Relevant mechanisms of reperfusion injury after liver transplantation are most likely mediated by activated Kupffer cells. Recently, it has been demonstrated that taurine prevents Kupffer cell activation in vitro. Thus, this study was designed to assess the effects of taurine after liver transplantation. Female Sprague-Dawley rats (210-240 g) were infused with taurine dissolved in normal saline, before organ harvest. Controls were infused with the same volume of normal saline without taurine. Following 4 hours of cold ischemia, liver transplantation was performed. Graft and animal survival, serum transaminases, liver histology, perfusion data of intravital microscopy, blood distribution at reperfusion, and both phagocytosis of Kupffer cells and expression of tumor necrosis factor α (TNF-α) to index cellular activation were investigated. For comparison, both, analysis of variance (ANOVA) and Fisher’s exact test were used as appropriate. Results are presented as mean ± SEM. Controls survived in 60% of cases. Taurine improved survival in a dose-dependent manner to 100% (P < 0.05). In controls, mean aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lactic dehydrogenase (LDH) serum levels increased to 3,260 ± 814; 1,703 ± 432; and 14,071 ± 3,177 U/L, respectively, after transplantation. In contrast, these values were between 20 and 45% of control values after taurine (P < 0.05). Histology taken after transplantation confirmed the significant protective effects of taurine, including the reduction of TNF-α expression. Time until homogeneous reperfusion of the graft improved (P < 0.05) to 50% of control values (P < 0.05). Further, taurine significantly decreased both phagocytosis of latex beads by Kupffer cells and leukocyte–endothelial cell interaction. In parallel, flow velocity of red blood cells as well as acinar and sinusoidal perfusion improved (P < 0.05). In conclusion, these data show for the first time in vivo that taurine minimizes reperfusion injury after liver transplantation. Decreased leukocyte–endothelial cell interaction and improved microcirculation are the proposed mechanisms, which are most likely Kupffer cell–dependent. (Liver Transpl 2005;11:950-959.)

The clinical relevance of ischemia/reperfusion (I/R) injury in liver transplantation remains evident considering its strong association with both poor initial graft function/primary dysfunction and primary nonfunction. Taking the various definitions of I/R injury and the number of grafts with high risk for failure into account, primary dysfunction and primary nonfunction occur in up to 88% of all cases.1,2 Numerous experimental and clinical studies revealed that activated Kupffer cells play a pivotal role in the development of I/R injury. Several lines of evidence have been proposed but the exact underlying mechanisms remain unclear.3 Disturbance of the hepatic microcirculation4 combined with cellular interaction between platelets,5 leukocytes,6 and endothelial lining cells,7 various Kupffer cell–derived proinflammatory mediators, such as tumor necrosis factor α (TNF-α), interleukin-1, and free radicals,8,9 and the expression of adhesion molecules are important determinants of the pathophysiological changes happening during I/R injury.3,10,11 It has been conclusively demonstrated that reperfusion injury predominates, rather than ischemic cell damage developing during cold storage.3,12 Further, donor-dependent factors, i.e., steatosis and surgical organ manipulation during the harvesting procedure, can be detrimental to the viability of the graft. This is most likely due to activation of Kupffer cells.13 Underlying mechanisms include the disturbances of hepatic microcirculation, the autonomous nerve system, and metabolic changes.14-20 During the last decade, a large variety of experimental approaches have been developed to prevent I/R injury, although most of them are inapplicable to clinical liver transplantation due to a potential toxicity of the hepatoprotective compound itself or a special administrative mode required for their successful application. Hence, none of them

Abbreviations: TNF-α, tumor necrosis factor α; ANOVA, analysis of variance; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactic dehydrogenase; I/R, ischemia/reperfusion.

From the 1Department of General Surgery, 2Department of Pathology, 3Department of Experimental Surgery, Ruprecht-Karls-University, Heidelberg, Germany, 4Department of Surgery, University of North Carolina at Chapel Hill, Chapel Hill, NC.

Received March 2, 2005; accepted May 3, 2005.

