Glyoxalase I overexpression ameliorates renal ischemia-reperfusion injury in rats

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Kumagai T, Nangaku M, Kojima I, Nagai R, Ingelfinger JR, Miyata T, Fujita T, Inagi R. Glyoxalase I overexpression ameliorates renal ischemia-reperfusion injury in rats. Am J Physiol Renal Physiol 296. F912–F921, 2009. First published February 11, 2009; doi:10.1152/ajprenal.90575.2008. – Methyglyoxal (MG), a highly reactive carbonyl compound generated by carbohydrate oxidation and glycolysis, is the major precursor of protein glycation and induces cytotoxicity leading to apoptosis. Although recent studies have emphasized that MG accumulates in not only chronic oxidative stress-related diseases but also acute hypoxic conditions, the pathogenic contribution of MG in acute diseases is unclear. MG is efficiently metabolized by the glyoxalase system, namely, glyoxalase I. We investigated the pathophysiological role of glyoxalase I as an MG detoxifier in rat renal ischemia-reperfusion (I/R) injury. I/R-induced tubulointerstitial injury was associated with a deterioration in renal glyoxalase I activity independent of its cofactor, GSH, as well as an increase in renal GSH level. In vitro studies, knockdown of glyoxalase I by small interference RNA transfection in rat tubular cells exacerbated cell death by hypoxia-reoxygenation compared with control cells. We also examined whether glyoxalase I overexpression prevented renal I/R damage in rats overexpressing human glyoxalase I with enzyme activity in the kidney 17-fold higher than in wild-type. The histological and functional manifestations of I/R in these rats were significantly ameliorated in association with a decrease in intracellular MG adduct accumulation, oxidative stress, and tubular cell apoptosis. In conclusion, glyoxalase I exerts renoprotective effects in renal I/R injury via a reduction in MG accumulation in tubular cells.

Acute renal failure; advanced glycation end products; methylglyoxal; oxidative stress; tubular cells

Acute kidney injury, which remains an important health concern with high morbidity and mortality rates, is most frequently caused by ischemia due to hypotension or sepsis. The mechanisms underlying renal ischemia-reperfusion (I/R) injury are most likely multifactorial and interdependent, involving hypoxia, inflammatory responses, and cellular damage by free radicals, which in turn strongly suggests the pathological contribution of oxidative stress. However, the involvement of methylglyoxal (MG) and its detoxification system, key players in the pathogenesis of oxidative stress-related diseases, in acute renal ischemia is not well understood.

MG, an α-oxoaldehyde generated from the oxidation of carbohydrates and glycolysis, has attracted considerable attention as a major, highly reactive precursor for protein glycation under hyperglycemic conditions (19). Among findings, proteins glycated by MG, a group of advanced glycation end products (AGE), interact with the receptor for AGE (RAGE) to thereby induce oxidative stress followed by cellular injury (24). Of note, MG itself is a strongly cytotoxic substance, which among other adverse effects induces the dysfunction of mitochondrial respiration and glycolysis in leukocytes (2). In this regard, MG has been shown to impair mitochondrial integrity and decrease intracellular ATP levels (5). MG also induces apoptosis through various putative mechanisms: MG-mediated apoptosis in retinal pericytes occurs via an oxidative stress mechanism involving NF-κB (7), while cisplatin-induced apoptosis in myeloma cells is enhanced by MG through the activation of protein kinase Cα (4).

MG is effectively detoxified by the glyoxalase system. This system consists of two enzymes, namely, glyoxalase I, which metabolizes MG to S-D-lactoylglutathione, and glyoxalase II, which converts S-D-lactoylglutathione to D-lactate (22). It is well conserved in various species. Glyoxalase I is a GSH-dependent enzyme, whose activity in situ is proportional to the cellular concentration of GSH (21). Overexpression of glyoxalase I prevents the accumulation of intracellular MG and AGE in endothelial cells exposed to high concentrations of glucose, indicating that it plays an important role in the suppression of AGE formation under hyperglycemic conditions (19). The glyoxalase system has been closely associated with chronic diseases caused by either or both hyperglycemia and oxidative stress, such as diabetic nephropathy (1), diabetic retinopathy (13), Alzheimer’s disease (8), and aging (15). The association of these diseases with the in situ accumulation of AGE precursors including MG or AGE mediated by chronic oxidative stress is well known.

