Intravenous glycine improves survival in rat liver transplantation

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Intravenous glycine improves survival in rat liver transplantation, Am J Physiol 276 (Gastrointest. Liver Physiol. 39): G924–G932, 1999.—In situ manipulation by touching, retracting, and moving liver lobes gently during harvest dramatically reduces survival after transplantation (P. Schemmer, R. Schoonhoven, J. A. Swenberg, H. Bunzendahl, and R. G. Thurman. Transplantation 65: 1015–1020, 1998). The development of harvest-dependent graft injury upon reperfusion can be prevented with GdCl3, a rare earth metal and Kupffer cell toxicant, but it cannot be used in clinical liver transplantation because of its potential toxicity. Thus the effect of glycine, which prevents activation of Kupffer cells, was assessed here. Minimal dissection of the liver for 12 min plus 13 min without manipulation had no effect on survival (100%). However, gentle manipulation decreased survival to 46% in the control group. Furthermore, serum transaminases and liver necrosis were elevated 4- to 12-fold 8 h after transplantation. After organ harvest, the rate of entry and exit of fluorescein dextran, a dye confined to the vascular space, was decreased about twofold, indicating disturbances in the hepatic microcirculation. Pimonidazole binding, which detects hypoxia, increased about twofold after organ manipulation, and Kupffer cells isolated from manipulated livers produced threefold more tumor necrosis factor-α after lipopolysaccharide than controls. Glycine given intravenously to the donor increased the serum glycine concentration about sevenfold and largely prevented the effect of gentle organ manipulation on all parameters studied. These data indicate for the first time that pretreatment of donors with intravenous glycine minimizes reperfusion injury due to organ manipulation during harvest and after liver transplantation.

organ harvest; hepatic microcirculation; hypoxia

GLYCINE, A NONESSENTIAL amino acid, is nontoxic and has been shown to protect proximal tubules and hepatocytes (19) against hypoxia. Glycine also prevents nephrotoxicity caused by cyclosporin A (31). Furthermore, glycine added to a graft rinse solution reduced reperfusion injury and also improved initial graft function and survival after liver transplantation (19). Moreover, glycine improved the hepatic microcirculation and reduced liver injury in a low-flow, reflow perfusion model (33). A diet containing glycine improved survival of rats given endotoxin, most likely by inactivation of Kupffer cells, since tumor necrosis factor-α (TNF-α) production was decreased (16). Recently, gentle in situ graft manipulation by touching, retracting, and moving liver lobes gently during harvest has been demonstrated to be detrimental for survival after liver transplantation via mechanisms involving microcirculation and Kupffer cell-dependent reperfusion injury (27). Increases of intracellular Ca2+ concentration in Kupffer cells are essential for the release of prostanoids and inflammatory cytokines in response to stimuli such as endotoxin (lipopolysaccharide [LPS]). Glycine prevented the increase of intracellular Ca2+ concentration by activating a glycine-gated chloride channel, which hyperpolarized the cell membrane and made Ca2+ influx via voltage-dependent Ca2+ channels more difficult. Thus glycine blunted activation of Kupffer cells by LPS. This effect is important since activated Kupffer cells are involved in regulation of hepatic microcirculation, and several lines of evidence suggest that microcirculatory disturbances are a key factor in enhanced donor liver susceptibility to cold and warm ischemia (14, 15, 29). Depletion of Kupffer cells with gadolinium chloride (GdCl3), which is potentially toxic, and dietary glycine for 5 days before harvest blunted harvest-related reperfusion injury and primary nonfunction after transplantation in rats (27). Because organ retrieval occurs in a few hours, it is unlikely that dietary glycine would be clinically applicable. Therefore, the aim of this study was to determine whether brief intravenous infusion of glycine to donors would blunt harvest-related injury developing upon reperfusion. This is important because primary organ manipulation during harvest cannot be prevented with standard harvesting techniques, graft nonfunction is still a major obstacle in clinical liver transplantation, and the number of donor organs is limited.

METHODS

Experimental animals and treatment. Female inbred Lewis rats (200–230 g) were allowed free access to standard laboratory chow (Agway PROLAB RMH 3000, Syracuse, NY) and tap water. Donor animals were given glycine by infusion (1.5 ml; 300 mM) for 1 h through the femoral vein before harvest. To test whether survival of manipulated livers after transplantation is dose dependent, donors were given infusions of various concentrations of glycine before organ harvest. Isonitrogenous controls were given valine, which does not have an effect on Kupffer cells (17). Experimental procedures were The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
approved by the Institutional Animal Care and Use Committee.

