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Peter Schemmer
Robert Schoonhoven
James A. Swenberg
Hartwig Bunzendahl
James A. Raleigh
John J. Lemasters
Ronald G. Thurman

Gentle organ manipulation during harvest as a key determinant of survival of fatty livers after transplantation in the rat

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P. Schemmer · R.G. Thurman (✉)
Laboratory of Hepatobiology and Toxicology, Department of Pharmacology, CB 7365, Mary Ellen Jones Bldg., University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA
e-mail: thurman@med.unc.edu

R. Schoonhoven · J.A. Swenberg
Department of Environmental Sciences and Engineering, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA

H. Bunzendahl
Department of Surgery, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA

J. A. Raleigh
Department of Radiation Oncology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA

J. J. Lemasters
Department of Cell Biology and Anatomy, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA

Abstract Both in situ organ manipulation during harvest and steatosis reduce survival after liver transplantation via mechanisms involving Kupffer cells; thus, their effect on survival was compared here. Moderate steatosis was induced by a single dose of ethanol to Lewis rats, while long-term administration of ethanol yielded severe steatosis in donor animals. After minimal dissection during the first 12 min, livers were either manipulated gently or left alone for 13 min subsequently. Orthotopic liver transplantation was performed after 1 h of cold storage in UW solution. Ethanol increased hepatic lipid content to a level of moderate or severe steatosis that reduced survival after transplantation from 100% to approximately 70% ($P < 0.05$). However, gentle manipulation decreased survival to approximately 30% ($P < 0.05$) in livers from normal, saline-treated rats and in livers from rats fed a high-fat control diet. Moreover, after short- or long-term ethanol administration, manipulation of fatty livers decreased survival from 70% to approximately 13% ($P < 0.05$). Further, manipulation elevated serum transaminases, total bilirubin, and necrosis significantly about 2- to 20-fold in fatty grafts after transplantation. At the end of harvest, trypan blue distribution time and hypoxia assessed from 2-nitroimidazole binding were elevated significantly about two- to fourfold by manipulation of fatty grafts. Gadolinium chloride, a Kupffer cell toxicant, blocked the detrimental effects of manipulation. These data demonstrate for the first time that, while steatosis is detrimental for survival, organ manipulation plays a much greater role than fat in mechanisms of primary nonfunction.

Key words Liver transplantation · Organ harvest · Kupffer cells · Ethanol · Gadolinium chloride

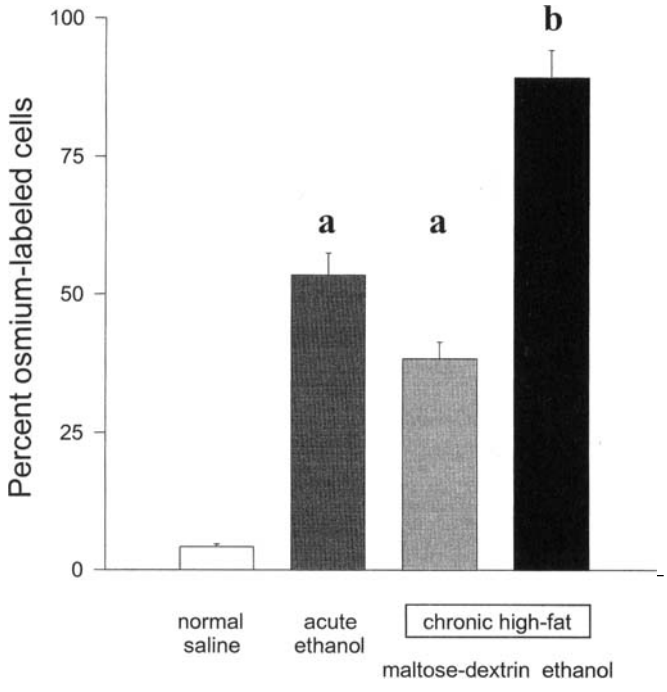

Fig. 1 Effect of short- and long-term ethanol administration on intracellular lipid accumulation. In a binge-drinking model, rats were gavaged with a single dose of ethanol or isovolemic normal saline in chow-fed controls before organ harvest. In a long-term ethanol-drinking model, animals were fed a liquid high-fat diet for 4 weeks, with 36% of calories from ethanol or maltose-dextrin. To compare fat content, livers were stained with osmium as described in Materials and methods. Values are mean ± SEM ($P < 0.05$ by two-way ANOVA with Student-Newman-Keuls post hoc test; $n = 5–10$). **a** $P < 0.05$ compared with saline controls; **b** $P < 0.05$ compared with moderate steatosis (acute ethanol, maltose-dextrin)

Introduction

Liver transplantation is the therapy of choice in an increasing number of liver diseases [28]; however, the organ pool is limited. Since primary nonfunction and dysfunction occur in 5–30% of human liver transplantation cases, for reasons that still remain unclear [29], there is an urgent need to understand the underlying mechanisms.