Address reprint requests to Peter Schemmer, MD, Department of General Surgery, Ruprecht-Karls-University, Im Neuenheimer Feld 110, 69120 Heidelberg, Germany. Telephone: (49)(0)6221/56-6110; FAX: (49)(0)6221/56-4215; E-mail: peter_schemmer@med.uni-heidelberg.de

Copyright © 2005 by the American Association for the Study of Liver Diseases

Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/lt.20501
has attained the level of clinical routine. Great effort has been invested into novel approaches to finally solve problems associated with I/R injury after liver transplantation.

Most recently, glycine, a nontoxic amino acid, has been reported to reliably protect livers from I/R injury and to improve graft function and survival after transplantation via prevention of Kupffer cell activation. Taurine, an ubiquitous sulfur-containing $\beta$-amino acid, acts similar to glycine in Kupffer cells. Taurine causes hyperpolarization of the Kupffer cell membrane, by mechanisms involving cellular chloride ion influx, which in turn blunts the lipopolysaccharide-induced increase of $[\text{Ca}^{2+}]_i$ and the subsequent TNF-$\alpha$ production. In addition to its great potential as a radical scavenger, these results support the hypothesis that taurine, like glycine, activates a chloride channel in Kupffer cells preventing their activation with subsequent I/R injury and failure of the graft.

Therefore, this study was designed to investigate the in vivo effect of taurine on microcirculation, leukocyte–endothelial cell interaction, signs for Kupffer cell activation and survival after liver transplantation.

Materials and Methods

Experimental Design and Treatment

Female Sprague-Dawley rats (210-240 g) were both organ donors and recipients. Before experiments all animals were allowed free access to standard laboratory chow (ssniff R/M-H; ssniff Spezialdiäten, Soest, Germany) and tap water. Donors and recipients were anesthetized with Narcoren (Merial, Hallbergmoos, Germany) (20 mg/kg of body weight; intraperitoneally) and Ketanest (Parke-Davis, Berlin, Germany) (100 mg/kg of body weight; intramuscularly). Donors were divided into control and treatment groups in a randomized, blinded fashion. Controls were given a single infusion with normal saline (1.5 mL) 10 minutes before harvesting. Donors of the treatment group were divided into 3 subgroups in which taurine (Synopharm, Barsbüttel, Germany), dissolved in normal saline, was given in different concentrations (1.5 mL; 30, 100, and 300 mmol/L). After organ harvest, livers were stored for 4 hours in histidine-tryptophan-ketoglutarate solution (HTK; Köhler-Chemie, Alsbach-Hähnlein, Germany) at 4°C. Subsequently, standardized liver transplantation was performed. At reperfusion, the time for blood to distribute completely and homogeneously into the graft was recorded. Immediately after complete reperfusion in vivo microscopy was performed to assess intrahepatic microcirculation, leukocyte–endothelial cell interaction, and phagocytosis of Kupffer cells. In vivo microscopy was repeated 8 hours after transplantation and blood was drawn for analysis of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lactic dehydrogenase (LDH) levels. Further, damage to liver was assessed in tissue samples taken 8 hours after transplantation. After an observation period of 1 postoperative week, survival was compared. Survival for more than 1 week was defined as permanent. All experiments were performed in accordance with institutional guidelines.

Harvest Procedure

Minimal surgical dissection was performed in a standardized fashion, including freeing the organ from ligaments and cannulation of the bile duct. In order to maintain the same conditions, all experiments were carried out by the same surgeon. At 12 minutes, perfusion with 10 mL of cold HTK solution was performed in situ via the portal vein until complete washout of blood was achieved. This volume of HTK, which is below the threshold for appropriate organ preservation in liver transplantation, was chosen to create a critical model for graft injury and survival after transplantation. Cuffs were attached in the cold to the infrahepatic vena cava and portal vein after explantation.

Transplantation

Orthotopic liver transplantation with arterialization was performed in rats as follows:27 Grafts were rinsed with 10 mL of Ringer’s solution (Braun, Melsungen, Germany) (18°C) and implanted by connecting the suprahepatic vena cava with a running 7/0 prolene suture (Ethicon, Hamburg, Germany), inserting cuffs into the corresponding vessels and anastomosing the bile duct and hepatic artery over an intraluminal polyethylene splint. Transplantation required less than 50 minutes; during which the portal vein was clamped for 18 to 20 minutes.