Harvest procedure. Livers were gently manipulated during harvest as described previously (27). Briefly, donor livers were harvested within 25 min before perfusion with cold University of Wisconsin cold storage (UW) solution. 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 last 13 min, livers were either left alone or were manipulated gently. Standardized gentle manipulation was carried out by touching, retracting, and moving the liver lobes in situ continuously. At 25 min, perfusion with 8 ml of cold Ringer 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. Both donors and recipients were anesthetized with methoxyflurane, and orthotopic liver transplantation was performed in rats using rearterialization (12). After explantation, livers were stored at 0–4°C for 1 h in UW solution. Grafts were rinsed with 10 ml of normal saline and were implanted by connecting the suprahepatic solution. Grafts were rinsed with 10 ml of normal saline exsanguination, livers were stored at 0–4°C for 1 h in UW solution. After organ manipulation, livers were perfused via the portal vein at 3–4 ml·min\(^{-1}\)·g\(^{-1}\) liver with oxygenated Krebs-Henseleit bicarbonate buffer (in mM: 118 NaCl, 25 NaHCO\(_3\), 1.2 KH\(_2\)PO\(_4\), 1.2 MgSO\(_4\), 4.7 KCl, and 1.3 CaCl\(_2\)) at pH 7.6, saturated with 95% O\(_2\) and 5% CO\(_2\) at 37°C in a nonrecirculating system (3).

Assessment of microcirculation. At the time perfusion with cold preservation solution would usually have been performed, some donor livers were perfused ex situ with fluorescein isothiocyanate-dextran (12 μM; mol wt 70,000, catalog no. FD-70S; Sigma) to assess microcirculation. A mercury arc lamp equipped with a glass filter was used to produce excitation wavelengths of 430 nm. Fluorescence of fluorescein dextran (560 nm) was measured via a light guide (tip diameter of 2 mm) placed on the surface of the perfused liver with a micromanipulator. The signal was amplified and recorded as described elsewhere (8). To normalize for day-to-day variation, all values were expressed as a percentage of basal values. Furthermore, at reperfusion after transplantation, the time for blood to distribute completely was recorded visually by observing when the liver turned homogeneously red, to index the quality of reperfusion and early microcirculation as is done in clinical transplantation (27).

Kupffer cell isolation. Kupffer cells were isolated by collagenase digestion and differential centrifugation using Percoll (Pharmacia, Uppsala, Sweden) as described elsewhere (17). After organ manipulation, livers were perfused via the portal vein with Ca\(^{2+}\)- and Mg\(^{2+}\)-free Hanks’ balanced salt solution containing collagenase IV (0.025%; Sigma Chemical, St. Louis, MO) at 37°C at a flow rate of 26 ml/min. After digestion, livers were cut into small pieces in collagenase buffer. The suspension was filtered through nylon gauze, and the filtrate was centrifuged at 450 g for 10 min at 4°C. Cell pellets were resuspended in buffer, parenchymal cells were removed by centrifugation at 50 g for 3 min, and the nonparenchymal cell fraction was washed two times with buffer. Cells were centrifuged on a density cushion of Percoll at 1,000 g for 15 min. The Kupffer cell fraction was collected and washed with buffer again. Viability of cells determined by trypan blue exclusion was >90%.

TNF-α and nitrate measurement. Isolated Kupffer cells were cultured for 24 h in 24-well culture plates (Sarstedt, Newton, NC) at a density of 1 × 10\(^6\) cells/well in DMEM supplemented with 10% FBS and antibiotics at 37°C in the presence of 5% CO\(_2\). Cells were incubated with fresh media containing LPS (100 ng/ml in 5% rat serum) for an additional 4 h. Nonadherent cells were removed after 1 h by replacing buffer, and cells were cultured for 24 h before experiments. All adherent cells phagocytized latex beads, indicating they were Kupffer cells. TNF-α concentrations were determined in the culture medium after using an enzyme-linked immunosorbent assay kit (ELISA; Genzyme, Cambridge, MA). TNF-α production after LPS (100 ng/ml) was compared with basal values. Furthermore, isolated Kupffer cells were cultured for 24 h in a saline-free medium or in medium containing glycine (10 mM). Nitrite concentration in media was measured colorimetrically by the Griess reaction after 48 h of culture (13). Briefly, 500 μl of medium was mixed with an equal volume of Griess reagent (1% sulfanilamide, 0.1% naphthalene-ethylenediamine dihydrochloride in 1% H\(_2\)PO\(_4\)) and incubated for 10 min at room temperature. The resulting product, N-(1-naphthyl) ethylenediamine, was quantitated spectrophotometrically at 550 nm. Nitrite levels were calculated using a standard curve generated with known concentrations of sodium nitrite.