Thus, although the pathophysiological role of MG in chronic oxidative stress disorders is well understood, little is known about its role in acute diseases such as acute hypoxic injury, or about the renoprotective effects of glyoxalase I as an MG detoxifier. Here, we investigated the pathophysiological role of glyoxalase I in renal I/R injury as an acute oxidative stress-related disease caused by hypoxia.

Materials and Methods

Animal experimental protocol. All experiments were conducted in accordance with the Guide for Animal Experimentation at the Uni-
versity of Tokyo. Male Wistar rats (Nippon Seibutsu Zairyu Center, Saitama, Japan) and human glyoxalase I-overexpressing (GLO I Tg) Wistar rats, generated as described previously (6), were used at the age of 6–8 wk. Rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (40 mg/kg, Nembutal, Dainippon Sumitomo Pharma, Osaka, Japan). One week before the induction of ischemic injury, a right nephrectomy was performed. Ischemic injury was induced by clamping of the left renal artery and vein for 45 min. Core body temperature was maintained at 37°C using a homeothermic table during surgery. Twenty-four hours after the induction of injury, blood and kidney samples were obtained. Blood urea nitrogen (BUN) levels were measured using a commercial kit (Wako, Osaka, Japan).

**Glyoxalase I activity and GSH concentration.** Whole kidneys (25 mg) or 2 x 10⁶ of immortalized rat cultured proximal tubular cells (IRPTC) were homogenized in 0.5 ml and 50 μl of NaPB, pH 7.0, containing 0.02% Triton X-100, respectively, and centrifuged at 20,000 g for 20 min at 4°C. The supernatant was used for assessment of glyoxalase I activity by spectrophotometry according to the method noted elsewhere (11), in which the increase in absorbance at 240 nm due to the formation of S-β-lactoylglutathione was monitored for 3 min at 25°C. GSH concentration in the kidney homogenates from the experimental rats was measured with Bioxytech GSH-400 Assay reagent (Oxis International, Portland, OR).

**Histological analysis.** Tissue fixed in methyl Carnoy’s solution was processed and paraffin-embedded. Four-micrometer sections were stained with periodic acid-Schiff (PAS) reagent and counterstained with hematoxylin.

The tubulointerstitial injury (TI) score was determined from the degree of tubular dilatation, tubular epithelial injury, debris accumulation, and cast formation and graded with an arbitrary score of 0–3, with 0, normal; 1, mild; 2, moderate; and 3, severe according to methods reported elsewhere (10).

**Immunohistochemistry.** Renal MG level was estimated by immunohistochemical detection of the MG-lysine adduct carboxyethyl lysine (CEL). Kidney samples were embedded in OCT compound (Sakura Fine Technical, Tokyo, Japan), frozen in liquid nitrogen, and stored at −80°C until use. The sections (4 μm) were incubated with monoclonal anti-CEL antibody (10 μg/ml) (16), washed, and reacted with a biotinylated, anti-mouse IgG (1:400; Vector Laboratories, Burlingame, CA) and horseradish peroxidase (HRP)-avidin (1:1,000; Vector). To confirm the specificity of the positive signal, the antibody was preincubated with CEL-modified bovine serum albumin (CEL-BSA, 0.1 mg/ml) or carboxymethyl lysine-modified BSA (CML-BSA, 0.1 mg/ml), which were prepared by the method previously reported (12), for 60 min at room temperature and then used.

Vimentin, a marker of tubular damage, was visualized with murine monoclonal IgG antibody V9 (1:500; Dako, Carpentaria, CA), and macrophage infiltration was detected with murine monoclonal IgG1 antibody ED-1 (1: 400; Chemicon, Temecula, CA) by an indirect immunoperoxidase method utilizing Carnoy’s fixed and paraffin-embedded kidney tissues.

For 4-hydroxyxenonalen (4-HNE) staining, Carnoy’s fixed and paraffin-embedded kidney sections were stained with monoclonal anti-4-HNE antibody (25 μg/ml; Japan Institute for the Control of Aging, Shizuoka, Japan) using a Histofine mouse stain kit (Nichirei Bi- science, Tokyo, Japan).