Histology

Livers were fixed by perfusion with 5% paraformaldehyde in Krebs-Henseleit bicarbonate buffer (118 mmol/L NaCl, 25 mmol/L NaHCO$_3$, 1.2 mmol/L KH$_2$PO$_4$, 1.2 mmol/L MgSO$_4$, 4.7 mmol/L KCl, and 1.3 mmol/L CaCl$_2$) at pH 7.6, embedded in paraffin, and processed for light microscopy (hematoxylin and eosin staining). Liver damage was assessed by estimating the proportion of necrotic to nonnecrotic areas, as described elsewhere. Briefly, 5 fields (20× magnifica-
Immunohistochemistry was performed on cryosections (severe infiltration). grade 4, 21-50 leukocytes/40 field (severe infiltration); grade 3, 10-20 leukocytes/40 field (moderate infiltration); grade 2, 1-10 leukocytes/40 field (mild infiltration); and grade 1, minimal or no evidence of injury. A total of 40 sections were investigated per slide to determine the percentage of necrotic cells. To index leukocyte infiltration to the hepatic vasculature, a semiquantitative scale for each hepatic zone from 0 to 4 was used: grade 1, <10 leukocytes/40 x field (focal infiltration); grade 2, 10-20 leukocytes/40 x field (mild infiltration); grade 3, 21-50 leukocytes/40 x field (moderate infiltration); grade 4, >50 leukocytes/40 x field (severe infiltration).

**Immunohistochemistry**

Immunohistochemistry was performed on cryosections (6 μm) of liver tissue taken 8 hours after reperfusion. Monoclonal anti-TNF-α antibody (R&D Systems, Minneapolis, MN) at dilution 1:100 and LSAB kit (DAKO, Carpinteria, CA) were used. TNF-α positive cells were counted in 10 microscopic fields per slide and slides were evaluated by the semiquantitative technique, relating the score of 0 to 4 points to the fraction of stained cells: scale 0, 0% cells; 1, <5% cells; 2, 5% to 20% cells; 3, 20% to 40% cells; 4, >40% positive cells.

**Reperfusion**

At the onset of liver reperfusion, time for blood to distribute completely was recorded to index the quality of hepatic reperfusion after transplantation.

**Intravital Fluorescence Microscopy**

Polypropylene catheters (Braun, Melsungen, Germany) were placed in the left jugular vein and carotid artery for intravenous application of contrast media and volume replacement and in the carotid artery for blood pressure monitoring. During in vivo microscopy, central body temperature was kept constant between 35.8 and 36.5°C by a heating pad. The hepatic microcirculation was observed in vivo at the surface of the left liver lobe with a modified inverted Leitz-Orthoplan microscope (Leica, Wetzlar, Germany) and epi-illumination technique as described previously. Fluorescein isothiocyanate–labeled erythrocytes were used for in vivo microscopy (3 μmol/kg of body weight; FITC Isomer 1; Sigma, Rödermark, Germany). At the same time, rhodamine 6G (0.05 mmol/kg of body weight; Sigma, Rödermark, Germany) was given intravenously to stain leukocytes. Acinar perfusion was assessed immediately and 8 hours after reperfusion and the perfusion index was calculated with the formula (N_C + 0.5 N_T)/N_T. N_C represents the number of well-perfused acini, N_T the number of irregularly perfused acini, and N_T the total number of analyzed acini. Further, the sinusoidal perfusion as well as temporary (rollers) and permanent adherent leukocytes (stickers) in 10 to 15 randomly selected acini (>5 venules were observed in each animal. Rollers were defined as leukocytes moving along the vessel wall of postsinusoidal venules with a velocity of less than 30% of central blood flow velocity. Stickers were defined as leukocytes adhering for more than 20 seconds to the endothelium. Red blood cell velocity within postsinusoidal venules was assessed as a mean value from 10 measurements (analyzed in 5 venules) in each animal.

At the end of in vivo microscopy, intraarterial injection of fluorescent latex beads (3 x 10^8 beads/kg of body weight; diameter = 1.1 μm; Polysciences, Warrington, PA) was performed to study their phagocytosis by Kupffer cells. Based on Uhlmann et al., the number of latex beads-positive, by Kupffer cells was counted per square millimeter within 300 seconds starting 10 seconds after the end of their injection.