Brain-dead accident victims are a major source of donor organs; since accidents are overwhelmingly associated with alcohol consumption, alcohol use is probably a common characteristic of organ donors [16, 27]. Unfortunately, ethanol causes hepatic fat infiltration, and approximately 35% of potential donor livers exhibit fat accumulation to some extent [3]. It is well known that livers with a high fat content are frequently associated with nonfunction or dysfunction after transplantation [29,39]. Livers with a mild fat accumulation have better rates of function and are reported to be suitable for transplantation [2, 35]; however, many transplant centers do not accept marginal livers as grafts because of the significantly higher risk of primary nonfunction, even though many fatty livers perform well after transplantation [2, 39]. To extend the donor pool, the incidence of primary nonfunction needs to be reduced, especially with fatty livers.

Recently, in situ manipulation by touching, retracting, and moving liver lobes gently during harvest, which cannot be prevented with standard harvesting techniques, has been demonstrated to dramatically reduce survival after transplantation [32]. Further, in clinical liver transplantation, it was observed that dissection of the liver during harvest reduced graft function after transplantation. Moreover, in pigs, preparation of the portal vein alone impairs the intrahepatic microcirculation [11, 18, 20, 34]. Since both in situ organ manipulation during harvest and steatosis reduce survival after liver transplantation via mechanisms involving Kupffer cells, their effect on survival was compared here. In addition, this study was designed to determine whether depletion of Kupffer cells could improve the overall outcome of liver transplantation by reducing the effect of manipulation.

Materials and methods

Experimental animals and treatment

Lewis rats (200–230 g) were allowed free access to laboratory chow and tap water. In one experimental series, fed female donor rats were treated with a single high dose of ethanol (binge-drinking model) by gavage 20 h before harvest [41]. Chow-fed control rats were given an equivalent volume of normal saline. In a second experimental series, female donor rats (4–5 weeks of age) were caged individually and pair-fed a modified liquid high-fat diet for 4–5 weeks (final weight 200–230 g) [23]. Both high-fat control and high-fat ethanol-treated groups received equivalent amounts of corn oil (35% of calories), with 36% of calories coming from ethanol (chronic ethanol model) or isocaloric maltose-dextrin. In both experiments, some donor animals were given a single injection of gadolinium chloride ($GdCl_3$; 10 mg/kg in acidic saline) through the tail vein 24 h prior to organ harvest. This treatment destroys all large ED2-positive Kupffer cells and minimizes ED1-positive Kupffer cells [15]. All animals were given humane care in compliance with institutional guidelines.

Organ harvest procedure

To determine the influence of gentle manipulation on fatty livers, donor livers were harvested within 25 min prior to perfusion with cold UW solution as described elsewhere [32]. Briefly, minimal dissection was performed in a standardized fashion during the first 12 min, including freeing the organ from ligaments and cannulation of the bile duct. During the next 13 min, livers were either left alone or manipulated gently. To standardize conditions, gentle manipulation was carried out by the same surgeon touching, retracting, and moving the liver lobes in situ for exactly 13 min uni-

formly and continuously [32]. At 25 min, perfusion with 8 ml of cold Ringer's solution followed by 3 ml of cold UW solution was performed in situ via the portal vein. Cuffs were attached in the cold to the infrahepatic vena cava and portal vein after explantation.

Transplantation

All animals were anesthetized with methoxyflurane. Orthotopic liver transplantation was performed using arterialization [13], since hepatic artery reconstruction is routinely used in clinical liver transplantation. After explantation, livers were stored at 0–4 °C for 1 h in UW solution to exclude effects of cold storage on graft viability [38]. Grafts were rinsed with 10 ml of normal saline (18 °C) and implanted by connecting the suprahepatic vena cava with a running 7/0 Prolene suture, inserting cuffs into the corresponding vessels and anastomosing the bile duct and hepatic artery over an intraluminal polyethylene splint. Transplantation required less than 40 min; during this time the portal vein was clamped for 12–15 min. After transplantation, recipients had free access to chow and tap water.