CEL-, vimentin-, ED-1-, 4-HNE-stained sections were quantitatively measured by computer-assisted morphometry. CEL- or 4-HNE-positive areas were measured in five randomly selected cortical fields (×200). Vimentin-positive cells were counted in five randomly selected cortical fields (×200). ED-1-positive cells were counted in five randomly selected fields (×200) including both cortex and outer medulla. All quantifications were performed in a blinded manner.

**Cell culture and induction of hypoxia-reoxygenation.** IRPTC is a cultured cell line derived from proximal tubular cells of male Wistar rats (20). Hypoxic conditions were provided using an Anaerocult A mini system (Merck, Darmstadt, Germany), which reduces the oxygen content to 0.2%, or exposure to 1% O₂/5% CO₂ with the balance as nitrogen in a multigas incubator, APC-30D (ASTEC, Fukuoka, Japan). Hypoxic stimulation was initiated at 70% confluence, and the cells were cultured in culture medium containing 1% FBS during treatment. After hypoxia, the cells were cultured under normoxia for 2 h and then used.

**Small interference RNA transfection.** The small interference (si) RNA oligonucleotides were synthesized by TaKaRa Bio (Shiga, Japan), transfected to IRPTC at 30% confluence using Lipofectamine 2000 (Invitrogen, Carlsbad, CA), and incubated for 24 h before induction of hypoxia-reoxygenation (H/R). To control for the off-target effects of siRNA, IRPTCs was also transfected with a control GC content-matched siRNA. The sequence of the GLO I siRNA duplex and scramble control duplex were as follows: GLO I: forward 5’-GUU CUC GCU CUA UUU CUU AGC-3’, reverse 5’-UAA GAA AUA GAG CGA GAA CUU-3’; and control: forward 5’-GUC UGG CGU UCG UUC AAG UAC-3’, reverse 5’-UCU AGG AAC GCC AGA CCCU-3’.

**Total RNA isolation and real-time quantification PCR.** Total RNA (1 μg) was isolated with Isogen (Nippon Gene, Tokyo, Japan), and reverse-transcribed with Im-Prom II (Promega, Madison, WI). cDNA was subjected to real-time quantitative PCR using iQ SYBR Green PCR supermix (Bio-Rad) and the iCyte PCR system (Bio-Rad). The primers used in this study were as follows: rat glyoxalase I, forward 5’-ATT TGG CCA CAT TGG GAT TGC-3’, reverse 5’-TCT AAC CTA GTA GCC ATC AGG-3’; and β-actin, forward 5’-CTT TCT ACA ATG AGC TGC GTG-3’, reverse 5’-TCA GGT AGT CTG CTA GG-3’. PCR was performed under the following conditions: 95°C for 15 min, followed by 40 cycles of denaturation at 94°C for 30s, annealing at 60°C for 30s, and extension at 72°C for 60s. The glyoxalase I mRNA level was normalized with β-actin, an internal control.

**Immunochemistry.** IRPTC was seeded on four-well Lab-Tek chamber slides (Nalge Nunc International, Rochester, IL) and cultured in normoxic or H/R conditions. The cells were fixed with acetone/methanol (1:1) at −20°C for 10 min, stained with anti-CEL monoclonal antibody (10 μg/ml) (16), and reacted with a biotinylated anti-mouse IgG (1:400; Vector Laboratories, Burlingame, CA) and anti-mouse IgG (1:400). After washing, the sections were incubated with a biotinylated, anti-mouse IgG (1:400). For quantitative computer-assisted morphometry, cells positive for CEL staining signal were counted in 15 randomly selected fields each.

**Cell viability test.** Cell damage was evaluated using the trypan blue exclusion test and LDH release. Unstained (viable) and stained (nonviable) cells suspended in 0.4% trypan blue were counted by hemacytometer, and death rate was expressed as the percentage of nonviable cells. LDH release from cells was calculated by determining the ratio of LDH in the culture medium compared with that in the lysed cells plus the culture medium (Kainos Laboratories, Tokyo, Japan) and expressed as a percentage.

**Western blot analysis.** Kidney tissue or IRPTC (20 μg) was homogenized in a sample buffer (0.35 M Tris·HCl, pH 6.8, 10% SDS, 36% glycerol, 5% β-mercaptoethanol, and 0.012% bromophenol blue), electrophoresed on 10–15% SDS-PAGE, and transferred to a polyvinylidene fluoride membrane (Amersham Biosciences, Piscataway, NJ).