**Enzyme Assays**

Blood samples were collected 8 hours after transplantation. Serum was obtained by centrifugation. Samples were stored at −20°C until analysis. AST, ALT, and LDH activity were determined by standard enzymatic methods.

**Statistics**

Mean ± standard error of the mean (SEM) values for various groups were compared using Fisher’s exact test, analysis of variance (ANOVA), or ANOVA on ranks, as appropriate, with P < 0.05 selected prior to the study as the criterion for significance of differences between groups.

**Results**

**General Data**

Blood pressure (104 ± 2 mmHg), hematocrit (46.4% ± 2.3%), and body temperature (36.0 ± 0.2°C) were comparable in all groups during all experimental phases.
studied. Sinusoidal diameters were measured in zone 2 of analyzed acini. Only a mild increase in sinusoidal width occurred in the taurine group 8 hours after transplantation, whereas no significant effect of taurine was present immediately after reperfusion (Table 1).

### Graft Survival

A total of 60% of grafts from controls, stored for 4 hours in cold HTK solution, survived for 1 week after liver transplantation (Fig. 1). With increasing concentrations of taurine, given as infusion to donors before harvest, survival rate improved to 80% (1.5 mL; 30 mmol/L) and to 100% (1.5 mL; 100 and 300 mmol/L) \( (P < 0.05) \) (Fig. 1). During the second week after transplantation, percent survival did not change within the groups.

### Liver Injury

In controls, 8 hours after transplantation AST, ALT, and LDH increased to 3,260 ± 814, 1,703 ± 432; and 14,071 ± 3,177 U/L, respectively (Fig. 2A-C). Infusion of 30 mmol/L taurine to the donors had no effects on all parameters after transplantation. In contrast, both 100 and 300 mmol/L taurine significantly reduced serum levels of AST, ALT, and LDH. Values were between 20% and 45% of control levels \( (P < 0.05) \) (Fig. 2A-C). An increase of taurine concentration from 100 to 300 mmol/L did not have a significant effect on serum parameters measured in this study (Fig. 2A-C).

No tissue injury was detectable prior to cold storage in all groups studied. At 8 hours after liver transplantation, the mean value of necrosis was 22.5% ± 2.4% in controls (Table 2; Fig. 3). This damage was largely reduced by infusion of taurine to the donor before harvest (Table 2; Fig. 3). Histology depicting pattern of injury revealed that 8 hours after transplantation necrosis developed in both pericentral and midzonal areas of

### Table 1. Acinar, Sinusoidal Perfusion and Adherence of Leukocytes

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control(_0)</th>
<th>Taurine(_0)</th>
<th>Control(_8)</th>
<th>Taurine(_8)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Acinar perfusion</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonperfused acini (%)</td>
<td>10 ± 8.2</td>
<td>9.2 ± 6.5</td>
<td>27.9 ± 9.9</td>
<td>3.7 ± 2.5*</td>
</tr>
<tr>
<td>Perfusion index</td>
<td>0.7 ± 0.1</td>
<td>0.7 ± 0.1</td>
<td>0.5 ± 0.08</td>
<td>0.8 ± 0.04*</td>
</tr>
<tr>
<td><strong>Sinusoidal perfusion</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonperfused sinusoids (%)</td>
<td>3.5 ± 0.9</td>
<td>3.6 ± 0.7</td>
<td>5.7 ± 1.1</td>
<td>2.3 ± 0.5*</td>
</tr>
<tr>
<td>Within perfused acini</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sinusoidal diameter (μm)</td>
<td>9.7 ± 0.2</td>
<td>10.3 ± 0.2</td>
<td>10.4 ± 0.4</td>
<td>11.9 ± 0.3†</td>
</tr>
<tr>
<td><strong>Adherence of leukocytes in venules</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stickers (n/mm(^2))</td>
<td>650 ± 41</td>
<td>197 ± 41*</td>
<td>733 ± 155</td>
<td>173 ± 31*</td>
</tr>
<tr>
<td>Rollers (n/mm(^2))</td>
<td>186 ± 42</td>
<td>94 ± 20*</td>
<td>455 ± 90†</td>
<td>166 ± 40*</td>
</tr>
</tbody>
</table>

