Bucillamine, a thiol antioxidant, prevents transplantation-associated reperfusion injury

Farin Amersi*, Sally K. Nelson†, Xiu Da Shen*, Hirohisa Kato*, Judy Melinek*, Jerzy W. Kupiec-Weglinski*, Lawrence D. Horwitz‡, Ronald W. Busuttil*, and Marcus A. Horwitz§¶

*The Dumont-University of California, Los Angeles Transplant Center, Division of Liver and Pancreas Transplantation, Department of Surgery, Los Angeles, CA 90095; †Webb–Waring Antioxidant Research Institute, Denver, CO, 80262; ‡Division of Cardiology, Department of Medicine, University of Colorado Health Sciences Center, Denver, CO 80262; and §Division of Infectious Diseases, Department of Medicine, 37-121 CHS, University of California, Los Angeles School of Medicine, 10833 Le Conte Avenue, Los Angeles, CA 90095

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Ischemia/reperfusion (I/R) injury is a serious potential threat to outcomes in organ transplantation and other clinical arenas in which there is temporary interruption of blood flow. I/R is a frequent cause of primary failure in organ transplantation. We hypothesized that the antioxidant bucillamine, a potent sulfhydryl donor, would protect against I/R injury in high-risk organ transplants. Because livers subjected to prolonged ischemia and very fatty livers are highly susceptible to severe I/R injury, we studied the effect of bucillamine in three animal models of liver transplantation: two *ex vivo* models of isolated perfused livers, either normal or fatty rat livers, and an *in vivo* model of syngenic orthotopic liver transplants in rats. In all models, livers were deprived of oxygen for 24 h before either *ex vivo* reperfusion or transplantation. In the *ex vivo* models, bucillamine treatment significantly improved portal vein blood flow and bile production, preserved normal liver architecture, and significantly reduced liver enzyme release and indices of oxidative stress. Moreover, bucillamine treatment significantly increased levels of reduced glutathione in the liver and lowered levels of oxidized glutathione in both liver and blood. In rats subjected to liver transplants, bucillamine significantly enhanced survival and protected against hepatic injury. Possible mechanisms of this protection include prevention of excessive accumulation of toxic oxygen species, interruption of redox signaling in hepatocytes, and inhibition of macrophage activation. This study demonstrates the potential utility of bucillamine or other cysteine-derived thiol donors for improving outcomes in organ transplantation and other clinical settings involving I/R injury.

When organs transiently deprived of oxygen are reoxygenated, an inflammatory cascade is triggered. Cytokine release, complement and macrophage activation, leukocyte invasion, and release of reactive oxygen species (ROS) result in ischemia/reperfusion (I/R) injury (1–3). During organ transplantation, donor grafts exposed to this stress may do poorly. After liver transplantation, hepatic I/R prolongs recovery and contributes to early and late graft rejection (4–5). Moreover, the high susceptibility to I/R injury of fatty livers and livers subjected to prolonged ischemia limits the number of donor livers suitable for transplantation. Very fatty (steatotic) livers, which account for 13–26% of livers donated for transplantation (6–8), are so susceptible to I/R injury and subsequent failure that they usually are not used.

Currently, there is no treatment available for I/R injury. Approaches that have been used in experimental animal models of liver transplantation include antibodies to leukocyte/endothelial cell adhesion sites, inhibitors of Kupffer cell activation, and various antioxidant measures. The latter have included superoxide dismutase, given by gene therapy or in liposomes, various scavengers of ROS, and up-regulation of heme oxygenase-1, a heat-shock protein (9–12). The effectiveness of these interventions has been variable.

ROS have several potential pathogenic effects during I/R. Through redox-mediated signaling pathways, they can activate cytokines, macrophages, and other components of the inflammatory process (1, 13, 14). They also can, when generated in large quantities, cause direct oxidative damage to cells through iron-mediated reactions (15). Thiol donors can interrupt redox signaling pathways and thereby reduce cytokine and macrophage activation (16). In addition, thiol donors can protect cells against oxidative injury by replenishing intracellular glutathione and other endogenous thiol compounds (17). Reduced glutathione (GSH) cannot enter cells readily and therefore has limited value as a treatment for I/R injury (18). However, L-cysteine is actively transported into cells. L-cysteine itself is toxic, but certain thiol-containing derivatives of cysteine are not toxic and are able to use the same transport pathway to enter cells (18).

