized warm hepatic ischemia (45 min) was induced after laparotomy in intubation narcoses (ITN) by Pringle-maneuver in pigs ($n = 12$). Animals were either pretreated with $GdCl_3$ (20 mg/kg i.v.) or sodium chloride 0.9% (control group) in a randomized manner 24 h before investigation. Relaparotomy was performed at day 7. Before, during ischemia and until 6 h after liver reperfusion, transhepatic blood flow (portal venous + hepatic artery flow) was defined by ultrasonic flow probes and hepatic parenchymous microcirculation evaluated by implanted thermodiffusion electrodes. ET plasma concentrations were analyzed (commercial RIA) at all time points in the hepatic veins after selective canulation. $GdCl_3$ pretreatment of animals markedly improved hepatic macro- and microperfusion before and particularly after warm ischemia. Mean ET plasma concentrations in the hepatic vein were significantly lower before, 6 h and 7 days after ischemia, compared with controls. Kupffer cell destruction by $GdCl_3$ pretreatment improves hepatic micro- and macroperfusion after warm ischemia, thus indicating reduced ischemia/reperfusion injury. Documented reduction of postischemic liver blood flow impairment after $GdCl_3$ pretreatment could be mediated by a decreased hepatic ET secretion, as hemodynamic effects were associated with significantly reduced ET plasma levels in hepatic veins.

Introduction

Primary nonfunction or dysfunction still is a major problem after liver transplantation (LTX). Severe preservation injury and graft dysfunction is encountered in up to 30% of patients. Up to 10% of patients have to be retransplanted during the first 4 weeks [1]. Ischemia/reperfusion injury is the main reason for hepatic dysfunction after transplantation, especially in preinjured organs from elderly donors, or grafts with fatty degeneration. Release of mediators by activated Kupffer cells and endothelial cells play a key role in the pathogenesis of hepatic ischemia/reperfusion injury. Oxygen radicals, proteolytic enzymes, thromboxane, prostaglandin, leukotrienes, tumor necrosis factor α and interleukin have been shown to be released by Kupffer cells. Endothelial cells are known to release

arachidonic acids, cytokines, oxygen radicals and endothelin [2,3]. A main function of Kupffer cells is the elimination of toxins, such as endotoxin (lipopolysaccharide; LPS) and bacteria from the splanchnic circulation. Kupffer cells further are of relevance for antigen presentation and specific immune responses [4,5].

Several approaches have been tested in experimental research to reduce hepatic ischemia/reperfusion injury in various settings. So far, none of them have found their way into clinical practice [1,6,7]. Pretreatment of liver donors with gadolinium chloride ($GdCl_3$) could possibly constitute a potentially feasible clinical approach in future. Treatment with $GdCl_3$ effectively destroys 80% of Kupffer cells within 24 h after application [8,9]. Infused $GdCl_3$ particles are incorporated by macrophages via phagocytosis, then ingested into lysosomes where acidic pH leads to free Gd^{3+} ions. This causes rapid destruction of cells. Hepatic cellular repopulation subsequently starts 4 days after $GdCl_3$ injection, primarily with undifferentiated blood monocytes.

Multiple data have previously stated a beneficial effect of $GdCl_3$ pretreatment in various animal models of experimental liver damage. Release of tumor necrosis factor α (TNF-α) and prostaglandin E2 could be prevented by prior Kupffer cell destruction following standardized surgical mechanical manipulation of rat livers [10]. Endotoxin clearance was found to improve after Kupffer cell destruction, probably because of increased liver perfusion and improvement of liver cell function. Kupffer cell destruction by $GdCl_3$ in rats further has proved to be successful for prevention of experimental alcoholic liver injury. Selective Kupffer cell destruction by $GdCl_3$ pretreatment of donors also proved to be effective in reducing ischemia/reperfusion injury after experimental LTX [5,7,9,11]. Kupffer cell depletion by $GdCl_3$ in liver transplants was associated with reduced serum transaminase levels, histological liver injury and mortality in a rat model compared with controls [12,13]. $GdCl_3$ pretreatment of liver donors also was shown to minimize hepatic ischemia/reperfusion injury, to optimize liver perfusion and to reduce the rate of liver graft failure after pig LTX [9,14]. Application of $GdCl_3$ or other faster acting agents as the amino acid glycine to potential donors thus could potentially enlarge the future pool of liver transplants by improvement of marginal organs [1]. Application of $GdCl_3$ could even potentially be indicated to reduce postoperative liver dysfunction after liver resection because of temporal hepatic ischemia or mechanical stimulation.