NOTE. Conditions as described in Figure 1. Before organ harvesting, some donor rats were infused with taurine (1.5 mL; 300 mmol/L) as described in Materials and Methods. Leukocyte-endothelium interaction was monitored to study temporal (roller) and permanent leukocyte adhesion (sticker) to endothelial lining cells in both sinusoids (Fig. 5) and venules. Further, sinusoidal and acinal perfusion were assessed. Control\(_0\), taurine\(_0\) immediately and control\(_8\), taurine\(_8\): 8 hours after transplantation. Values are mean ± SEM \( (P < 0.05) \) by 2-way ANOVA with Student-Newman-Keuls post-hoc test, \( n = 10 \).

Abbreviations: SEM, standard error of the mean; ANOVA, analysis of variance.

* \( P < 0.05 \) for comparison to corresponding controls.

† \( P < 0.05 \) for comparison to corresponding groups immediately after reperfusion.

No tissue injury was detectable prior to cold storage in all groups studied. At 8 hours after liver transplantation, the mean value of necrosis was 22.5% ± 2.4% in controls (Table 2; Fig. 3). This damage was largely reduced by infusion of taurine to the donor before harvest (Table 2; Fig. 3). Histology depicting pattern of injury revealed that 8 hours after transplantation necrosis developed in both pericentral and midzonal areas of

![Figure 1. Effects of taurine on survival rates after liver transplantation. Before organ harvesting, some donor rats were infused with taurine (1.5 mL; 30, 100, or 300 mmol/L) as described in Materials and Methods. Subsequently, liver grafts were stored in HTK solution at 4°C for 4 hours, and transplantation was performed using arterialization. * \( P < 0.05 \) compared control by Fisher’s exact test, \( n = 5\text{-}15 \).](image-url)
controls (Fig. 3A), which was largely prevented with taurine (Fig. 3B). Analogous to the findings above, both the index of liver damage and leukocyte infiltration were significantly reduced with taurine (Table 2).

**Taurine Decreases Intrahepatic Expression of TNF-α After Liver Transplantation**

To evaluate the effect of taurine on Kupffer cell–dependent TNF-α production, liver tissue was taken 8 hours after reperfusion and immunohistochemistry was performed. Taurine (300 mmol/L) given to donors before organ harvest significantly reduced intrahepatic expression of TNF-α to about 70% of controls (Table 2).

**Reperfusion**

To determine the influence of taurine on quality of reperfusion after cold storage, the time for the liver to turn uniformly red from inflow of blood after removing the vascular clamps was recorded. In controls, it took $181 \pm 53$ seconds for the blood to distribute homogeneously and completely. The blood distribution time decreased in recipients of taurine treated grafts in a dose-dependent manner (Fig. 4). Donors treated with 300 mmol/L of taurine before organ harvest; blood distributed more rapidly, within $51 \pm 9$ seconds after transplantation ($P < 0.05$) (Fig. 4).

**Taurine Decreases Leukocyte–Endothelium Interaction After Liver Transplantation**

For the following experiments a taurine concentration of 300 mmol/L was chosen based on results from the dose-finding experiments described above. In vivo microscopy revealed leukocyte accumulation within sinusoids in all subacinar zones as well as in postsinusoidal venules in both experimental groups (Table 1; Fig. 5). However, taurine given to donors prior to harvest significantly reduced the number of permanent adherent leukocytes in both sinusoids and venules from $107 \pm 8$/mm² and $650 \pm 41$/mm² to about 30% of controls immediately after reperfusion (Table 1). At 8 hours after transplantation quantitative analysis of leukocyte adherence demonstrated no significant changes in all groups studied compared to early after reperfusion (Table 1). Separate analysis within the 3 zones revealed a significant increase of stickers from zone 1 to zone 2, 8 hours after transplantation in controls. Taurine effectively prevented permanent adhesion in all zones (Fig. 5).