One such compound is bucillamine, *N*-(2-mercapto-2-methylpropionyl)-L-cysteine (Fig. 1), which has been used to treat rheumatoid arthritis in Japan and Korea (19). It contains two donatable thiol groups and is more potent than other cysteine-derived agents such as *N*-acetylcysteine and *N*-(2-mercaptopropionyl)glycine, both of which contain only a single donatable thiol group (20, 21). Horwitz and Sherman demonstrated in isolated rat cardiac myocytes that bucillamine is a potent antioxidant (21). Moreover, in a canine model of 90 min of coronary artery occlusion followed by 48 h of reperfusion, bucillamine, given only during reperfusion, reduced myocardial infarct size by 41% (21). The effect of bucillamine on oxidative stress and I/R injury in models of liver transplantation has not been investigated previously.

In this study we investigated the capacity of bucillamine to reduce I/R injury in three well defined rat liver models of cold ischemia followed by either *ex vivo* perfusion or orthotopic liver transplantation (OLT). In two *ex vivo* models, one with normal rat livers and the other with steatotic rat livers from genetically obese Zucker rats, we studied the effects of bucillamine on hepatic blood flow, function, injury, and cell architecture. In addition, we quantitated oxidative stress by measuring hepatic carbonyl proteins, lipid peroxides, GSH, and oxidized glutathione (GSSG). Subsequently, we assessed survival, hepatic cell injury, and hepatic cell architecture in rats subjected to syngenic OLT. Bucillamine treatment markedly reduced I/R injury and oxidative stress in *ex vivo* models of both normal and steatotic liver transplantation and enhanced survival after OLT *in vivo*.

Materials and Methods

Animals. Male Sprague–Dawley rats (250–300 g) and male genetically obese (*fa/fa*) Zucker rats (275–350 g) were purchased

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Abbreviations: ROS, reactive oxygen species; I/R, ischemia/reperfusion; OLT, orthotopic liver transplantation; GSH, reduced glutathione; GSSG, oxidized glutathione; GOT, glutamic oxaloacetic transaminase.

¶To whom reprint requests should be addressed. E-mail: mhorwitz@mednet.ucla.edu.

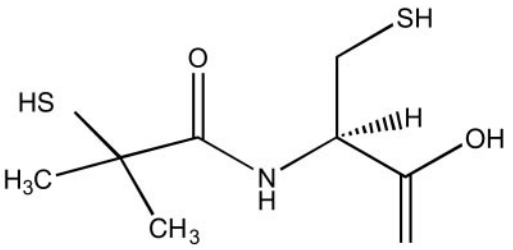

Fig. 1. Structure of bucillamine.

from Harlan–Sprague–Dawley. Animals were fed standard rodent chow and water *ad libitum* and cared for according to guidelines approved by the American Association of Laboratory Animal Care.

Bucillamine. Lyophilized bucillamine (purity >99.9%) was provided by Keystone Biomedical (Los Angeles). Just before use, it was reconstituted with sterile water, diluted in 0.45% (wt/vol) saline to a concentration of 50 mg/ml, and diluted further in University of Wisconsin (UW) solution. A placebo formulation containing the same excipients as in the bucillamine formulation was diluted in the same way such that the final concentration of excipients in the two formulations was identical.

***Ex Vivo* Normal Liver Cold Ischemia Model.** Sprague–Dawley rats underwent isofluorane anesthesia and systemic heparinization. After skeletonization of the liver, the portal vein, inferior vena cava, and common bile duct were cannulated, and the liver was flushed with 10 ml of UW solution and stored for 24 h at 4°C in UW solution. After cold storage, livers were perfused with syngenic rat blood diluted with Krebs-Ringer bicarbonate medium to a hematocrit of 15% (total volume 90 ml) on an isolated perfusion rat liver apparatus for 2 h as described previously (12). During each experiment, pH, temperature, and oxygenation were kept constant.

Livers were divided into three groups ($n = 6$ livers per group). One control group was untreated. A second control group was treated with placebo (vehicle only). The third group was treated with bucillamine. Treatment groups received 10 mg of bucillamine or placebo intraportally at the time of harvest and 90 mg of bucillamine or placebo in the rat blood perfusate. During *ex vivo* reperfusion, portal vein blood flow and pressure were recorded every 30 min. Bile was collected at 30-min intervals from the common bile duct. Blood perfusate samples were collected at 30-min intervals to obtain glutamic oxaloacetic transaminase (GOT) levels, measured with an autoanalyzer from ANTECH Diagnostics (Irvine, CA). At the conclusion of each experiment, a portion of the liver was snap-frozen for measurement of protein carbonyl, lipid peroxide, and glutathione levels. The remaining tissue samples were fixed and stained for histologic evaluation by an investigator who was blinded as to the treatment status of the samples.