Vasoactive peptides are involved in liver blood flow autoregulation [15,16]. Physiological mechanisms of the liver perfusion increment detected after $GdCl_3$ treatment have not yet been clearly specified. Endothelin-1 (ET) is a very potent vasoconstrictor peptide, synthesized and secreted by endothelial cells. ET is known to be an important factor in splanchnic and hepatic blood flow regulation [17,18]. We therefore tested the hypothesis that hemodynamic effects of $GdCl_3$ pretreatment in the liver are mediated by hepatic endothelin secretion in a well-established porcine model of warm liver ischemia and reperfusion. This large animal model has not been used to examine the hemodynamic effects of $GdCl_3$ pretreatment before.

Materials and methods

Experimental protocol/induction of warm liver ischemia

Experiments were performed in young pigs (German domestic pig). All investigations were performed under deep general anesthesia with Ketamin (Ketanest-S®, 8–10 mg/kg/h) and Piritramid (Dipidolor®, 0.6–0.7 mg/kg/h) and during continuous cardiopulmonary monitoring (Systemic arterial and central venous blood pressure, oxygen saturation, pH, blood gases and hemoglobin concentration).

After median laparotomy, the liver hilus was surgically prepared and measurement of hepatic perfusion (see below) performed. Both common hepatic artery and portal vein were temporarily occluded by a tourniquet application around the hepatoduodenal ligament in both groups (Pringle maneuver). Blood flow was kept completely interrupted for exactly 45 min, reperfusion was then instituted by tourniquet removal. After reperfusion, animals were further ventilated with opened abdomen for an observation period of 6 h under general anesthesia. Then the abdomen was closed. Pigs were re-operated after 7 days, graft perfusion was re-evaluated and blood samples and biopsies taken. After the experiments animals were killed by infusion of potassium in deep anesthesia.

Groups of animals

Two experimental groups were formed in a randomized manner.

Control group

In the control group (A; $n = 6$), warm ischemia was performed without $GdCl_3$ donor pretreatment. Group A animals received an infusion of acidic 0.9% NaCl solution (pH = 3.0) in a comparable volume with group B 24 h before surgical procedure.

$GdCl_3$ therapy group

In the therapy group (B; $n = 6$) animals 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), slowly infused over a 5-min period into the central caval vein 24 h before surgical procedure.

Before any treatment, baseline measurements of all parameters, except the histological evaluation and liver perfusion, were not significantly different between groups.

Laboratory parameters

Blood samples for biochemical and hematological parameters were drawn from a central venous line before the procedure and at the following predefined time points: 2 h, 6 h, 2 days and 7 days postoperatively. Blood samples from the hepatic vein were directly taken before ischemia and at time points 6 h and 7 days after reperfusion in both groups. During ischemia no hepatic vein blood samples were collected. ET plasma concentrations were analyzed in all samples using a commercial radioimmunoassay (RIA) [19]. Additionally, cellular blood counts, liver transaminase enzymes aspartate transmerase (AST) and alanine transaminase (ALT), bilirubin levels, prothrombin coagulation time (PT) and partial thromboplastin time (PTT) were determined.

Liver perfusion measurement

Hepatic microperfusion and macroperfusion was evaluated before warm ischemia and at time points 1, 2, 4, 6 h and 7 days after postischemic reperfusion. Blood flow in both the common hepatic artery and portal vein was measured by implanted Doppler flow probes around vessels (Transonic Systems Inc., Ithaca, NY, USA). Total transhepatic blood flow (THBF) was calculated by addition of both parameters. Thermodiffusion (TD) electrodes (Thermal Technologies Inc., Cambridge, MA, USA) were implanted in the liver parenchyma to evaluate parenchymous liver microperfusion [20]. The technique of TD has been previously described in detail [21].