### Table 2. Histology 8 Hours and After Transplantation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Taurine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Index of liver damage</td>
<td>2.2 ± 0.07</td>
<td>1.5 ± 0.05*</td>
</tr>
<tr>
<td>Index of leukocyte infiltration</td>
<td>1.8 ± 1.3</td>
<td>1.3 ± 0.04*</td>
</tr>
<tr>
<td>Necrosis (%)</td>
<td>22.5 ± 2.4</td>
<td>9.8 ± 0.5*</td>
</tr>
<tr>
<td>TNF-α score</td>
<td>2.0 ± 0.1</td>
<td>1.4 ± 0.2*</td>
</tr>
</tbody>
</table>

**NOTE.** Conditions as described in Figure 3. Before organ harvesting, some donor rats were infused with taurine (1.5 mL; 300 mmol/L) as described in Materials and Methods. Leukocyte–endothelium interaction was monitored to study temporal (roller) and permanent leukocyte adhesion (sticker) to endothelial lining cells in both sinusoids and venules. Further, sinusoidal and acinar perfusion were assessed. Moreover, expression of TNF-α was evaluated with immunohistochemistry. Controls, taurine; 8 hours after transplantation. Values are mean ± SEM ($P < 0.05$ by ANOVA on ranks). Abbreviations: TNF, tumor necrosis factor; SEM, standard error of the mean; ANOVA, analysis of variance. *$P < 0.05$ for comparison to corresponding controls.
Comparable effects were observed on rollers in venules (Table 1). In contrast to permanent leukocyte adhesion, temporal adhesion in venules increased significantly over time in controls (Table 1).

**Acinar and Sinusoidal Perfusion**

Immediately after reperfusion, disturbances in acinar perfusion were observed in both groups (Table 1). While acinar perfusion rapidly decreased within the following 8 hours in controls, perfusion of acini of taurine-treated grafts increased over time and acini were perfused significantly better than controls (Table 1). In parallel, taurine significantly decreased the percentage of nonperfused sinusoids within perfused acini by 60% compared to controls 8 hours after transplantation (Table 1).

The velocity of red blood cells and blood flow was comparable in both groups immediately after reperfusion. However, while blood flow and red blood cell velocity decreased in venules of controls, taurine markedly increased the velocity of red blood cells in both grafts. The velocity of red blood cells and blood flow was comparable in both groups immediately after reperfusion. However, while blood flow and red blood cell velocity decreased in venules of controls, taurine markedly increased the velocity of red blood cells in both

---

**Figure 3.** Histology 8 hours after transplantation. Conditions as described in Figure 2. Liver tissue was taken 8 hours after transplantation and was processed for light microscopy by hematoxylin and eosin staining. (A) Taurine. (B) Control. Necrosis was focused pericentral and midzonal. Pictures depicting typical pattern of injury.

**Figure 4.** Effects of taurine on microcirculation. Conditions are as described in Figure 1. Before organ harvesting, some donor rats were infused with taurine (1.5 mL; 300 mmol/L) as described in Materials and Methods. The time for blood to distribute completely was recorded during reperfusion. Values are mean ± SEM ($P < 0.05$ by 2-way ANOVA with Student-Newman-Keuls post-hoc test, $n = 10$). *$P < 0.05$ for comparison to control.

**Figure 5.** Permanent leukocyte adherence in hepatic sinusoids in all subacinar zones. Conditions are as described in Table 1. Acinar zones have been differentiated into portal, midzonal, and central areas to describe differences in sinusoidal perfusion. (A) Immediately after reperfusion. (B) Eight hours after transplantation. Values are mean ± SEM ($P < 0.05$ by 2-way ANOVA with Student-Newman-Keuls post-hoc test, $n = 10$). *$P < 0.05$ for comparison to control. #$P < 0.05$ for comparison between zones.
postsinusoidal venules and sinusoids from 0.45 ± 0.02 and 0.26 ± 0.01 mm/second in controls to 0.86 ± 0.05 mm/second (P < 0.05) and 0.41 ± 0.01 mm/second (P < 0.05), respectively, at 8 hours after transplantation (Fig. 6). In parallel, 8 hours after transplantation, taurine increased the blood flow in both postsinusoidal venules and sinusoids from 171 ± 28 and 26 ± 5 nm²/second in controls to 693 ± 130 mm/second (P < 0.05) and 54 ± 8 mm/second (P < 0.05), respectively (Fig. 6).