Histological procedures

Some rats were killed 8 h after transplantation, and livers were fixed by perfusion with 4 % paraformaldehyde in Krebs-Henseleit bicarbonate buffer (118 mM NaCl, 25 mM $NaHCO_3$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 4.7 mM KCl, 1.3 mM $CaCl_2$) at pH 7.6, embedded in paraffin, and processed for light microscopy. Liver damage was assessed by estimating the proportion of necrotic to nonnecrotic areas in H&E-stained sections as described elsewhere [38]. Briefly, five fields (× 100 magnification) were selected at random from at least four different sections per sample, and mean values were calculated. Further, osmium staining was used to detect lipids as described elsewhere [24]. A Universal Imaging Corporation image acquisition and analysis system (Image-1/AT; Chester, Pa.) incorporating an Axioskop 50 microscope (Carl Zeiss, Thornwood, N. Y.) was used to capture and analyze osmium-stained tissue sections at × 100 magnification [5].

Assessment of hepatic microcirculation

Prior to organ removal, when perfusion with cold preservative solution would normally have been performed, some donor livers were perfused in situ via the portal vein at 3–4 ml/min per gram of liver with oxygenated Krebs-Henseleit bicarbonate buffer at pH 7.6, saturated with 95 % O_2 and 5 % CO_2 at 37° C. Trypan blue (500 µM; Aldrich, Milwaukee, Wis.) was infused for 10 min, and the time for the dye to distribute completely was recorded to index the hepatic microcirculation [19].

Determination of reduced, protein-bound pimonidazole by enzyme-linked immunosorbent assay and immunohistochemistry

Pimonidazole, a 2-nitroimidazole hypoxia marker, binds to hypoxic liver cells in vivo [6]. Pimonidazole was given intravenously to donors 5 min before organ harvest. Pimonidazole adduct accumulation was measured in tissue homogenates with a competitive enzyme-linked immunosorbent assay (ELISA) procedure, described previously [30] and modified for liver tissue [6]. Protein levels in tissue homogenates were determined with the bicinchoninic acid assay using a commercially available kit (Pierce Chemical Company, Rockford, Ill.). Paraffin blocks of formalin-fixed liver tissue were sectioned at 6 µm, and pimonidazole was detected with a biotin-streptavidin-peroxidase indirect immunostaining method using diaminobenzidine as chromogen as described previously [6]. The Image-1/AT imaging system was used [5].

Kupffer cell number was assessed in vivo immunohistochemically [10]. Immediately after harvesting and 8 h after transplantation, liver was collected. Sections (6 µm) were cut on a rotary microtome and stained for ED1-positive Kupffer cells immunohistochemically using the DAKO Envision System and a primary anti-ED1 antibody (Biosource International, Camarillo, Calif.). Subsequently a counterstain of hematoxylin was applied.

Assays

Blood samples were collected from the tail vein 8 h after transplantation. Serum was obtained by centrifugation and stored at –80 °C until analysis. Aspartate immunotransferase (AST) and alanine immunotransferase (ALT) were determined by standard enzymatic methods [7]. Eight hours after transplantation, total bilirubin was determined in sera by direct spectrophotometry at 454 nm as described elsewhere [31].

Statistics

Mean values ± SEM for various groups were compared using Fisher's exact test or two-way analysis of variance with Student-Newman-Keuls post hoc test as appropriate. $P < 0.05$ was selected prior to the study as the criterion for significance.

Results

Effects of gentle organ manipulation on survival after transplantation of fatty livers

To compare lipid accumulation, donor livers were stained with osmium. Livers from rats given saline displayed less than 5 % osmium-positive cells, while short-term ethanol administration increased the number of positive cells to about 50 % (moderate steatosis). Further, about 40 % (moderate steatosis) of cells were stained with osmium in rats treated with a long-term high-fat control diet alone. In contrast, long-term ethanol administration yielded values of about 90 % (severe steatosis; $P < 0.05$; Fig. 1). In saline and high-fat controls receiving maltose-dextrin, survival was 100 % after transplantation; however, fat induced with short- or long-term ethanol decreased survival slightly from 100 % to approximately 70 % ($P < 0.05$). Further, gentle in situ manipulation decreased survival significantly from 100 % to 30 % and from 70 % to 13 % in both control groups and fatty livers, respectively (Fig. 2). Moreover, total bilirubin was increased significantly about 5-fold after transplantation of fatty livers, while manipulation increased values three- to ninefold ($P < 0.05$; Table 1). Gadolinium chloride ($GdCl_3$), which decreased

Table 1 *Effect of gentle organ manipulation on serum transaminases and total bilirubin levels after transplantation. Hepatic fat was induced as described in Materials and methods. Eight hours after transplantation blood was collected from the tail vein. Serum AST, ALT and total bilirubin were determined as described in the Materials and Methods. (A) Chow-fed (normal saline) and high-fat controls, (B) fatty livers induced with acute and chronic ethanol*