Liver histology

Liver wedge biopsies were taken immediately before warm ischemia and at time points 6 h and 7 days after reperfusion. Biopsies were cut into blocks of <1 mm diameter and immediately stored in formalin. Paraffin sections were stained with hematoxylin and eosin. All histologic examinations were performed with the use of a computer-assisted histology analyzer and a video microscope (Leitz, Wetzlar, Germany). Numbers of macrophages, necrotic hepatocytes and intracellular vacuoles were analyzed in five random high power fields (magnification 400×). Average numbers were calculated in all specimens.

Statistics

Data are given as mean ± SD. Fisher's exact test or Student's *t*-test were used to test statistical significance where appropriate. $P < 0.05$ were accepted to define statistical significance of differences.

Ethics and animal rights

Approval for the experimental procedures was obtained by the German Committee for Animal Care, Regierungspraesidium Karlsruhe, Germany. During the experiments all animals received humane care in compliance with the United States National Research Council's criteria for human care, as outlined in 'Guide for the Care and Use of Laboratory Animals', prepared by the National Institution of Health (NIH publication no. 86-23, revised 1985).

Results

Kupffer cell depletion

The $GdCl_3$ treatment was very effective for destruction of Kupffer cells. Number of macrophages in liver biopsies of treated animals (group B) was reduced by 80% compared with controls. Macrophages of 13.5 ± 1.5 per high power field were seen in control liver biopsies, while $GdCl_3$ treated animals only showed 3.9 ± 0.1 macrophages per field in liver tissue ($P < 0.05$; Fig. 1).

Hepatic cellular injury

Hepatocellular injury, reflected by serum AST, was significantly reduced in $GdCl_3$ treated livers during early postischemic reperfusion (time point, 6 h: 126 ± 14 U/l vs. 39 ± 11 U/l; 2 days: 216 ± 14 U/l vs. 124 ± 25 U/l; $P < 0.05$). No significant difference between mean serum AST levels was detected on the seventh postoperative day (47 ± 58 U/l vs. 31 ± 11 U/l, $P = 0.6$; Fig. 2). Biopsies of $GdCl_3$ pretreated livers taken 6 h, 2 and 7 days after transplantation had less severe signs of ischemia/reperfusion injury compared with non-treated organs, as reflected by numbers of necrotic cells, infiltrating leukocytes and vacuoles (Table 1; Fig. 1). Control animals had a significantly higher number of serum leukocytes 6 h and 2 days after reperfusion (time point, 6 h: 26.5 ± 4.2 leukocytes/nl vs. 19.6 ± 2.7 leukocytes/nl; 2 days: 15.2 ± 2.5 leukocytes/nl vs. 8.3 ± 1.7 leukocytes/nl; $P < 0.05$). No difference was seen 7 days after reperfusion.

Liver excretory function and synthesis

No differences were seen with regard to hepatic excretory liver function (bilirubin plasma levels) between controls and treated animals. Quick value (PT) was significantly higher in Kupffer cell depleted organs compared with controls 6 h and 2 days after LTX

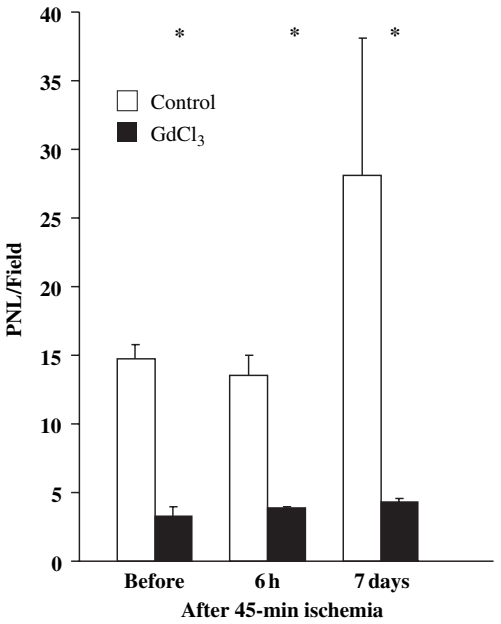

Figure 1 Number of polymorph nucleated leukocytes (PNL)/field (magnification 400×) in biopsies of pig livers before warm ischemia and 6 h and 7 days after reperfusion. PNL were counted in hematoxylin and eosin stained liver biopsies and are shown to represent mainly Kupffer cells in the liver. *$P < 0.05$; Student's *t*-test.