***Ex Vivo* Steatotic Liver Cold Ischemia Model.** Livers from 12 fatty Zucker rats were processed, divided into two treatment groups ($n = 6$ each), and treated with bucillamine or placebo as described above for normal rat livers. The investigators were blinded to the treatment status.

***In Vivo* Syngenic OLT Model.** Syngenic OLTs were performed by using livers that were harvested from Sprague–Dawley donors and stored for 24 h at 4°C in UW solution before being transplanted into syngenic recipients with revascularization without hepatic artery reconstruction (22). The treatment group received 2 doses of bucillamine: 15 mg/kg in 0.5 normal saline (NS) intraportally 10 min before reperfusion (final concentration 2.5 mg/ml, ≈1.8 ml per rat) and 10 mg/kg in 0.5 NS intravenously 30 min after reperfusion (final concentration 2.5 mg/ml, ≈1.2 ml per rat). Control animals received 1.8 ml of 0.5 NS 10 min before reperfusion and 1.2 ml of 0.5 NS 30 min after reperfusion. OLT recipients were followed for survival for 30 days. In a second set of experiments, a sufficient number of bucillamine-treated and untreated animals were subjected to OLT until three animals in each group survived for 1, 2, or 3 days, at which point survivors were killed. Because 100% of bucillamine-treated animals survived, only three animals per time point were required for this group. Because mortality was high in the control group, seven, eight, and six control animals were required at days 1, 2, and 3, respectively, to obtain three survivors per time point. Blood and liver samples were obtained from the three bucillamine-treated and the three control animals that were killed on day 1 post-OLT and analyzed for serum GOT levels and histologic status, respectively.

Estimation of Liver Carbonyl Protein Content and Lipid Peroxides. Liver homogenates were assayed for protein carbonyl groups by using the method of Oliver *et al.* in which carbonyl proteins are detected after a reaction with 2,4-dinitrophenylhydrazine to form protein hydrazones (23). Lipid peroxide levels were estimated by a method based on the rapid peroxide-mediated oxidation of ferrous to ferric iron under acidic conditions (24).

Histology. Liver specimens were fixed in a 10% buffered formalin solution and embedded in paraffin. Sections were made at 4 μm and stained with hematoxylin and eosin. The histologic severity of I/R injury in the *ex vivo* perfusion models was graded by using international Banff criteria (25). By using these criteria, lobular disarray and ballooning changes are graded from 1 to 4, where no change is given a score of 1 and severe disarray or ballooning is given a score of 4. The previously published Suzuki's criteria (26) were modified to evaluate the histologic severity of I/R injury in the OLT model. In this classification, sinusoidal congestion, hepatocyte necrosis, and ballooning degeneration are graded from 0 to 4. No necrosis, congestion, or centrilobular ballooning is given a score of 0, and severe congestion and ballooning degeneration as well as >60% lobular necrosis is given a value of 4.

Measurement of Glutathione Levels. GSH and GSSG were determined in hepatic tissue and the blood perfusate by using the enzymatic cycling method described by Tietze (27). All measurements were made by using a diode array spectrophotometer at 412 nm and 25°C. Levels were quantitated against a standard curve generated with known concentrations of GSH and GSSG. In a control study, bucillamine at the concentrations used did not interfere with these measurements.

Statistics. Data are presented as the mean ± SE in the figures. Except as noted, tests for significance were made by using a one-way ANOVA, and the differences between specific groups were determined by the Tukey–Kramer multiple comparisons test. Differences between groups in GSH and GSSG levels were determined by using the Wilcoxan rank sum test.

Results

Effect of Bucillamine in an *ex Vivo* Normal Liver Cold Ischemia Model Followed by Reperfusion. Three groups of livers were studied: an untreated control group, a control group in which livers were treated with vehicle only administered intraportally at the time of harvest and into the blood perfusate at the time of reperfusion (placebo group), and a group in which livers were treated with bucillamine administered intraportally at the time of harvest and into the blood perfusate at the onset of reperfusion (bucillamine

group). Livers were reperfused for 2 h in a recirculating system and portal venous blood flow, bile production, and blood perfusate GOT levels were measured.

I/R injury reduces portal venous blood flow because of increased resistance caused by hepatocyte swelling, sinusoidal congestion, and lobular ballooning. Portal venous blood flow in the bucillamine-treated livers was substantially higher than in either the untreated or placebo-treated control livers during the 2-h reperfusion period ($P < 0.0001$ at 120 min; Fig. 2*a Left*).

Bile production was quantitated to assess hepatic function. Livers treated with bucillamine produced much more bile during 2 h of reperfusion (0.39 ± 0.01 ml) than untreated (0.14 ± 0.02 ml) or placebo-treated (0.07 ± 0.02 ml) controls ($P < 0.0001$ for bucillamine-treated livers vs. either untreated or placebo-treated controls; Fig. 2*b Left*).