Phagocytosis
To index the activation of Kupffer cells, the number of latex beads-positive Kupffer cells were compared between groups. Taurine significantly reduced phagocytosis after transplantation to about 50% of control values (Fig. 7).

Discussion
Reperfusion Injury to Liver is Associated With Activated Kupffer Cells, Disturbed Microcirculation, and Adhesion of Leukocytes
Recent studies have clearly demonstrated that disturbed microcirculation, adhesion of leukocytes to the endothelium, and activated Kupffer cells play a pivotal role for the development of reperfusion injury with subsequent failure of the graft.3,13,17,19,29,32,33

The major finding of this study is that taurine improves graft survival and reduces injury to liver, most likely via mechanisms including improved hepatic microcirculation, decreased leukocyte-endothelial cell interactions and most likely inactivation of Kupffer cells. The latter is reflected by decreased phagocytosis of latex beads, which is associated with a decreased TNF-α expression in liver tissue34 after taurine. How can these protective effects of taurine be explained?

Taurine Prevents Reperfusion Injury to Liver After Transplantation
There is evidence from animal models that taurine protects cell lines and organs from I/R injury in in vitro animal models.35-37 Clinically, taurine has been used with varying degree of success in patients with congestive heart failure, cystic fibrosis, toxic exposure (i.e.,
alcohol), and hepatic disorders, including acute hepatitis.\textsuperscript{38} In previous studies, taurine was given in the diet, intravenously, and as a supplement to preservation solutions to successfully reduce I/R injury.\textsuperscript{35-38} Whether taurine has the same protective effects as glycine given to recipients prior to reperfusion can only be speculated. However, assuming that taurine has the same Kupffer cell–dependent mechanism as glycine, taurine would most likely also be beneficial if given to recipients before reperfusion.\textsuperscript{22,23} It would be difficult to give dietary taurine to cadaveric human donors because of their medical condition and the very limited time for treatment before organ retrieval. Further, recently it has been suggested that organ donors should be preconditioned to protect from harvest-related I/R injury.\textsuperscript{13,14} Therefore, in this study taurine was given intravenously before organ harvest.

Based on in vitro studies,\textsuperscript{39} several lines of evidence have been developed that taurine protects livers from injury after ischemia and reperfusion;\textsuperscript{35-37} however, protective mechanisms of taurine in I/R injury have not yet been completely clarified. In a rat liver perfusion model of warm I/R injury, it was shown that protective effects of taurine may be due to modulation of Kupffer cell and endothelial cell function.\textsuperscript{35} Indeed, while Kupffer cells showed enhanced capacity for phagocytosis of latex beads after transplantation in controls, Kupffer cell–phagocytosis was largely reduced after reperfusion in recipients from taurine-treated donors and Kupffer cell–dependent reperfusion injury to liver decreased.\textsuperscript{3,35} Since Kupffer cells can be inactivated with taurine and significantly fewer cells were latex beads–positive, it can be assumed that the predominant latex beads–positive cell type was the Kupffer cell in our model. Although it is not possible to directly prove with in vivo microscopy that the observed latex beads are taken up only by Kupffer cells, it was demonstrated that these particles are phagocytized exclusively by Kupffer cells,\textsuperscript{40} which was most recently confirmed with electron micrographs by Yano et al.\textsuperscript{41} In their study, no latex particles of different sizes were taken up by the sinusoidal endothelial cells of the rat liver.