(Time point, 6 h: 68 ± 3,4% vs. 81 ± 6,1%; 2 days: 74 ± 3.8% vs. 88 ± 4.1%, $P < 0.05$). No difference was seen in PTT.

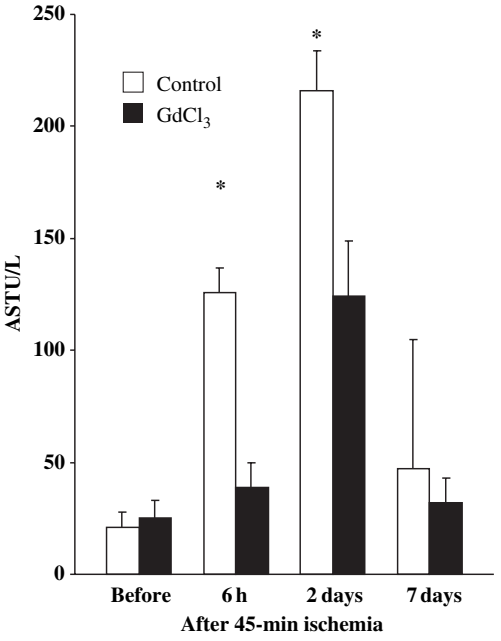

Figure 2 Serum transaminases 6 h, 2 days and 7 days after 45 min of warm ischemia in pigs with and without $GdCl_3$ treatment. *$P < 0.05$; Student's *t*-test.

Table 1. Liver histology 6 h and 7 days after warm ischemia.

	(Number per field 400×)			
	Necroses		Vacuoles	
	6 h	7 days	6 h	7 days
Controls	9.2 ± 1.8	1.5 ± 0.5	12.7 ± 0.4	7.2 ± 0.9
$GdCl_3$ group	2.3 ± 1.1	1.5 ± 0.3	2.9 ± 1.1	2.9 ± 0.8
P-value	<0.01	1.0	<0.05	<0.01

Histology of liver biopsies taken 6 h and 7 days after 45 min warm liver ischemia of control or $GdCl_3$ treated livers. Biopsies were formalin fixed, paraffin embedded and hematoxylin–eosin stained. All histologic examinations were performed using a computer-assisted histology analyzer and a video microscope (Leitz). In every biopsy five randomized high power fields (magnification 400×) were examined for necrotic hepatocytes and vacuoles in hepatocytes by two different analyzers. Student's *t*-test was used to calculate significant differences.

Systemic macrocirculation

Systemic macrocirculation was not changed in anyway by $GdCl_3$ treatment. Continuous cardiopulmonary monitoring revealed no differences in heart rate, mean arterial or central venous pressure at any time point before or after ischemia (shortly after ischemia heart rate of animals was 88 ± 15 in controls and 88 ± 11 in $GdCl_3$ treated animals and mean arterial pressure was 56 ± 7 in controls vs. 60 ± 13 in Kupffer cell depleted animals.)

Hepatic macro- and microcirculation

After ischemia, hepatic arterial and portal blood flow and hepatic microcirculation were improved in Kupffer cell depleted animals compared with controls at all time points later than 2 h after reperfusion. Hepatic blood flow and parenchymal microcirculation was significantly enhanced 4 h, 6 h and 7 days after reperfusion. THBF was 516 ± 106 (4 h), 527 ± 72 (6 h) and 638 ± 55 ml/min (7 days) in controls versus 696 ± 163 (4 h), 817 ± 107 (6 h) and 1000 ± 156 ml/min (7 days) in $GdCl_3$ treated pigs after reperfusion ($P < 0.05$, Fig. 3). At all time points hepatic arterial blood flow almost doubled, 76 ± 25 ml/min (6 h, controls) vs. 136 ± 50 ml/min (6 h, $GdCl_3$ treated animals), while portal venous flow was increased by more than 30% in treated livers, 535 ± 126 ml/min (6 h, controls) vs. 816 ± 170 ml/min (6 h, $GdCl_3$ treated animals). TD was 54 ± 6.8 (4 h), 54 ± 8.8 (6 h) and 66 ± 13 ml/min/100 g (7 days) in untreated animals versus 76 ± 12.3 (4 h), 91 ± 18.3 (6 h) and 88 ± 8.4 ml/min/100 g (7 days) in Kupffer cell depleted animals ($P < 0.001$; Fig. 4). Differences between THBF and TD mean values were insignificant 1 and 2 h after reperfusion.