Levels of GOT were quantitated in the blood perfusate to assess hepatic cell viability. The bucillamine-treated livers released considerably less GOT than either the untreated or placebo-treated controls (Fig. 2*c Left*). After 2 h of reperfusion, GOT levels were 124.8 ± 7.6 in the bucillamine-treated group vs. 206.0 ± 5.5 and 218.0 ± 25.4 in the untreated and placebo-treated control groups, respectively ($P < 0.004$ for bucillamine-treated livers vs. untreated controls, and $P < 0.0002$ for bucillamine-treated livers vs. placebo-treated controls).

Carbonyl proteins and lipid peroxides were assayed to assess oxidative stress. Free radical oxidation of proteins results in the formation of carbonyl groups in quantities that reflect the intensity of the oxidative stress. Protein carbonyl content was much lower in livers treated with bucillamine than in either the untreated ($P < 0.0001$) or placebo-treated ($P < 0.003$) livers (Fig. 2*d Left*). Thus, bucillamine markedly reduced oxidative degradation of proteins.

Lipid peroxidation is an important consequence of oxidant stress. Lipid peroxides were much lower in the bucillamine-treated livers than in either the untreated ($P < 0.0001$) or placebo-treated ($P < 0.004$) controls (Fig. 2*e Left*). Therefore, bucillamine markedly attenuated this index of oxidant injury.

Hepatocyte injury was graded by using the Banff criteria. In the untreated control livers, there was severe vascular and sinusoidal congestion, centrilobular necrosis, and ballooning, all evidence of severe ischemic injury (Banff score 3.3 ± 0.5). Similarly, in the placebo-treated livers, there was moderate vascular congestion with areas of bridging, central ballooning, and necrosis (Banff score 3.2 ± 0.7). In contrast, bucillamine-treated livers had only mild focal congestion with minimal hepatocyte necrosis, and there was significantly less sinusoidal congestion than in either the untreated or placebo-treated controls (Banff score 1.7 ± 0.5).

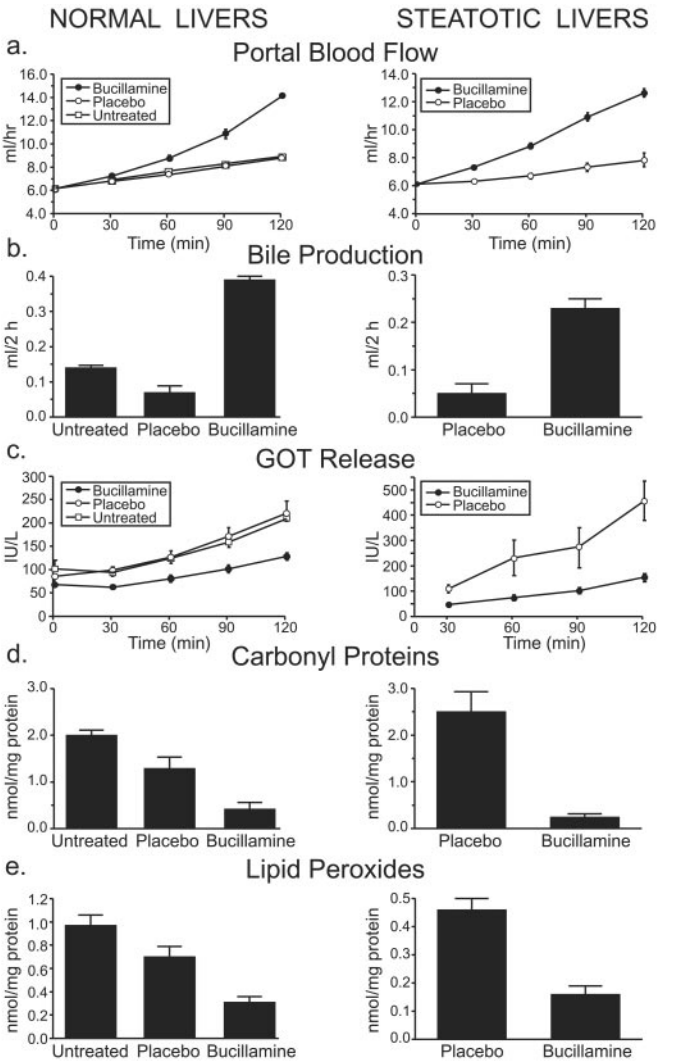

Fig. 2. Bucillamine treatment protects against liver injury and oxidant stress in *ex vivo* models of I/R injury involving normal (*Left*) or steatotic (*Right*) livers. Bucillamine treatment increases portal vein blood flow (*a*), enhances bile production (*b*), decreases hepatic cell injury as reflected by decreased release of GOT into the perfusate (*c*), and reduces the level of carbonyl proteins (*d*) and lipid peroxides (*e*) in the liver. Data are the mean ± SE of six independent perfusions for each group. IU/L, units/liter.