Trypan blue infusion and histology. After 24 h of storage in cold UW solution, trypan blue (500 μM; Aldrich, Milwaukee, WI) was infused in the liver to assess viability of cells. Livers were then flushed with additional perfusate to remove excess dye and were fixed by perfusion with 4% paraformaldehyde in Krebs-Henseleit bicarbonate buffer at pH 7.6, embedded in paraffin, and processed for light microscopy using an eosin counterstain. The presence of trypan blue in the nuclei is indicative of irreversible loss of cell viability (3). Five pericentral and five perilobular fields (×100 magnification) were selected at random from at least four different sections per sample, and mean values of stained nuclei from nonparenchymal and parenchymal cells were calculated. Furthermore, some livers were evaluated for histology either before cold storage or after removal from rats killed 8 h after transplantation. Livers were fixed by perfusion with 4% paraformaldehyde in Krebs-Henseleit bicarbonate buffer at pH 7.6, embedded in paraffin, and processed for light microscopy after hematoxylin and eosin staining. Liver damage was assessed by estimating necrotic areas as described elsewhere (30). Briefly, five fields (×100 magnification) were selected at random from at least four different sections per sample, and mean values were calculated.

Determination of reduced, protein-bound pimodalone by ELISA and immunohistochemistry. Pimodalone is a 2-nitromidazole that detects hypoxia in liver tissue (2). Pimodalone (120 mg/kg, 5 min before donor operation) was given intravenously, and adducts were measured in tissue homogenates with a competitive ELISA procedure previously described (26) and modified for liver tissue (2). Protein levels in tissue homogenates were determined with the bicinchoninic acid assay using a commercially available kit (Pierce Chemical, Rockford, IL). Pimonidazole blocks of Formalin-fixed liver tissue were sectioned at 6 μm, and pimodalone adducts were detected with a biotin-streptavidin-peroxidase indirect immunostaining method using diaminobenzidine as a chromogen as described previously (2). After the immunostaining procedure, a counterstain of hematoxylin was applied. A Universal Imaging Image-1/AT image acquisition and analysis system (Chester, PA) incorporating an Axioskop 50 micro-
scope (Carl Zeiss, Thornwood, NY) was used to capture the immunostained tissue sections at \(\times 100\) magnification (1).

Enzyme assays. Blood samples were collected from the tail vein 8 h after transplantation. Serum was obtained by centrifugation and was stored at \(-80^\circ\text{C}\) until analysis. Aspartate aminotransferase and alanine aminotransferase activity was determined by standard enzymatic methods, whereas total bilirubin was determined in sera by direct spectrophotometry at 454 nm (4). To detect proteolytic activity after harvest, livers were rinsed with 2 ml of UW solution before cold storage. Arginine-specific proteolytic activity was measured in the effluent using H-D-Ile-Pro-Arg-p-nitroaniline-2HCl (KabiVitrum). Activity was determined from the rate of formation of p-nitroaniline at 405 nm spectrophotometrically at 37°C (18).

Glycine measurements. Blood was taken before experiments for glycine determination in serum as described previously (4). Briefly, glycine was extracted and benzolated, and the resulting hippuric acid was extracted and dried. Subse-

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Table 1. Effects of intravenous glycine on liver injury after transplantation of manipulated grafts

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Values are means ± SE. Conditions as described in Fig. 1. After transplantation (8 h), blood and liver tissue were collected. Serum transaminases, total bilirubin, and liver necrosis were determined as described in METHODS. Valine, the isonitrogenous control, or glycine (1.5 ml; 300 mM) was infused over 1 h before organ harvest. AST, aspartate aminotransferase; ALT, alanine aminotransferase. \(P < 0.05\) by 2-way ANOVA with Student-Newman-Keuls post hoc test, \(n = 4–8\). *\(P < 0.5\) for comparison with no manipulation; †\(P < 0.05\) compared with manipulation after valine infusion.