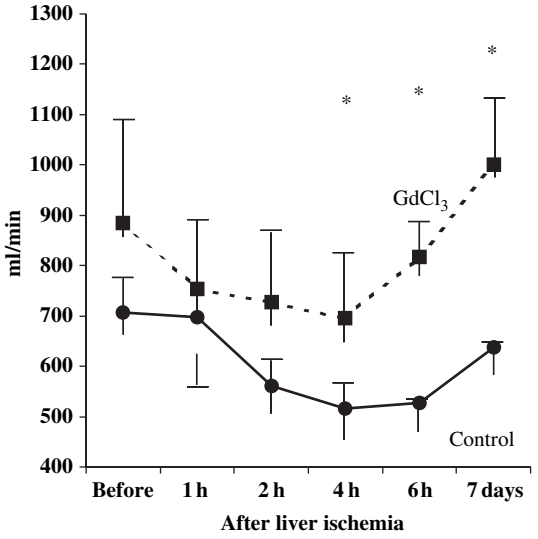

Figure 3 Transhepatic blood flow (THBF) before warm ischemia and 1, 2, 4, 6 h and 7 days after reperfusion in pigs with and without $GdCl_3$ treatment. *$P < 0.05$; Student's *t*-test.

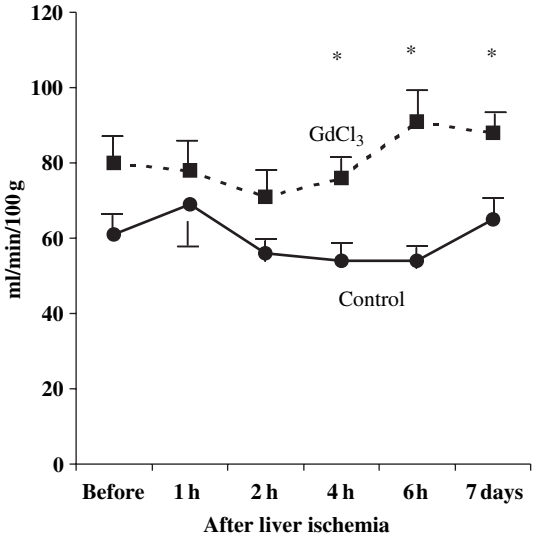

Figure 4 Parenchymous liver microcirculation (TD) in control or Kupffer cell depleted livers before warm ischemia and 1, 2, 4, 6 h and 7 days after reperfusion. *$P < 0.05$; Student's *t*-test.

Posthepatic endothelin plasma levels

Mean posthepatic endothelin plasma concentration in the hepatic vein already was markedly reduced in $GdCl_3$ treated animals at all time points compared with controls (time point, 6 h: 0.9 ± 0.3 pg/ml vs. 0.3 ± 0.1 pg/ml; 2 days: 1.0 ± 0.3 pg/ml vs. 0.4 ± 0.2 pg/ml, $P < 0.05$). Levels were already increased in treated animals before the induction of ischemia (0.3 ± 0.2 pg/ml vs. 0.2 ± 0.1 pg/ml, $P = 0.6$; Fig. 5).