Effects of Bucillamine in an *ex Vivo* Steatotic Liver Cold Ischemia Model Followed by Reperfusion. Steatotic livers from fatty Zucker rats were studied under the same conditions as normal livers except that only one control group, a placebo-treated group, was used. The results with the steatotic livers were very similar to the results with normal livers (Fig. 2 *Right*). Livers treated with bucillamine had (*i*) substantially higher portal venous blood flow (Fig. 2*a*; $P < 0.0003$ for difference between bucillamine and placebo at 120 min); (*ii*) more than 4-fold greater bile production (Fig. 2*b*; $P < 0.0003$); and (*iii*) 3-fold less GOT released into the blood perfusate (Fig. 2*c*; $P < 0.002$ at 120 min). Furthermore, bucillamine-treated livers had 10-fold less protein carbonyl content (Fig. 2*d*; $P < 0.0001$) and 3-fold lower lipid peroxide levels (Fig. 2*e*; $P < 0.0001$). On histologic examination, bucillamine-treated steatotic livers had nearly normal architecture and no evidence of ballooning degeneration or necrosis (Banff score 1.7 ± 0.5), whereas placebo-treated livers had severe disruption of the lobular architecture with marked zone III ballooning and extensive hepatocyte necrosis (Banff score 3.3 ± 0.5).

Bucillamine Greatly Enhances GSH Levels and Blocks the Formation of GSSG. During oxidative stress, the tripeptide glutathione is converted from its reduced to oxidized form. Because bucillamine is a thiol donor, we hypothesized that bucillamine may preserve intracellular GSH levels in the face of oxidative stress. To explore this hypothesis, we assayed GSH and GSSG levels in the livers and blood perfusates in the *ex vivo* models of I/R injury (Fig. 3). The mean level of GSH in the bucillamine-treated normal livers was 8-fold greater than in the untreated or placebo-treated normal livers and almost 3-fold greater in bucillamine-treated steatotic livers than in placebo-treated steatotic livers (Fig. 3*a*). In normal livers, these differences were statistically significant ($P = 0.03$ for bucillamine vs. untreated or placebo controls and $P = 0.008$ for bucillamine vs. the two control groups combined; statistical analysis showed that the results of these two control groups were not significantly differ-