For detection of glyoxalase I and CEL, the rabbit polyclonal antibody to rat glyoxalase I peptide GIAPDVYVEA (5 μg/ml) and mouse anti-CEL monoclonal antibody (16) (CEL-SP, 2.5 μg/ml) were used as the first antibody, respectively, and rabbit anti-actin polyclonal antibody (1:1,000; Sigma, St. Louis, MO) was used as the control. The bands were detected by an enhanced chemiluminescence system (Amersham Biosciences) and subject to quantitative densitometry using National Institutes of Health ImageJ software.

**Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling assay.** Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL)-positive cells were identified using a TACS 2 TdT-Blue Label In Situ Apoptosis Detection Kit ( Trevigen, Gaithersburg, MD). For each renal section, five randomly selected
Fig. 1. Effect of renal ischemia-reperfusion (I/R) on histology, glyoxalase I activity and expression, GSH level, and the level of methylglyoxal (MG)-lysine adduct CEL in rats. A: representative light microscopy of periodic acid-Schiff (PAS)-stained kidney section from sham-operated and I/R-induced rats. Original magnification, ×200. B: glyoxalase (GLO) I activity and its expression level of mRNA and protein in renal tissue lysate from experimental rats (n = 6 for each). Expression level of glyoxalase I mRNA or protein was normalized using that of actin as an internal control. **P < 0.01 vs. sham-operated rats. C: GSH level in renal homogenate obtained from experimental rats (n = 6 for each). Immunohistochemistry (D; n = 6 each) and Western blot analysis (E) for detection of MG-lysine adduct carboxyethyl lysine (CEL) in kidney tissue and lysate from experimental rats are also shown. CEL-BSA, immunohistochemistry utilizing anti-CEL antibody preincubated with CEL-BSA; CML-BSA, immunohistochemistry utilizing anti-CEL antibody preincubated with CML-BSA, another lysine-adduct modified by glyoxal. Original magnification, ×200. A vertical black bar and an arrow indicate the bands, the molecular masses of which corresponded to 24–30 and 14 kDa, respectively.
Fig. 2. Glyoxalase I (GLO I) activity and MG-lysine adduct CEL in cultured rat tubular cells with hypoxia-reoxygenation (H/R). A: change in glyoxalase I activity and its mRNA or protein expression in rat proximal tubular cells, IRPTC, with H/R (n = 6 each). B: immunocytochemistry followed by quantitative computer-assisted morphometry for CEL detection in IRPTC with normoxia or H/R. Original magnification, ×400. **P < 0.01 vs. normoxia. C: Western blot analysis for CEL detection in IRPTC treated with normoxia or H/R. Representative bands, the intensity of which was enhanced by H/R, are indicated by a black bar and arrows.
fields (×200) were examined and the numbers of positive cells were counted.

Statistical analysis. Statistical analysis was performed using SAS software version 5.1.2 (SAS Institute, Cary, NC). Data are expressed as means ± SE. Statistical differences were assessed using Student’s paired t-test. All reported P values are two-sided. Nonparametric data were analyzed with the Mann-Whitney test when appropriate. Values of P < 0.05 were considered statistically significant.

RESULTS

Renal glyoxalase I activity decreases and MG content increases following renal I/R injury. We evaluated changes in intrarenal glyoxalase I activity and the level of GSH, a cofactor of glyoxalase I, in I/R injury in rats. Histological examination demonstrated that I/R induced tubulointerstitial injury, including the loss of tubular epithelial cells, dilation of tubules, macrophage infiltration, and cast formation (Fig. 1A). Interestingly, this tubulointerstitial damage was associated with a significant decrease in renal glyoxalase I activity without any change in mRNA or protein expression level (Fig. 1B). Further, renal I/R injury had no statistically significant effect on levels of GSH, which acts as a cofactor in the regulation of glyoxalase I activity (Fig. 1C). We then evaluated the renal level of MG, a substrate of glyoxalase I, by indirect immunohistochemistry of a MG-lysine adduct (CEL) and found that the renal level of MG estimated by CEL staining was also markedly augmented in the tubulointerstitium (Fig. 1D). The CEL-positive signal was undetectable when the monoclonal anti-CEL antibody was preincubated with CEL-BSA but not CML-BSA, which is an adduct modified by another reactive carbonyl compound, glyoxal. These results indicated that the positive signal is specific for CEL. We confirmed these results by Western blot analysis for CEL: the intensity of bands with a molecular weight corresponding to 24–30 and 14 kDa was increased in lysate from I/R-injured compared with sham-operated kidneys (Fig. 1E).