A	No manipulation		Manipulation		Manipulation + $GdCl_3$	
	Normal saline	High-fat maltose-dextrin	Normal saline	High-fat maltose-dextrin	Normal saline	High-fat maltose-dextrin
AST (U/l)	312 ± 27	452 ± 97	1490 ± 157[a]	1750 ± 132[a]	563 ± 92[b]	472 ± 52[b]
ALT (U/l)	176 ± 25	193 ± 46	871 ± 57[a]	981 ± 77[a]	321 ± 33[b]	282 ± 43[b]
Bilirubin (mg%)	0.1 ± 0.05	0.1 ± 0.05	0.9 ± 0.02[a]	0.7 ± 0.04[a]	0.2 ± 0.05[b]	0.3 ± 0.01[b]
B	**Acute ethanol**	**High-fat chronic ethanol**	**Acute ethanol**	**High-fat chronic ethanol**	**Acute ethanol**	**High-fat chronic ethanol**
AST (U/l)	2268 ± 489[c]	905 ± 221[c]	4447 ± 1095[a,c]	4301 ± 738[a,c]	2581 ± 741[b,c]	2052 ± 252[b,c]
ALT (U/l)	645 ± 161[c]	640 ± 175[c]	2486 ± 121[a,c]	2874 ± 299[a,c]	683 ± 323[b,c]	1296 ± 148[b,c]
Bilirubin (mg%)	0.5 ± 0.1[c]	0.4 ± 0.05[c]	1.5 ± 0.03[a,c]	1.2 ± 0.05[a,c]	0.4 ± 0.1[b,c]	0.2 ± 0.01[b]

Values are mean ± SEM ($P < 0.05$ by two-way ANOVA with Student-Newman-Keuls post-hoc test, $n = 5$–8). [a] $P < 0.05$ for comparison with appropriate groups not receiving manipulation; [b] $P < 0.05$ comparison with appropriate groups with manipulation but without pretreatment with $GdCl_3$; [c] $P < 0.05$ compared with corresponding controls

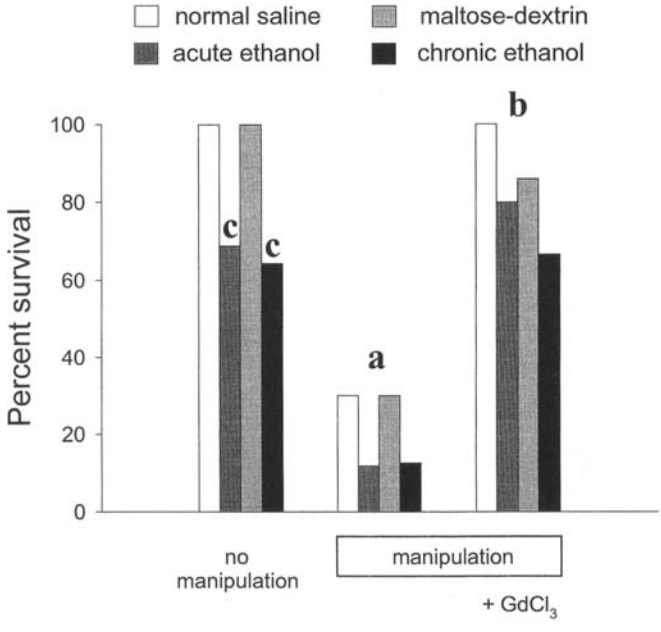

Fig. 2 Effect of gentle organ manipulation on survival rates of fatty livers after transplantation. Hepatic fat was induced as described in Fig. 1. Donor livers were harvested with or without gentle manipulation during harvest. Some donors from each group were pretreated with $GdCl_3$ as described in Materials and methods. After harvest, grafts were stored in UW solution at 0–4 °C for 1 h and transplantation was performed using arterialization. **a** $P < 0.05$ for comparison with appropriate groups not receiving manipulation; **b** $P < 0.05$ for comparison with appropriate groups with manipulation but without pretreatment with $GdCl_3$; **c** $P < 0.05$ compared with saline controls by Fisher's exact test; $n = 14$–18

the number of ED1-positive Kupffer cells by 70%, blocked the effect of gentle manipulation on graft viability after transplantation in all groups. $GdCl_3$ improved survival nearly to control values ($P < 0.05$; Fig. 2). The same protective effect due to Kupffer cell depletion was observed in total bilirubin (Table 1).