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Fig. 1. Intravenous glycine improves survival of gently manipulated livers. Donor livers were harvested within 25 min with or without gentle in situ organ manipulation before perfusion with cold University of Wisconsin cold storage (UW) solution. Livers were stored in UW solution at 0–4°C for 1 h, and transplantation was performed using rearterialization. Top: survival of manipulated livers is compared after transplantation. Valine, the isonitrogenous control, or glycine (1.5 ml; 300 mM) was infused over 1 h before organ harvest. Bottom: dose dependence. *\(P < 0.05\) compared with no manipulation; †\(P < 0.05\) compared with manipulation after valine infusion by Fisher’s exact test, \(n = 10–16\) rats. Fractions indicate survival per total number of transplantations studied.

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Fig. 2. Intravenous glycine prevents increase of proteolytic activity in rinse solution by gentle organ manipulation. Livers were rinsed with cold UW solution after explantation, and proteolytic activity was measured in the effluent using H-D-Ile-Pro-Arg-p-nitroaniline-2HCl (KabiVitrum). Activity was determined from the rate of formation of p-nitroaniline at 405 nm spectrophotometrically at 37°C (18).

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sequently, the concentration of hippuric acid was determined spectrophotometrically at 458 nm (22).

Statistics. Mean values ± SE for groups were compared using Fisher's exact test or ANOVA (2-way ANOVA) with Student-Newman-Keuls post hoc test as appropriate with P < 0.05 selected before the study as the criterion for significance.

RESULTS

Intravenous glycine prevents effects of gentle organ manipulation on graft viability. In livers that were not manipulated, survival was 100% after transplantation; however, gentle manipulation decreased survival by ~50% in controls given valine (isonitrogenous control). In contrast, rats receiving manipulated livers from glycine-infused donors survived as well as nonmanipulated controls (Fig. 1). Furthermore, manipulation elevated transaminases and total bilirubin six- to sevenfold 8 h after transplantation (Table 1) and doubled proteolytic activity in the valine group (Fig. 2). In contrast, manipulated livers pretreated with glycine,
which increased glycine serum levels about sevenfold (1.7 ± 0.2 mM), reduced transaminases and proteases with values similar to unmanipulated controls (Table 1 and Fig. 2). Furthermore, survival after transplantation, defined as living for 7 days, improved as the dose of glycine given was increased (Fig. 1). Elevation of glycine to 1.7 mM by infusion totally prevented the effect of manipulation on survival (Fig. 1).

Intravenous glycine blunts liver necrosis after reperfusion. In all groups studied, tissue injury was undetectable before cold storage. In addition, nonmanipulated grafts developed <1% necrosis 8 h after transplantation (Table 1 and Fig. 3). However, after graft manipulation of controls, ~14% of the tissue was necrotic 8 h after transplantation (Table 1 and Fig. 3). Fourteen percent necrosis is considerable after 8 h since animals do not begin to die until 1 day after transplantation. This damage was largely prevented by infusion of glycine before the donor operation (Table 1 and Fig. 3). Moreover, necrosis progressed rapidly in the manipulated valine group, yielding values of >60% (P < 0.05) on the first day after transplantation, whereas <5% of the cells were necrotic in the glycine group, values not different from unmanipulated controls.

Intravenous glycine prevents disturbances to the hepatic microcirculation. Livers were perfused with fluorescein isothiocyanate-dextran (12 μM) for 3 min to index the hepatic microcirculation. Because fluorescein isothiocyanate-dextran is confined to the vascular space in liver, the rate of fluorescence wash in and washout as well as the percent increase of surface fluorescence over basal are indicative of microcirculation and vascular space, respectively (8). Surface fluorescence was maximal and stable within 1 min. Indeed, microcirculation was disturbed significantly when grafts were perfused...
after manipulation, reflected by a two- to fourfold decreased rate for fluorescein isothiocyanate-dextran entry and exit from the vascular space (Figs. 4 and 5). Moreover, manipulation decreased surface fluorescence nearly twofold \((P < 0.05; \text{Figs. 4 and 5})\). Intravenous glycine infusion before manipulation totally prevented the effects of manipulation on surface fluorescence and rates of changes in fluorescence (Figs. 4 and 5). To determine the influence of glycine in manipulated livers on microcirculation at reperfusion after transplantation, the time for the organ to turn uniformly red due to the hemoglobin pigment upon completion of implantation was recorded. In unmanipulated controls, blood was distributed completely in 30 ± 2 s. This time was prolonged more than fivefold by gentle manipulation in the valine group. However, when glycine was infused before manipulation, blood distributed rapidly and was not different from unmanipulated controls (Fig. 6).