Glyoxalase I activity decreases and intracellular MG accumulates in cultured tubular epithelial cells undergoing H/R. To confirm our in vivo results, we investigated the change in glyoxalase I activity in cultured tubular epithelial cells, namely, IRPTC, that were exposed to H/R (0.2% O2 for 24 h followed by reoxygenation for 2 h). H/R produced a significant decrease in glyoxalase I activity with no effect on the expression of its mRNA or protein (Fig. 2A). Immunocytochemistry and Western blot analysis showed that this deterioration in glyoxalase I activity was associated with intracellular accumulation of CEL, especially that with a molecular weight of 48, 24–30, or 14 kDa, in the H/R-exposed IRPTC (Fig. 2, B and C). These data are consistent with our in vivo findings.

Knockdown of glyoxalase I exacerbates H/R-induced cellular injury in cultured tubular cells. To confirm the functional contribution of glyoxalase I in H/R-induced cellular injury, we performed siRNA transfection specific for the knockdown of the glyoxalase I gene in IRPTC. Glyoxalase I-specific siRNA transfection to IRPTC decreased its gene expression by ~60% compared with control siRNA transfection. This enzymatic activity was also decreased by 38% in IRPTC transfected with glyoxalase I siRNA (Fig. 3A). We then used trypan blue exclusion and LDH release assays to assess whether knock-
down of glyoxalase I aggravates H/R-induced cell damage. To enhance the difference in cell death by H/R, this experiment employed relatively mild H/R conditions, namely, hypoxia by 1% O2 for 40 h followed by reoxygenation for 2 h. Survival of glyoxalase I-knocked down IRPTC was similar to that of IRPTC transfected with control siRNA under normoxia during the experimental period. Under H/R conditions, in contrast, cells in which glyoxalase I was knocked down had significant exacerbation of cell death compared with control cells (Fig. 3B).

Glyoxalase I overexpression ameliorates I/R-induced renal injury. Given our in vitro findings that glyoxalase I contributes to cell survival in H/R, we hypothesized that overexpression of glyoxalase I in the kidney might ameliorate renal I/R injury. To test this, we assessed the renoprotective effects of glyoxalase I against I/R injury in GLO I Tg rats, which overexpress human glyoxalase I (6). GLO I Tg rats showed a high level of glyoxalase I activity in various organs, including the kidney, without induction of any change in histology (Fig. 4A). Renal glyoxalase I activity in GLO I Tg rats was ~17-fold higher than that in wild-type rats (18.7 ± 0.3 U/g in wild-type rats vs. 268.6 ± 22.4 in GLO I Tg rats; Fig. 4B).

Results showed that the I/R-induced suppression of glyoxalase I activity observed in wild-type rats was prevented by glyoxalase I overexpression in GLO I Tg rats (P = NS; Fig. 4B). Importantly, when I/R injury was induced in GLO I Tg rats, the severity of TI, as represented by tubular dilatation and tubular epithelial loss, was markedly attenuated [score for TI damage, GLO I Tg rats with I/R (n = 11), 1.32 ± 0.21 vs. wild-type rats with I/R (n = 8), 2.36 ± 0.18; P < 0.01; Fig. 4A]. Pathological change in glomeruli was not observed during the period of the experiments in this I/R model; hence, glomeruli did not differ pathologically between wild-type and GLO I Tg rats. Renal function, estimated by BUN levels, was also better in the GLO I Tg compared with wild-type rats (Fig. 4C). Furthermore, the attenuation of TI damage observed in