Effect of gentle organ manipulation on liver injury in fatty livers

In all groups studied, tissue injury was undetectable prior to cold storage, but mild necrosis developed 8 h after transplantation in chow-fed and high-fat controls. Hepatic lipid increased necrosis about five- to twenty-fold ($P < 0.05$), while serum transaminases were increased significantly two- to sevenfold (Table 1, Fig. 3). Moreover, fatty grafts induced with short-term ethanol administration released about twofold more transaminases than fatty livers from rats treated chronically with ethanol. Hepatic manipulation aggravated reperfusion injury significantly reflected by about 20% necrotic tissue (Fig. 3) and a fourfold increase in serum transaminases (Table 1) 8 h after transplantation. Gentle manipulation of fatty grafts induced with ethanol further increased necrosis after transplantation to about 40% ($P < 0.05$; Fig. 3) and increased serum transaminases two- to fourfold (Table 1). $GdCl_3$ given to donors prior to harvest blunted these pathological changes ($P < 0.05$; Table 1, Fig. 3).

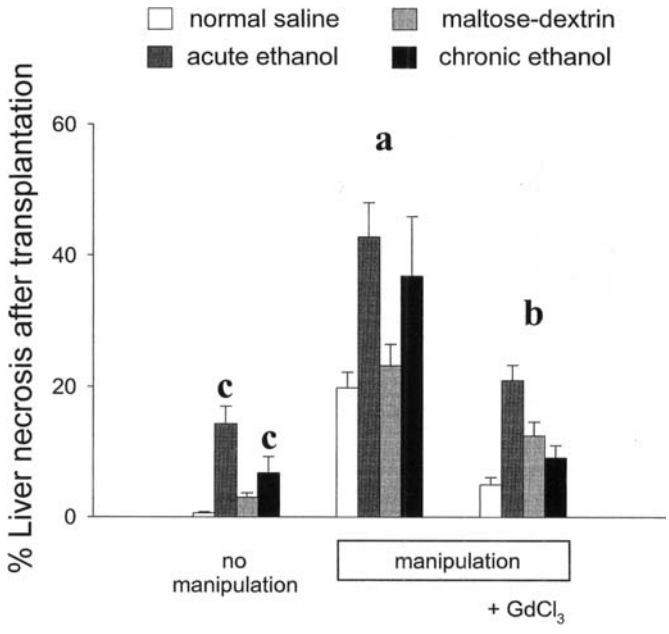

Fig. 3 Effect of gentle organ manipulation on necrosis in fatty livers. Hepatic fat was induced as described in Fig. 1. Liver damage was assessed by estimating the proportion of necrotic to nonnecrotic areas in five fields (× 100 magnification) selected at random from at least four different sections per sample 8 h after transplantation. Some donors were treated with $GdCl_3$ before harvest. Values are mean ± SEM ($P < 0.05$ by two-way ANOVA with Student-Newman-Keuls post hoc test; $n = 4$–8). **a** $P < 0.05$ for comparison with appropriate groups not receiving manipulation; **b** $P < 0.05$ for comparison with appropriate groups with manipulation but without pretreatment with $GdCl_3$; **c** $P < 0.05$ compared with saline controls

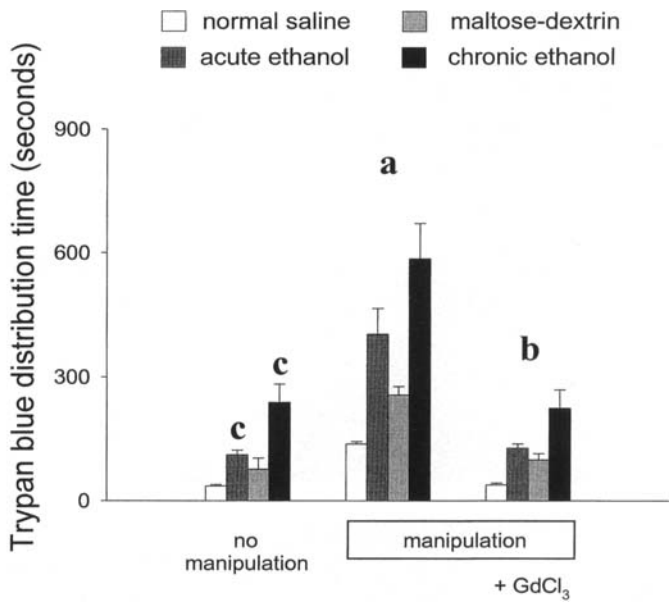

Fig. 4 Effect of gentle organ manipulation on microcirculation in fatty livers. Hepatic fat was induced as described in Fig. 1. Some donors from each group were pretreated with $GdCl_3$. After harvest, livers were perfused in situ with trypan blue to index microcirculation as described in Materials and methods. Values are mean ± SEM ($P < 0.05$ by two-way ANOVA with Student-Newman-Keuls post hoc test; $n = 5$–10). **a** $P < 0.05$ for comparison with appropriate groups not receiving manipulation; **b** $P < 0.05$ for comparison with appropriate groups with manipulation but without pretreatment with $GdCl_3$; **c** $P < 0.05$ compared with saline controls

Effect of gentle organ manipulation on microcirculation in fatty livers

Prior to organ removal, donor livers were perfused in situ with trypan blue via the portal vein. The time for the dye to distribute completely was used as an index of changes in intrahepatic microcirculation [19]. Overall, hepatic fat increased the time for trypan blue to distribute homogeneously about two- to sevenfold ($P < 0.05$); however, manipulation increased this time by a further two- to fourfold ($P < 0.05$; Fig. 4). When Kupffer cells were destroyed by $GdCl_3$, the microcirculation was not different from controls (Fig. 4). Distribution of blood at reperfusion followed the same pattern (data not shown).