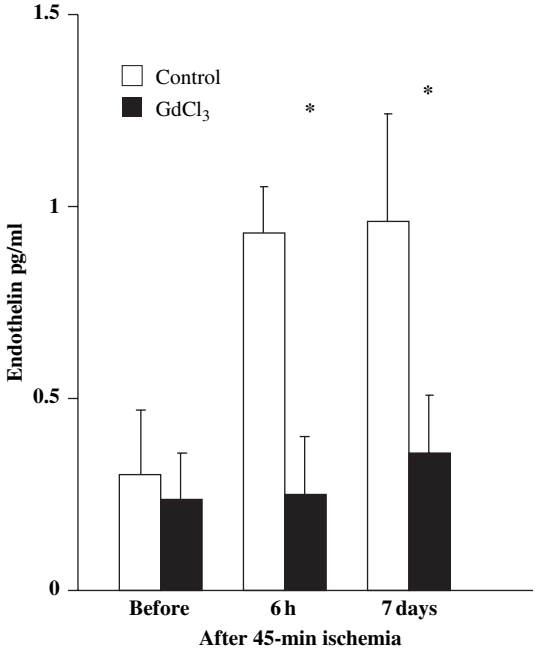

Figure 5 Posthepatic endothelin concentrations in the suprahepatic caval vein before warm liver ischemia and 6 h and 7 days after reperfusion of livers from pigs with and without $GdCl_3$ preconditioning. *$P < 0.05$; Student's *t*-test.

Endothelin levels 6 h and 7 days after ischemia also were significantly increased in saline treated animals ($P < 0.05$).

Discussion

Kupffer cells play a crucial role in the mediation of reperfusion injury by production of toxic substances like reactive oxygen species, proteases and release of mediators such as interleukin, adhesion molecules and TNF-α [5,7,11,13,22,23]. Destruction of Kupffer cells by $GdCl_3$ can prevent these events and minimize hepatic ischemia/reperfusion injury in various rat models after warm and cold ischemia, toxic injury by LPS, acute and chronic ethanol intoxication [3,11,24–26]. Furthermore, pretreatment of alcohol fed donors by $GdCl_3$ prevents failure of fatty marginal grafts after experimental LTX in rats [26,27]. It was previously further shown that Kupffer cell depletion could also reduce ischemia/reperfusion injury after LTX in both rats and pigs [3,7,9,23]. Our current study has now demonstrated for the first time, that also severe hepatic injury after a prolonged period of warm ischemia and subsequent reperfusion can be markedly reduced by $GdCl_3$ pretreatment in a large animal model, as reflected by both postischemic AST

plasma kinetics and liver histology. Histologic examination of biopsies at different time points after warm ischemia revealed a significantly reduced injury after pretreatment. Mean numbers of necrotic cells and areas 6 h after warm ischemia were significantly reduced. Six hours and 7 days after warm liver ischemia significantly less vacuoles were detected in hepatocytes. Numbers of polymorph nucleated leukocytes (PNL) were reduced in livers before ischemia because of destruction of Kupffer cells by $GdCl_3$. This effect was still detectable 7 days after warm ischemia. In contrast, control animals showed a significant rise in PNL in liver tissue 7 days after warm ischemia and reperfusion.

Disturbance of macroperfusion and microperfusion is a crucial factor and clearly defines the extent of hepatic damage, also of ischemia/reperfusion injury. Perfusion disturbances in parenchymous organs not only reflect injury, but also can be responsible for any damage itself. Previous experiments have also shown an increase in liver perfusion after $Gdcl_3$ pretreatment. After experimental LTX macroperfusion as well as microperfusion was significantly improved in Kupffer cell depleted livers compared with controls [9]. In our current model of warm hepatic ischemia, macroperfusion (reflected by THBF) as well as microperfusion (reflected by TD) were significantly improved in Kupffer cell depleted livers 4 h, 6 h and 7 days after temporal warm ischemia, while both groups showed a marked impairment of hepatic blood flow 1 h after reperfusion. THBF showed minimum values 4 h and microperfusion already 2 h after reperfusion. In treated animals blood flow reduction was less pronounced at all time points. Recovery of macroperfusion was first detected 6 h after reperfusion and progressive. THBF even exceeded preischemic baseline liver perfusion values 7 days after reperfusion in pretreated animals. In contrast, THBF was still reduced by more than 10% compared with baseline values at this time point in controls. Hepatic microperfusion also first recovered 4 h after reperfusion. Corresponding to THBF values, peak hepatic microcirculation flow values at the time points 6 h and 7 days after ischemia exceeded baseline preischemic flow values.