**Effect of intravenous glycine on hypoxia in manipulated grafts.** Pimonidazole, a 2-nitroimidazole hypoxia marker, binds to hypoxic liver cells in vivo (2). Gentle liver manipulation increased hypoxia about twofold in the valine control group before cold storage \((P < 0.05; \text{Figs. 7 and 8})\). As expected, pimonidazole binding predominated in pericentral regions where \(O_2\) supply is naturally low (Fig. 7). Binding of pimonidazole in manipulated livers from donors given glycine intravenously was not different from unmanipulated controls (Figs. 7 and 8).

**DISCUSSION**

Glycine infusion to donors minimizes harvest-related reperfusion injury. Previous studies have shown that addition of amino acid mixtures to the perfusate of isolated kidneys preserves tubular integrity and prolongs renal function (10). Weinberg et al. (32) were the first to relate this protective effect to the simplest amino acid, glycine. Glycine has been demonstrated to be protective in vitro against tissue damage caused by ischemia, ischemia-reperfusion, or toxicants in a variety of species (19, 20, 32, 33). Furthermore, Carolina rinse solution containing glycine prevents reperfusion injury in livers in both experimental and clinical liver transplantation (19). Moreover, dietary glycine prevents cyclosporin A-induced nephrotoxicity after transplantation (31).

Injury developing at reperfusion, mediated by activated Kupffer cells, is one of the most important events leading to early dysfunction or nonfunction of grafts, which remains a major obstacle in transplantation. Most recently, gentle in situ graft manipulation by touching, retracting, and moving liver lobes gently during harvest has been demonstrated to be detrimen-

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**Fig. 7.** Effect of intravenous glycine on pattern of pimonidazole binding after manipulation. Conditions are as described in Fig. 1. Photomicrographs depict patterns of pimonidazole binding in livers after harvest. Immunohistochemistry using antibodies to bound pimonidazole is described in METHODS. Top: unmanipulated livers after harvest; middle donors undergoing graft manipulation during harvest were infused with valine; bottom: glycine before donor operation. Typical photomicrographs are shown. Magnification = ×100.
tal for survival after liver transplantation via mechanisms involving microcirculation and Kupffer cell-dependent reperfusion injury. The effects of manipulation were prevented by GdCl3, a rare earth metal that depletes Kupffer cells, and with glycine, which prevents activation of Kupffer cells (27). GdCl3 cannot be used clinically because of its potential toxicity, and it would be difficult to give dietary glycine to human donors before organ retrieval; therefore, in this study, donors were given a brief intravenous infusion of glycine before organ harvest. Gentle in situ graft manipulation during harvest dramatically decreased survival (Fig. 1) and elevated serum transaminases, bilirubin, and necrosis (Table 1 and Fig. 3) after transplantation (27). Furthermore, proteolytic activity was elevated after harvest in the graft rinse solution (Fig. 2), and microcirculation was disturbed by manipulation (Figs. 4–6). This effect is important since manipulation of the liver during harvest with standard techniques cannot be prevented completely in clinical liver transplantation. Glycine given intravenously to donors before harvest prevented all of the detrimental effects of manipulation (Table 1 and Figs. 1–8). Therefore, it is concluded that intravenous glycine prevents harvest-related reperfusion injury after liver transplantation.