Effect of gentle organ manipulation on hypoxia in fatty livers

Here, binding of pimonidazole reflecting hypoxia was increased more than twofold in fatty livers induced with ethanol prior to cold storage (Fig. 5). Gentle liver manipulation during harvest elevated binding of pimonidazole in normal livers about twofold ($P < 0.05$) and in fatty livers by a further two- to fourfold ($P < 0.05$), while $GdCl_3$ blunted the effect of manipulation on tissue hypoxia in all groups studied (Fig. 6). Pimonidazole binding predominated in pericentral regions, where oxygen supply is naturally low (Fig. 5).

Discussion

Gentle in situ organ manipulation during harvest is a key determinant for survival of fatty livers

Many patients die each year before a suitable organ becomes available. Of those receiving livers, primary nonfunction and dysfunction still occur in 5–30% of cases [29], leading to significant morbidity and mortality [29, 35]. Approximately 35% of potential donor livers exhibit elevated lipid levels to some extent, most frequently caused by short- or long-term ethanol consumption [3, 16, 21, 27]. Previous clinical and experimental studies have linked intrahepatic lipid content and ethanol con-

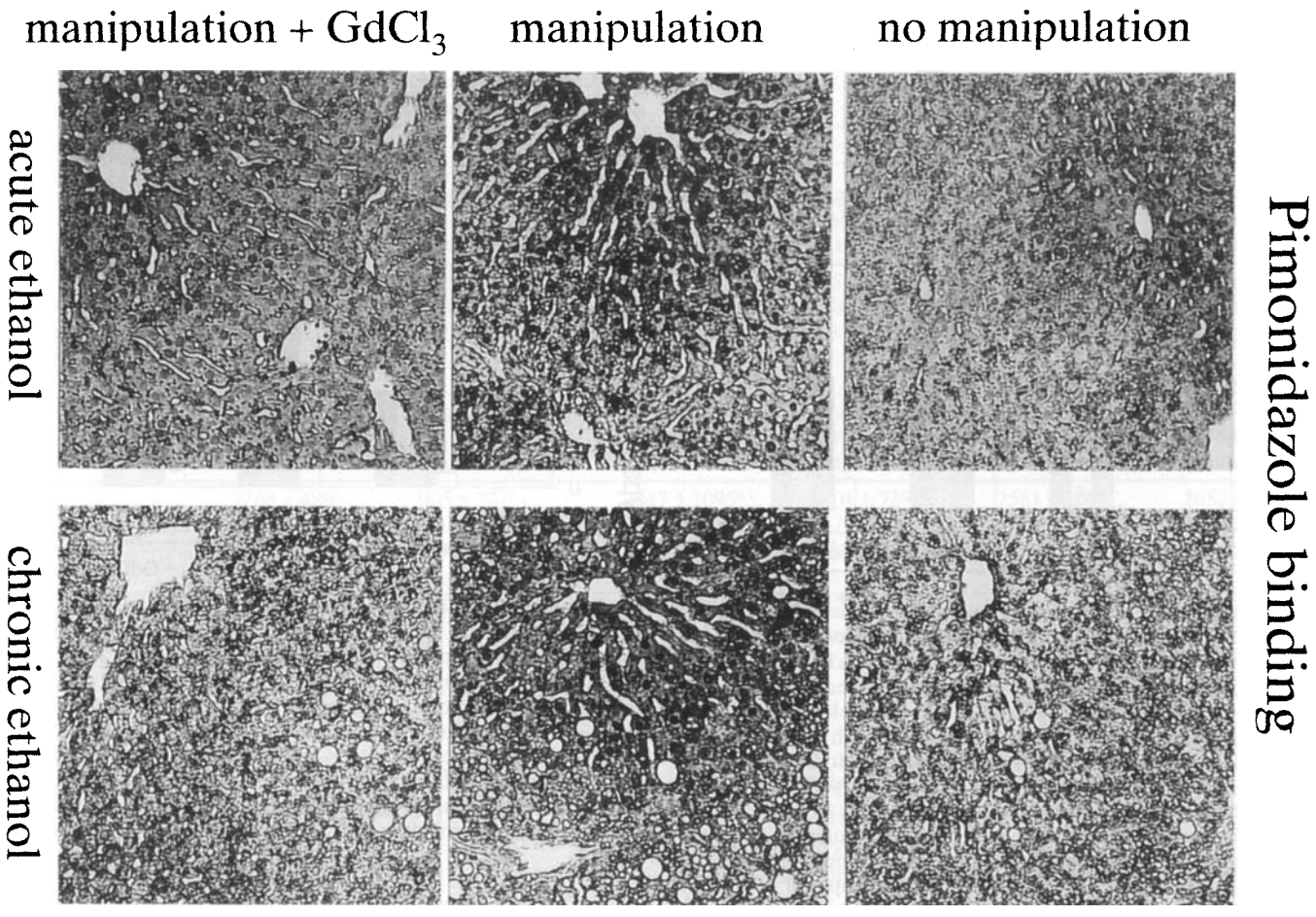

Fig. 5. Effect of gentle organ manipulation on pattern of pimonidazole binding in fatty livers. Conditions as in Fig. 4. Photomicrographs depict patterns of pimonidazole binding, which reflect hypoxia, in fatty livers after harvest. In all groups studied, pimonidazole binding predominated in pericentral regions of the liver lobule. Immunohistochemistry using antibodies to bound pimonidazole is described in Materials and methods. Some donors from each group were pretreated with $GdCl_3$. Photos in *upper row*, short-term ethanol; *lower row*, long-term ethanol. Typical experiments

sumption to a higher incidence of early graft injury and reduced graft function after transplantation; however, mechanisms of primary nonfunction of fatty livers remain unclear [2, 25, 35, 39].

Recently, in situ manipulation by touching, retracting, and moving liver lobes gently during harvest has been demonstrated to dramatically reduce survival of nonfatty grafts after transplantation by mechanisms involving priming or activation of Kupffer cells [32]. Graft manipulation during harvest cannot be prevented with standard harvesting techniques [34]. Since primary graft nonfunction is increased both by manipulation and steatosis, their effect on survival was compared here. Further, this study was designed to determine whether depletion of Kupffer cells, which play a major