**Possible Site of Action of Taurine**

Kupffer cells are known to release proteases and TNF-\(\alpha\) upon activation.\textsuperscript{8} The destruction of Kupffer cells, which are a major source of eicosanoids in the liver,\textsuperscript{42} is widely known to reduce I/R injury.\textsuperscript{3,13,14,19} Recent studies have shown that taurine reduces TNF-\(\alpha\) production and minimizes death induced by endotoxin, a known activator of Kupffer cells.\textsuperscript{25} Indeed, data of this study confirm these findings since taurine dramatically reduced expression of TNF-\(\alpha\) in liver tissue after reperfusion, while liver injury is blunted; although Kupffer cells are not the only source of TNF-\(\alpha\) they are the predominant one. Thus taurine has been proposed to prevent activation of Kupffer cells, thereby minimizing I/R injury after transplantation. These effects are most likely associated with its binding on glycine-gated chloride channels of Kupffer cells, since taurine blunts the lipopolysaccharide-induced increase in intracellular calcium ions [Ca\(^{2+}\)], in Kupffer cells. Indeed, taurine significantly blunted the lipopolysaccharide-induced increase in [Ca\(^{2+}\)], in a dose-dependent manner and TNF-\(\alpha\) production was reduced by more than 40%.\textsuperscript{25} Moreover, taurine increased \(^{36}\)Cl\(^{-}\) uptake by Kupffer cells in a dose-dependent manner.\textsuperscript{25} The hypothesis that taurine activates a chloride channel in Kupffer cells, preventing their activation, is supported by these results.

**Hepatic Microcirculation and Leukocyte–Endothelial Cell Interaction**

A characteristic phenomenon during the early stage of reperfusion is the adherence of white blood cells to the endothelium and their subsequent activation and release of reactive oxygen species and various mediators.\textsuperscript{3,4,10,32,43} These processes are mediated in several steps, including expression of selectins, followed by integrins and adhesion molecules.\textsuperscript{32,33,43} Kupffer cells are activated upon reperfusion after ischemia.\textsuperscript{3} Once activated, Kupffer cells impair the intrahepatic circulation by releasing vasoactive mediators.\textsuperscript{44} Similar to adhesive and activated white blood cells, activated Kupffer cells are one and perhaps the most important source of reactive oxygen species and various mediators involved in the development of reperfusion injury.\textsuperscript{3}

This is consistent with disturbances of microcirculation at reperfusion, demonstrated by increased leukocyte–endothelial cell interaction after transplantation. This is thought to play a major role in the development of liver injury. Reduced microcirculation leads to aggravated reperfusion injury after transplantation. Little necrosis, injury to the liver, leukocyte infiltration, and minimal disturbances of the microcirculation were observed in transplanted livers from donors pretreated with taurine, which supports the hypothesis that Kupffer cells are pivotal in transplantation-related injury. In addition, accumulation of leukocytes in both hepatic sinusoids and in postsinusoidal venules has been reported following reperfusion of ischemic liver.\textsuperscript{3,29,32} Adhesion molecules, such as selectins and
integrins, are expressed on vascular and leukocyte membrane surfaces and turn to mediate leukocyte adhesion in venules, which can be induced after ischemia by mediators like TNF-α, some of which derived from activated Kupffer cells. Interestingly, in zones where adherent white blood cells accumulated (i.e., zone 2, 3) necrosis was detectable after transplantation. In this study taurine improves reperfusion, reflected by the blood distribution time at reperfusion.

Data of this study, using a most clinically relevant transplant model, demonstrate a hepatoprotective effect of taurine in vivo, reflected by significantly increased survival and improved hepatic microcirculation. With taurine, levels of transaminases, LDH, and the number of adherent white blood cells are markedly lower than controls. However, the predominant mechanisms of how taurine protects from I/R injury have not been completely clarified.

**Conclusion and Clinical Implication**

These in vivo data indicate for the first time that taurine increases survival after rat liver transplantation, most likely via mechanisms including Kupffer cells. This finding is of clinical relevance since taurine possesses a high degree of chemical stability, very low metabolic reactivity, and no known major adverse side effect. Its potential usefulness as a therapeutic modality in transplant conditions warrants clinical trials. If the hepatoprotective effect of taurine is confirmed in humans, donor pretreatment would be important for liver transplantation to lower the rate of primary graft nonfunction and dysfunction, increase survival, and lead to more efficient utilization of transplantable organs.

**Acknowledgment**

We thank Genevieve Dei-Anane for helping us to edit the manuscript.

**References**

35. Wettstein M, Haussinger D. Cytoprotection by the osmolytes betaine and taurine in ischemia-reoxygenation injury in the perfused rat liver. Hepatology 1997;26:1560-1566.