![Fig. 4. Effect of glyoxalase I overexpression on renal injury induced by I/R in vivo. A: representative light microscopy of periodic acid-Schiff-stained kidney from wild-type (WT) and human glyoxalase I-overexpressing (GLO I Tg) rats with or without I/R. WT + I/R, WT with I/R; Tg + I/R, Tg with I/R. Original magnification, ×200. B: glyoxalase I activity in kidney lysate of experimental rats. **P < 0.01 vs. wild-type rats without I/R. WT + I/R, WT with I/R; Tg + I/R, Tg with I/R. Original magnification, ×200. C: glyoxalase I activity in kidney lysate of experimental rats. **P < 0.01 vs. wild-type rats without I/R. WT + I/R, WT with I/R; Tg + I/R, Tg with I/R. Original magnification, ×200. D: significant positive correlation between morphological severity (TI score) and BUN level. ●, WT rats with I/R; ▲, GLO I Tg rats with I/R.](http://ajprenal.physiology.org/content/296/4/F917)
GLO I Tg rats after I/R injury was positively correlated with the improvement in renal function (Fig. 4D).

To confirm less severe renal injury in GLO I Tg rats after I/R, we immunohistochemically analyzed the detection of vimentin, a marker of TI injury, and ED-1, a macrophage marker. Vimentin-positive staining in the tubulointerstitium was barely detected in either wild-type or GLO I Tg rats without I/R. In contrast, while expression was remarkably increased in injured tubular cells or interstitium in wild-type rats with I/R, this increase was significantly attenuated in GLO I Tg rats [wild-type rats with I/R (n = 8), 42.3 ± 2.6 vimentin-positive tubules per field vs. GLO I Tg rats with I/R (n = 11), 19.5 ± 2.5; P < 0.01; Fig. 5, A and B]. Furthermore, whereas I/R injury was associated with an increase in ED-1-positive cell number in the interstitium in wild-type rats, significantly fewer infiltrating macrophages were seen in GLO I Tg rats [wild-type rats with I/R (n = 8), 115.0 ± 9.4 ED-1-positive cells per field vs. GLO I Tg rats with I/R (n = 11), 73.9 ± 4.3; P < 0.01, Fig. 5, A and B].

Improvement in I/R injury by glyoxalase I overexpression is associated with a decrease in MG adduct accumulation, oxidative stress, and apoptosis in the tubulointerstitium. We then investigated whether the improvement in renal I/R injury in GLO I Tg rats was associated with a decrease in MG level in the kidney. Immunohistochemistry showed minimal detection of CEL, a measure of an MG-lysine adduct, in both wild-type and GLO Tg rats at baseline. In contrast, while accumulation was increased in the tubulointerstitium in wild-type rats after I/R, this increase was significantly attenuated in GLO I Tg rats (P < 0.05; Fig. 6, A and B). Given that CEL is an oxidative stress marker derived from glycation or glycoxidation, this decrease in I/R-induced CEL accumulation suggests that the increased intrarenal glyoxalase I in the transgenic animals protects against I/R-induced oxidative stress.

We further evaluated the status of oxidative stress in the kidney of I/R-injured GLO I Tg rats by immunohistochemical analysis of 4-HNE, an oxidative stress marker derived from lipid peroxidation. Results showed a remarkable increase in area positively stained for this oxidative marker in the tubulointerstitium of wild-type rats after I/R. In contrast, this increase was attenuated in GLO I Tg rats following I/R (P < 0.05; Fig. 6, A and B).

Furthermore, since MG itself induces apoptosis in various cells, we examined the degree of apoptosis in tubular epithelial cells by TUNEL staining. The number of TUNEL-positive nuclei was increased in wild-type rats after I/R compared with that seen in sham-operated control rats. This increase was attenuated in GLO I Tg rats with I/R (Fig. 6C). These results were confirmed by computer-assisted morphometry (P < 0.05; Fig. 6D).