role in reperfusion injury and primary nonfunction, could improve the overall outcome of liver transplantation. To simulate the clinical situation, both a binge-drinking model and chronic ethanol treatment were used to induce steatosis. In clinical liver transplantation, accumulation of hepatic fat has been categorized into three groups: minimal (less than 33 % of hepatocytes containing fat), moderate (33 %–66 %) and severe (more than 66 %) [35]. In this study, a single dose of ethanol caused moderate fat accumulation, while long-term ethanol induced severe steatosis (Fig. 1). Interestingly, both moderate and severe steatosis induced with ethanol reduced survival after transplantation slightly from 100 % in saline and high-fat controls to about 70 %; however, the major finding of this study was the considerably greater impact of in situ graft manipulation compared with the effect of steatosis on survival (Figs. 1, 2). Manipulation of fatty grafts reduced survival by 60–70 % (Fig. 2). The impact of manipulation on reperfusion injury and graft function was about threefold greater than the effect of steatosis, reflected by increases in necrosis, serum transaminases, and bilirubin (Tables 1, 2, Fig. 3). Depletion of Kupffer cells blunted effects of manipulation on all

parameters studied. How can this be explained? These findings are consistent with the hypothesis that Kupffer cells become activated by ethanol [1, 12, 33] and are stimulated further by in situ organ manipulation [32]. Activation of Kupffer cells enhances the production of vasoactive mediators, which cause constriction of intrahepatic vessels [4], thus disturbing hepatic microcirculation (Fig. 4), leading to hypoxia (Figs. 5, 6). Several lines of evidence suggest that microcirculatory disturbances are a key factor in enhanced donor organ susceptibility to cold and warm ischemia in fatty livers [17, 37]. In this study, manipulation caused two- to fourfold greater disturbances in microcirculation and hypoxia prior to cold storage than steatosis due to ethanol per se (Figs. 4–6). This early Kupffer cell-dependent effect of manipulation may be responsible for reduced survival and graft viability after reperfusion compared with steatotic, nonmanipulated livers. Manipulation during harvest might further stimulate Kupffer cells [32], leading to release of toxic mediators [36], which could effect the later fate of the graft [8, 26]. In support of this hypothesis, outcome after transplantation of fatty grafts was markedly improved by $GdCl_3$, a Kupffer cell toxicant [15], given to donors prior to harvest (Table 1, Figs. 2–6).