The pathophysiological mechanisms of gadolinium chloride which lead to increased liver perfusion have not been fully clarified so far. A relevant pathophysiological mechanism might be the decreased intrahepatic production of reactive oxygen species after $GdCl_3$ induced Kupffer cell depletion. Reactive oxygen species are known to block the enzyme guanylat cyclase. This effect would then lead to lower local cGMP concentrations in hepatic tissue which then will decrease local NO production. This pathophysiological sequence is known to be markedly enhanced by different mediators released from activated Kupffer cells. By knocking out Kupffer cells, this pathway might be reduced in activity or even blocked. Intrahepatic NO production and NO release could thereby be increased after $GdCl_3$ application and as a consequence lead to hepatic vascular relaxation [6,14,23,27].

As Kupffer cells also play an important role in intercellular hepatic communication, a decreased release of mediators like TNF-α, platelet activating factor (PAF) and leukotriene could also lead to decreased platelet aggregation, which also will improve hepatic sinusoidal perfusion. Furthermore, particularly a diminished release of TNF-α, which is known to be a major stimulus for endothelin-1 generation by endothelial cells, could be responsible for reduction of ET synthesis and secretion, as demonstrated in our current experiments [4–6,11,20,23,24]. This could be responsible for a decreased contraction of the intrahepatic vascular system or of stellate cells [28].

The peptide ET is one of the most potent vasoconstrictors know so far. ET not only causes constriction of arterioles and venoles, but also can lead to a contraction of intrahepatic stellate cells [29]. This may increase intrahepatic resistance up to a complete stasis in sinusoids. Hypoxia again leads to decreased oxidative phosphorylation and impairment of ATP-dependent electrolyte pumps. Secondary transcellular electrolyte shifts lead to swelling of hepatocytes and may increase resistance in liver sinusoids. This can constitute a vicious circle. Disturbances of macro- and microperfusion after warm ischemia of the liver were found to be associated with a significant increase of ET-1 plasma concentration in the hepatic vein 6 h and 7 days after reperfusion in our current experiments. Furthermore, in $GdCl_3$ treated animals endothelin levels in the hepatic vein, which should be characteristic for intrahepatic ET secretion, were significantly reduced at all time points compared with controls. In contrast, a boost of endothelin secretion after postischemic liver reperfusion was detected in untreated control livers. ET serum levels in the hepatic vein were still elevated 7 days after reperfusion, compared with preischemic baseline levels in untreated animals. This could be effectively prevented by $GdCl_3$ induced destruction of the Kupffer cells and clearly has significantly reduced disturbances in liver perfusion, which were noticed in untreated animals. Two pathways may explain this interesting phenomenon, which has not been described so far. First, as explained above, activated Kupffer cells release different mediators which may activate endothelial cells and lead to TNF-α-dependent endothelin production and release. By knocking out Kupffer cells, this pathway is blocked. Secondly, minimizing ischemia/reperfusion injury should furthermore be associated with less hepatic endothelial damage leading to subsequent activation of endothelial cells, again reducing the release of endothelin [3,6,7,14,20,23,30,31].

Summarizing the results of the current experimental study we can conclude that $GdCl_3$ treatment leads to destruction of Kupffer cells in the liver in pigs. As was previously shown in a porcine model of cold hepatic storage and subsequent LTX, destruction of Kupffer cells by $GdCl_3$ also minimizes hepatic ischemia/reperfusion injury and improves hepatocellular function in the porcine model with warm liver ischemia/reperfusion. Decreased endothelin concentration detected in the hepatic vein point to a reduced intrahepatic ET secretion after $GdCl_3$ pretreatment. This factor may have contributed to enhance liver perfusion, compared with the control setting as demonstrated in our current experiment.

Therapeutic Kupffer cell destruction with $GdCl_3$ before liver surgery appears to be very effective in reduction of postoperative ischemia/reperfusion injury in the liver. The concept of $GdCl_3$ pretreatment can potentially be transferred into the clinical practice. Research must now focus to exclude toxic side effects before using $GdCl_3$ in humans.