![Fig. 5. Effect of glyoxalase I overexpression on tubular damage and macrophage infiltration in renal I/R injury. A: immunohistochemical analysis for detection of tubulointerstitial injury estimated by vimentin staining (top) and macrophage infiltration estimated by ED-1 staining (bottom). Original magnification, ×200. B: computer-assisted morphometry of the immunohistochemical stained slides. **P < 0.01 vs. WT with I/R. WT, n = 3; Tg, GLO I Tg rats, n = 4; WT + I/R, n = 8, Tg + I/R, n = 11.](http://ajprenal.physiology.org/...
Fig. 6. A: effect of glyoxalase I overexpression on CEL accumulation, oxidative stress, and renal tubular apoptosis in renal I/R. Immunohistochemical analysis for CEL, a marker of intracellular MG accumulation, and for 4-hydroxynonenal (4-HNE), an oxidative stress marker by lipid peroxidation. Original magnification, ×300. B: computer-assisted morphometry of immunohistochemical stained slides of kidney from experimental rats. *P < 0.05 vs. WT with I/R. C: terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) staining in kidney tissues of the experimental rats. Original magnification, ×200. D: computer-assisted morphometry of TUNEL-stained slides. WT, n = 3; Tg, GLO I Tg rats, n = 4; WT + I/R, n = 8; Tg + I/R, n = 11. *P < 0.05 vs. WT with I/R.
DISCUSSION

Here, we demonstrate that renal glyoxalase I activity is decreased in response to I/R in vivo and to H/R in vitro. These results indicate that the decrease in situ glyoxalase I activity is an important factor in the increase in MG accumulation in acute hypoxic lesions. While previous studies focused primarily on MG in chronic diseases, more recent studies on cardiac and cerebral I/R injury have shown the accumulation of MG and the MG-arginine adduct argpyrimidine in the hypoxia-damaged heart and brain, respectively (3, 17). While these reports suggested the pathogenic contribution of MG to the development of I/R injury in these models, the mechanism of MG accumulation in these I/R injury models has remained unclear. Given that glyoxalase I activity was decreased in our experimental animals and in cultured tubular cells subject to I/R and H/R, we speculate that the accumulation of MG in acute hypoxia may be due to a decrease in glyoxalase I activity, in part at least.

Among our main findings, we observed that the decrease in renal glyoxalase I activity induced by hypoxia did not correlate with the mRNA or protein expression level of this enzyme or of the glyoxalase I co-factor GSH. This finding is consistent with a previous finding that glyoxalase I activity in Caenorhabditis elegans is reduced with age despite unchanged levels of its mRNA (15). One possible mechanism of the deterioration in glyoxalase I activity in renal I/R injury might be the formation of as yet undefined substances or posttranslational protein modification, which in turn would modulate glyoxalase I activity. For example, S-nitrosoglutathione, which is formed from nitric oxide and GSH, interacts with glyoxalase I to convert it into the inactive form (14, 18). The phosphorylation of glyoxalase I may also cause a change in its activity and contribute to TNF-induced cell death in murine fibrosarcoma cells (23). The mechanism of this decrease in glyoxalase I activity in hypoxia requires clarification.

The biological significance of glyoxalase I was emphasized by our functional studies, which included knockdown experiments in cultured tubular cells and overexpression studies in transgenic rats. Of marked interest, we found that overexpression of glyoxalase I ameliorated the pathological and functional manifestations of I/R in vivo. To our knowledge, this is the first report to demonstrate an effect of glyoxalase I against acute kidney injury such as renal I/R injury.

With regard to potential mechanisms of the amelioration of renal I/R injury by overexpression of glyoxalase I, we propose two distinct but not mutually exclusive possibilities (9). The first is a decrease in the cytotoxicity of MG per se. This is supported by our observation that I/R-induced tubular cell apoptosis, which might be mediated by MG itself, was reduced by glyoxalase I overexpression. The second is suppression of the vicious cycle of oxidative stress and MG formation. This is supported by our immunohistochemical studies of 4-HNE, which showed that I/R-induced lipid peroxidation was also reduced by glyoxalase I overexpression.

GLO I Tg rats showed histological and functional improvement in I/R-induced renal damage with statistical significance. However, the degree of functional improvement was milder than that of the histological improvement. One possible explanation for this phenomenon is that dehydration during the I/R operation may have caused an increase in BUN concentration, thereby overestimating renal dysfunction and resulting in the apparent mild functional improvement.

In conclusion, our findings show that glyoxalase I exerts renoprotective effects in renal I/R injury via reduction of MG levels in tubular cells, causing a subsequent interruption of the events that lead to oxidative stress, hypoxia, and tubular cell apoptosis. These findings provide an important insight into the pathophysiology of renal I/R injury and suggest that targeting the glyoxalase system might be a fruitful avenue for therapy in acute kidney injury.

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