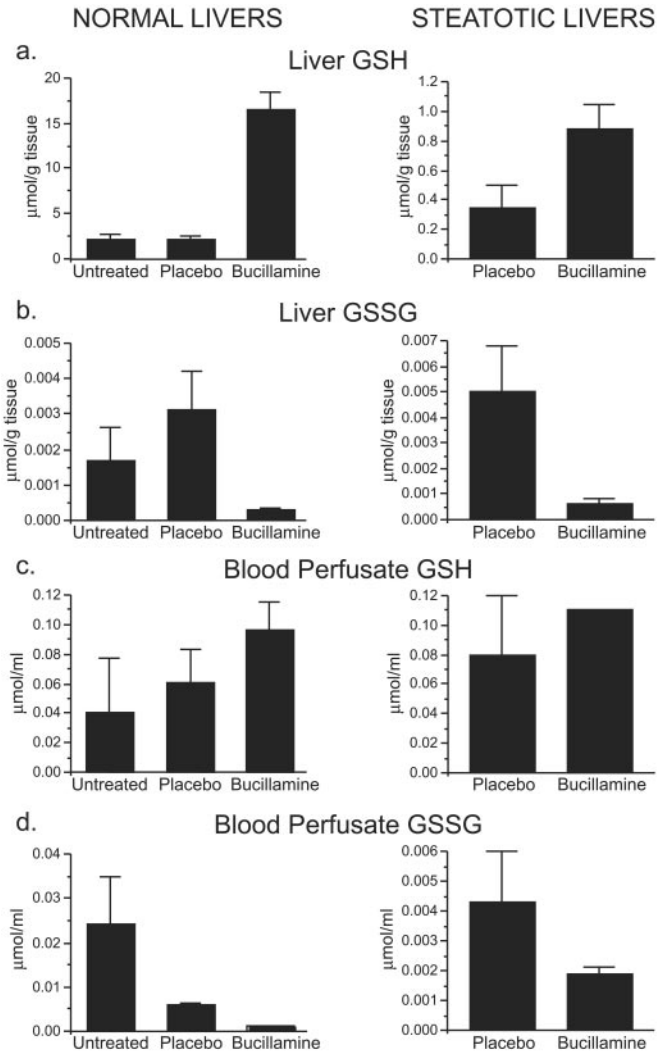

Fig. 3. Bucillamine treatment increases GSH levels in tissue and lowers GSSG levels in tissues and the blood perfusate in the *ex vivo* models of I/R injury. GSH (*a* and *c*) and GSSG (*b* and *d*) levels were assayed in the tissues (*a* and *b*) and blood perfusate (*c* and *d*) in the same models as those described for Fig. 2. Data are the mean ± SE ($n = 4$ for normal livers and $n = 5$ for steatotic livers, $n = 4$ for normal and steatotic blood perfusates).

ent from each other and therefore could be combined for statistical purposes). Conversely, the mean level of GSSG was 6- and 11-fold lower in the bucillamine-treated normal livers than in the untreated ($P = 0.03$) or placebo-treated ($P = 0.03$) controls, respectively ($P = 0.008$ for bucillamine vs. combined controls), and 8-fold lower in the bucillamine-treated steatotic livers than in the placebo-treated steatotic livers ($P = 0.01$; Fig. 3*b*). Hence, bucillamine had a major impact on glutathione redox in the tissues.

Interestingly, the level of GSH in the placebo-treated steatotic livers was 6-fold less than the level in the untreated or placebo-treated normal livers. A previous study similarly observed a significant reduction in the level of GSH in steatotic livers induced by a choline-methionine-deficient diet compared with that in normal livers (28). Low GSH levels may underlie the greater susceptibility of steatotic livers to reperfusion injury.

GSSG formed during oxidative stress may be reconverted to GSH if thiol groups are available or may be exported into the blood. We measured blood perfusate levels of glutathione in normal and fatty livers subjected to reperfusion injury *ex vivo*. Mean levels of GSSG in the blood perfusate of untreated and placebo-treated normal livers were 20- and 5-fold greater, respectively, than the level in the blood perfusate of bucillamine-treated normal livers ($P = 0.03$ for bucillamine-treated livers vs. untreated or placebo-treated controls and $P = 0.008$ for bucillamine-treated livers vs. combined controls), and the level of GSSG in the blood perfusate of placebo-treated steatotic livers was 2.3-fold greater than the level in the blood perfusate of bucillamine-treated steatotic livers ($P = 0.08$; Fig. 3*d*). Bucillamine did not significantly change the levels of GSH in the blood perfusate in normal or steatotic livers (Fig. 3*c*). Hence, bucillamine enhanced levels of GSH in the liver and decreased levels of GSSG in both the liver and blood perfusate in both normal and steatotic livers.

Bucillamine Confers Protection in an *in Vivo* Syngenic OLT Model. To determine the effect of bucillamine in an *in vivo* transplant setting, we performed OLTs using livers that were harvested from Sprague–Dawley donors, stored at 4°C for 24 h, and then transplanted into syngenic recipients. The treatment group was given two boluses of bucillamine, the first intraportally 10 min before reperfusion and the second intravenously 30 min after reperfusion. The control animals were sham-treated in the same way with vehicle only.

Four cohorts of bucillamine-treated and control animals were monitored for survival for various periods of time. In the first study, animals were monitored for survival for 30 days and then killed. Recipients of liver isografts treated with bucillamine had 100% survival at day 30 (6 of 6; Table 1). In contrast, untreated controls had 50% survival at day 30 (3 of 6). The three control animals that did not survive died in the first 24 h after transplantation. In a second study, cohorts of bucillamine-treated and control animals were killed at day 1, 2, or 3 after transplantation. In this study, 100% of nine bucillamine-treated animals survived the predetermined observation period compared with 43% of 21 controls (Table 1). Combining all studies, 100% of 15 bucillamine-treated animals survived compared with 44% of 27 control animals ($P = 0.0005$, χ^2 exact test). This enhanced survival of bucillamine-treated animals correlated with improved liver viability, as assessed by serum GOT levels. Serum GOT levels 1 day posttransplant in the three of eight controls that survived was 9,072 ± 4,170 (SE) units/liter compared with 2,336 ± 374 (SE) units/liter in the three bucillamine-treated animals, all of which survived.