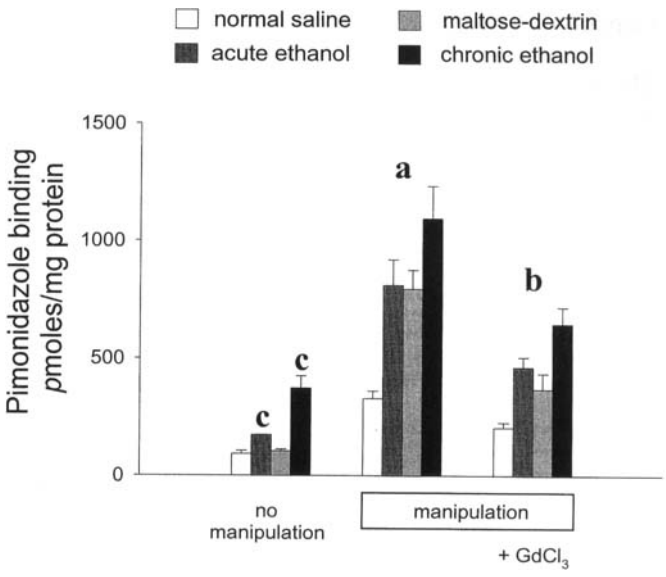

Fig. 6 Effect of gentle organ manipulation on hypoxia in fatty livers. Hepatic fat was induced as in Fig. 1. Some donors from each group were pretreated with $GdCl_3$. Competitive ELISA of pimonidazole is described in Materials and methods. Results are means ± SEM ($P < 0.05$ by two-way ANOVA with Student-Newman-Keuls post hoc test, $n = 5$). **a** $P < 0.05$ for comparison with appropriate groups not receiving manipulation; **b** $P < 0.05$ for comparison with appropriate groups with manipulation but without pretreatment with $GdCl_3$; **c** $P < 0.05$ compared with saline controls

Graft viability does not correlate with hepatic fat content

Since survival was not different in livers with moderate or severe steatosis (Fig. 2), it can be concluded that fat content had little effect on graft viability in these experiments (Fig. 1, 2). Indeed, fat droplets in hepatic cells may increase lipid peroxidation, which could be linked with injury to the graft; however, parenchymal cells, which contain most of the hepatic fat, have radical scavengers that can protect cells from injury. Further, lipid peroxidation may actually be prevented by lipid-soluble antioxidants. Moreover, intracellular fat droplets in the liver are most probably metabolically inert [14, 40]. In addition, ruptured parenchymal cells in severe steatotic livers could release fat droplets into the intrahepatic microcirculation, which may result in microcirculatory disturbances [39]. Indeed, microcirculation was disturbed in fatty livers from ethanol-treated donors; however, microcirculation was similar in donors which displayed marginal or extensive steatosis (Fig. 4). Further, reperfusion injury reflected by increased necrosis and serum transaminases also did not correlate with steatosis [42]. In fact, there was twofold more necrosis (Fig. 3) and serum enzyme release (Table 1) after transplantation of unmanipulated livers with marginal compared with severe steatosis. Taken together, results of this study do not support the hypothesis that lipid accumulation is itself responsible for graft injury and primary nonfunction of fatty livers.

The role of ethanol treatment before harvest

Results of this study suggest a role for ethanol in mechanisms of primary nonfunction. Although fat content was similar in livers from rats given a high-fat control diet or short-term ethanol treatment (Fig. 1), graft survival was reduced exclusively by short-term ethanol administration(Fig. 2). Moreover, both short- and long-term ethanol administration caused markedly different degrees of steatosis (Fig. 1), yet graft survival was reduced equally (Fig. 2). Thus, an effect of ethanol per se on survival is possible [41, 43]. This phenomenon could be explained by the fact that Kupffer cells become sensitized to gut-derived endotoxin in cells isolated 24 h after short-term ethanol administration,reflected by increased $[Ca^{2+}]_i$, tumor necrosis factor-α (TNF-α) production, and large increases in the endotoxin receptor CD14 [12]. Ethanol also may deplete glutathione, which is important in the protection of cells against reactive oxygen species [22], and reduce hepatic glycogen stores, an energy source used during anoxia [9]. This could increase susceptibility to oxidative stress on reperfusion. In this study, Kupffer cell-dependent reperfusion injury was increased by both short- and long-term ethanol treatment (Table 1, Fig. 3), supporting this hypothesis.

Conclusion and clinical implications

These data indicate for the first time that brief, gentle organ manipulation during harvest is a key factor in Kupffer cell-dependent graft injury of fatty livers. Modulating Kupffer cell function could improve the overall outcome of liver transplantation, because it reduces the effects of gentle organ manipulation during harvest.

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