Mechanisms by which glycine reduces harvest-related reperfusion injury. Nichols et al. (21) found that glycine inhibited nonlysosomal Ca\(^{2+}\)-dependent proteases and concluded that this action was responsible for protection of hepatocytes from anoxic injury. Furthermore, proteolysis has been shown to contribute to graft injury after transplantation of livers (7) and protease inhibitors improve graft function (19). Indeed, in this study, glycine prevented increased proteolytic activity by manipulation (Fig. 2). Glycine activates a glycine-gated chloride channel in Kupffer cells, and influx of chloride hyperpolarizes the cell membrane, making Ca\(^{2+}\) channels more difficult to open. Therefore, glycine blunts the increase of intracellular Ca\(^{2+}\), thus minimizing activation of Kupffer cells and increases in Ca\(^{2+}\)-dependent proteases (17). Kupffer cell-dependent reperfusion injury after cold storage, a key event in primary nonfunction, is also characterized by death of endothelial lining cells (19). Furthermore, apoptosis of sinusoidal endothelial cells occurs (11). Indeed, gentle organ manipulation dramatically increased the number of dead sinusoidal lining cells per field after 24 h of cold storage more than fivefold (P < 0.05), which was prevented by glycine (P < 0.05; no manipulation, 4 ± 1; manipulation + valine, 23 ± 3; manipulation + glycine, 6 ± 1), as assessed by nuclear staining with trypan blue in perfused livers. Because Kupffer cells and stellate cells initially retain viability (19), the exacerbated damage in manipulated livers corresponds to death of the endothelium. Qu et
al. (25) showed that activated Kupffer cells release prostaglandin E2, which then stimulates parenchymal cell O2 consumption, most likely creating hypoxia, and Bradford et al. (6) demonstrated that GdCl3 blocks elevated O2 uptake due to Kupffer cell activation with ethanol. In addition, Qu et al. (24) demonstrated that O2 uptake is nearly doubled after liver transplantation, and this effect is also blocked by GdCl3. Because increased O2 uptake leads to hepatic hypoxia (1), it was evaluated in this study. Indeed, binding of the hypoxia marker pimonidazole was increased more than twofold after manipulation, an effect blunted by infusion of glycine (Figs. 7–9). Thus the rapid development of hypoxia in the liver due to gentle in situ manipulation can be prevented by intravenous glycine (Fig. 9).

Ozaki et al. (23) recently demonstrated that glycine could also protect livers in situ from reperfusion injury by reducing lipid peroxidation, an effect that was not due to alteration of glutathione in the liver tissue. Furthermore, Schilling et al. (28) found that glycine stabilized the cell membrane by inhibition of phospholipase A2, which releases arachidonic acid, leading to eicosanoid production. It is possible that vasoconstrictive eicosanoids lead to the altered microcirculation observed here (Figs. 4–6). Indeed, both during harvest and at reperfusion after transplantation, microcirculation was improved in manipulated grafts from donors given glycine intravenously before the donor operation. Furthermore, glycine did not increase the production of nitric oxide (NO), a potent vasodilator, by Kupffer cells; in fact, it caused a small but significant reduction of NO production (unpublished data). A number of studies have shown that hepatic microcirculation plays an important role in development of reperfusion injury. Several lines of evidence suggest that microcirculatory disturbances are a key factor in enhancing donor organ susceptibility to both cold and warm ischemia in livers (14, 15, 29). Kupffer cells may be responsible for reducing survival and graft viability after transplantation as a result of manipulation. This idea is supported by the fact that GdCl3, a selective Kupffer cell toxicant, reduced harvest-related reperfusion injury, most likely via mechanisms involving the hepatic microcirculation (27). Taken together, these data clearly indicate that glycine improves survival in manipulated grafts by prevention of Kupffer cell activation via mechanisms involving hepatic microcirculation.

Possible site of action of glycine. It is known that destruction of Kupffer cells, the major source of eicosanoids in the liver (9), reduces reperfusion injury (33). Moreover, Kupffer cells release proteases and TNF-α upon activation (5). A recent study has shown that glycine reduces TNF-α production and minimizes death induced by endotoxin, a known activator of Kupffer cells (16). Indeed, TNF-α production after LPS (100 ng/ml), expressed as percentage of increase over basal, increased significantly in Kupffer cells from manipulated livers; however, this effect was blunted when glycine was infused to the donor before manipulation (P < 0.05; no manipulation, 11 ± 0.5; manipulation + glycine, 34 ± 4; manipulation + glycine, 21 ± 3). Accordingly, it is proposed that glycine prevents activation of Kupffer cells at harvest, thereby minimizing reperfusion injury after transplantation. This effect is most likely related to actions on glycine-gated chloride channels of Kupffer cells (Fig. 9).

Clinical implications. Reperfusion injury is linked to primary graft nonfunction, which is still a major problem after clinical liver transplantation. Harvest-related reperfusion injury and primary nonfunction were prevented by intravenous glycine in this study. This is important since gentle organ manipulation cannot be prevented with standard harvesting techniques. Based on the data presented here, clinical trials are warranted to determine if a similar effect of glycine occurs in human liver transplantation.

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REFERENCES