Hepatic I/R injury in the OLT model was assessed at day 1 by using Suzuki's classification. Livers from the three of the eight animals in the control group that survived had moderate to severe hepatocyte necrosis (>60%) with disruption of hepatic architecture and sinusoidal congestion (Fig. 4*a*; Suzuki score 3.5 ± 0.2). In contrast, livers from the three animals in the

Table 1. Survival after OLT in rats treated with bucillamine and in control rats

	Survivors/total observation period				
Treatment status	30 days	1 day	2 days	3 days	All periods
Bucillamine	6/6 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)	15/15 (100%)
Controls	3/6 (50%)	3/7 (43%)	3/8 (37.5%)	3/6 (50%)	12/27 (44%)

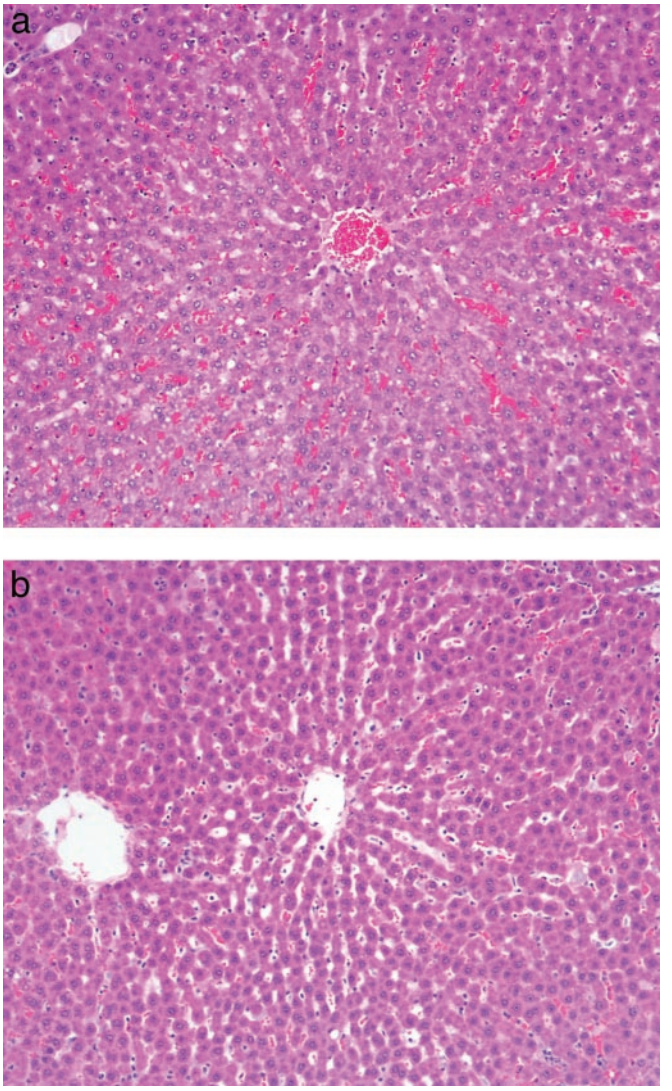

Fig. 4. Histopathology after OLT: representative sections. (*a*) Control group. Section shows disruption of the hepatic architecture and sinusoidal congestion. (*b*) Bucillamine-treated group. Section shows essentially normal hepatic architecture.

bucillamine-treated group had minimal ballooning, single-cell necrosis, and no sinusoidal congestion (Fig. 4*b*; Suzuki score 1.8 ± 0.3).

Discussion

Early organ-donor failure during transplantation is one example of a myriad of clinical settings including early reperfusion after myocardial infarction, stroke, limb ischemia, and acute pulmonary syndromes in which I/R injury contributes to adverse outcomes. In liver transplantation, the definitive therapy for patients with end-stage liver disease (29), I/R damage has been shown to be of major importance in both the short-term and long-term function of transplanted livers (30). In addition, I/R injury contributes to the acute shortage of livers available for transplantation, because the specter of severe I/R injury and failure in marginal livers frequently precludes their use. Extending the inclusion criteria to livers with greater than 30% macrovesicular steatosis or livers that have had markedly prolonged ischemia would increase the number of donor livers available for transplantation substantially.

The mechanisms of liver injury caused by I/R resemble those observed in other organs that are transiently deprived of oxygen. These mechanisms involve a series of events that include Kupffer cell activation, cytokine release, neutrophil activation, increased expression of adhesion molecules, sinusoidal endothelial cell death, and hepatocyte injury (1–3, 31, 32). There is evidence that oxidative stress from exposure to excess quantities of ROS plays a major role (2, 11, 13, 14). Redox signaling can activate macrophages including Kupffer cells and play a role in the release of cytokines that in turn influence leukocyte activation, transmigration, and target cell adhesion (13, 16). The enormous numbers of leukocytes that enter the extravascular space immediately after transplantation release large quantities of ROS capable of overwhelming hepatic cell antioxidant defenses (14). Furthermore, increased mitochondrial production of ROS also may have harmful effects (33).