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References

1. Selzner M, Clavien P-A. Fatty liver in liver transplantation and surgery. *Semin Liver Dis* 2001; **21**: 105.
2. 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.
3. Suzuki K, Konno T, Nakamura B. Regulation of pro-inflammatory and anti-inflammatory cytokine responses by Kupffer cells in endotoxin-enhanced reperfusion injury after total hepatic ischemia. *Transpl Int* 2003; **16**: 231.
4. Nathan CF. Secretory products of macrophages. *J Clin Invest* 1987; **79**: 319.
5. Wardle EN. Kupffer cells and their function. *Liver* 1987; **7**: 63.
6. Serracino-Inglott F, Habib NA, Mathie RT. Hepatic ischemia–reperfusion injury. *Am J Surg* 2001; **181**: 160.
7. Olthoff KM. Molecular pathways of regeneration and repair after liver transplantation. *World J Surg* 2002; **26**: 831.
8. 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.
9. Frankenberg MV, Golling M, Mehrabi A, Nentwich H, Klar E, Kraus TW. Donor pretreatment with gadolinium chloride improves early graft function and survival after porcine liver transplantation. *Transpl Int* 2003; **16**: 806.
10. Schemmer P, Connor HD, Arteel GE, *et al.* Reperfusion injury in livers due to gentle in situ organ manipulation during harvest involves hypoxia and free radicals. *J Pharmacol Exp Ther* 1999; **290**: 235.
11. 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.
12. 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.
13. 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.
14. Kimura H, Katsuramaki T, Isobe M, *et al.* Role of inducible nitric oxide synthase in pig liver transplantation. *J Surg Res* 2003; **111**: 28.
15. Kraus T, Mehrabi A, Golling M, *et al.* Effects of exogenous endothelin-1 application on liver perfusion in native and transplanted porcine livers. *J Surg Res* 2000; **93**: 272.
16. Kraus T, Mehrabi A, Klar E, *et al.* Intraoperative evaluation of big-endothelin plasma levels during liver transplantation in different vascular compartments. *Transpl Int* 1994; **7**(Suppl. 1): S144.
17. Mizunuma K, Ohdan H, Tashiro H, Fudaba Y, Ito H, Asahara T. Prevention of ischemia-reperfusion-induced hepatic microcirculatory disruption by inhibiting stellate cell contraction using rock inhibitor. *Transplantation* 2003; **75**: 579.
18. Krejci V, Hiltebrand L, Erni D, Sigurdsson G. Endothelin receptor antagonist bosentan improves microcirculatory blood flow in splanchnic organs in septic shock. *Crit Care Med* 2003; **31**: 203.
19. Yanagisawa M, Kiruhara H, Kimura S, *et al.* A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature* 1988; **332**: 411.
20. Klar E, Kraus T, Bredt M, *et al.* First clinical realization of continuous monitoring of liver microcirculation after transplantation by thermodiffusion. *Transpl Int* 1996; **9**(Suppl. 1): S140.
21. 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.

22. Decker K. Biologically active products of stimulated liver macrophages (Kupffer cells). *Eur J Biochem* 1990; **192**: 245.
23. Lentsch O. Cytokine cascades and the hepatic inflammatory response to ischemia and reperfusion. *J Invest Surg* 2003; **16**: 141.
24. 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.
25. Zhong Z, Li X, Yamashina S, *et al.* Cyclosporin A causes a hypermetabolic state and hypoxia in the liver: prevention by dietary glycine. *J Pharmacol Exp Ther* 2001; **299**: 858.
26. 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.
27. Frankenberg Mv, 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.
28. Sakamoto M, Ueno T, Kin M, *et al.* Ito cell contraction in response endothelin-1 and substance P. *Hepatology* 1993; **18**: 978.
29. Schemmer P, Enomoto N, Bradford BU, *et al.* Activated Kupffer cells cause a hypermetabolic state after gentle in situ manipulation of liver in rats. *Am J Physiol Gastrointest Liver Physiol* 2001; **280**: G1076.
30. Oikawa K, Ohkohchi N, Sato M, Masamume A, Satomi S. Kupffer cells play an important role in the cytokine production and activation of nuclear factors of liver grafts from non-heart-beating donors. *Transpl Int* 2002; **15**: 397.
31. Jin M, Todo S. The role of endothelin-1 in hepatic ischemia and reperfusion injury. *J Gastroenterol* 2002; **37**: 763.