Ex vivo and *in vivo* models of animal liver transplantation offered excellent paradigms for testing the potential value of a potent thiol donor for prevention of I/R injury. Bucillamine shares with other cysteine derivatives the ability to enter cells rapidly and interrupt redox signaling and oxidative injury. Bucillamine contains two donatable thiol groups and is considerably more potent as an antioxidant than the single-thiol donors *N*-acetylcysteine and *N*-(2-mercaptopropionyl)glycine (19, 20). In our study, treatment with bucillamine significantly decreased I/R-mediated hepatocyte injury in three models of cold ischemia followed by either *ex vivo* reperfusion or OLT. In both normal and steatotic rat livers, bucillamine treatment significantly improved portal venous blood flow, markedly enhanced liver function as evidenced by increased bile production, and substantially reduced liver injury as evidenced by decreased GOT levels and preservation of normal liver architecture.

We attempted to determine whether the protective effect of bucillamine was related to its antioxidant properties by measuring carbonyl proteins and lipid peroxides. Enzymes and structural proteins may be attacked whenever free radicals are generated. As a consequence, oxidative modification of proteins is a marker of reperfusion injury. Livers treated with bucillamine had significantly lower levels of carbonyl protein than either untreated or placebo-treated livers. Lipid peroxidation involves the production of peroxides from free radical intermediates produced by direct or indirect reaction of oxygen-derived reactive metabolites with unsaturated lipids. Lipid peroxidation proceeds by a free radical chain-reaction mechanism that may persist for some time after initiation. The bucillamine-treated livers had markedly reduced levels of lipid peroxides. We did not attempt to assess the effect of bucillamine on redox signaling, but others have demonstrated such effects with other cysteine-derived thiol donors (16).

The efficacy of bucillamine was correlated with a remarkable capacity to enhance the level of GSH and suppress the level of GSSG in the liver. It seems likely that the high potency of bucillamine in combating I/R injury relates to its capacity to donate thiol groups readily to counteract oxidative stress. In addition to its direct effect on oxidative stress, as a result of its capacity to scavenge oxygen radicals (21), bucillamine likely indirectly counters oxidative stress by supplying excess thiol groups that promote the rapid conversion of GSSG to GSH. *N*-acetylcysteine, a less potent thiol donor, also has been found to counter the effects of I/R injury on GSH and GSSG levels in hepatic tissue (34–38). Thus, thiol donors that can be transported into cells such as bucillamine and other cysteine derivatives may be particularly attractive for preventing adverse oxidative processes during reperfusion.

The efficacy of bucillamine in preventing hepatic damage caused by I/R injury was confirmed in our study of syngenic OLTs. In this model, treatment with bucillamine correlated with

improved recipient survival, decreased serum GOT levels, and decreased severity of histological injury.

The marked protection against I/R injury afforded by bucillamine in our models of liver transplantation contrasts favorably with previous putative treatments. The antioxidant enzyme superoxide dismutase had variable effectiveness in previous studies and has been most impressive when administered by gene therapy with viral vectors, an as-yet-unproven technology (12). *N*-acetylcysteine, especially at high doses, has been successful in preserving microcirculatory function in some studies but has been ineffective in others (28, 34–45). In a pilot clinical study, it also seemed to improve outcomes in patients (46). A P-selectin antibody has been effective, presumably by preventing or reducing leukocyte infiltration (47). However, this agent would be unlikely to counteract oxidant injury caused by Kupffer cell activation, hepatic mitochondrial ROS release, or endothelial ROS release. In contrast, bucillamine probably prevents injury caused by ROS generation from both leukocyte and nonleukocyte sources. Indeed, bucillamine alone in this study was at least as effective as the combination of a P-selectin antibody and a potent iron chelator that reduced oxidant injury in a previous study that used the same *ex vivo* model of I/R injury in normal rat livers (48). In conclusion, bucillamine significantly reduces hepatic I/R injury in *ex vivo* models involving both normal and steatotic rat livers and markedly improves outcomes after syngenic OLTs. Thus, bucillamine seems to be an attractive candidate for use in preventing I/R injury in liver transplantation. If its effects in humans resemble its effects in animal models, bucillamine could increase the liver transplant donor pool by allowing the use of marginal steatotic livers or livers subjected to prolonged ischemia. Cysteine-derived thiol donors may have potential as therapeutic agents for prevention of I/R injury in numerous other clinical settings in which there is a period of ischemia or hypoxia followed by the onset of inflammatory cascades during the ensuing period of reperfusion.

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