

Delayed DMSO Administration Protects the Kidney from Mercuric Chloride–Induced Injury

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Abstract. Reactive oxygen species are implicated as mediators of tissue damage in ischemic and toxic acute renal failure. Whereas many agents can inhibit renal ischemic injury, only hepatocyte growth factor, melatonin, N-acetylcysteine, and DMSO inhibit injury after mercuric chloride administration. Although it has been suggested that DMSO may chelate the mercuric ion, more recent studies suggest that it has anti-inflammatory and antioxidant effects. Acute renal failure was induced by 5 mg/kg subcutaneous injection of mercuric chloride in BALB/c mice. DMSO (3.8 ml/kg, 40% in PBS) or vehicle (PBS) was injected intraperitoneally at 0 and 24 h after mercuric chloride injection, or DMSO treatment was delayed 3 or 5 h. DMSO prevented increases in serum creatinine and tubular damage at 24 and 48 h. When DMSO treatment was

delayed by 3 h, it was still beneficial; however, with a 5-h delay, the histology score and serum creatinine were not significantly decreased. DMSO partially prevented a mercuric chloride–induced decrease in glutathione peroxidase activity and completely prevented the transient decrease in superoxide dismutase activity. Neither mercuric chloride nor DMSO affected catalase activity significantly. For investigating possible effects of DMSO on cellular mercuric ion uptake, MDCK cells that were transfected with human organic anion transporter-1 were used. ²⁰³Hg uptake was inhibited 90% by N-acetylcysteine but only 5% by DMSO, indicating that the effect of DMSO is not related to chelating mercuric ion or inhibiting its uptake. It is concluded that DMSO acts in part as an antioxidant to inhibit mercuric chloride–induced acute renal injury.

Human acute renal failure (ARF) is often caused by ischemic and nephrotoxic insults, commonly acting in combination. Whereas many agents are protective in animal models of renal ischemic-reperfusion injury (I/R) (1), only a few agents inhibit renal injury in the widely used mercuric chloride (HgCl₂) model of nephrotoxic ARF. The agents that have been demonstrated to reduce HgCl₂-induced renal injury include DMSO (2), dimethylthiourea (DMTU) (2), N-acetylcysteine (NAc) (3), spironolactone (4), hepatocyte growth factor (HGF) (5,6), superoxide dismutase (SOD) (7), and melatonin (8). Because it is often difficult clinically to differentiate ischemic from nephrotoxic ARF, it would be helpful to have agents that work in both ischemic and toxic injury. The anti-inflammatory agents α -melanocyte stimulating hormone (α -MSH) and IL-10 and antibodies to intracellular adhesion molecule-1, lymphocyte function-associated antigen 1 (LFA-1), and TNF- α inhibit renal injury after ischemia but are ineffective as treatments for HgCl₂-induced renal failure (9,10). This is possibly because

the inflammatory phase of the nephrotoxic model occurs late, after the renal injury is established. Only a few agents, such as HGF, NAc, and DMSO (2,11–13), protect in both types of renal injury.

Recent work has focused on the role of free radicals and oxidants in the renal injury induced by HgCl₂. For example, HgCl₂ increases the production of many endogenous oxidants such as hydrogen peroxide (14), depletes protective antioxidants such as glutathione (GSH), and reduces free radical scavenging systems such as SOD and GSH peroxidase (GPx) (15). Indeed, HgCl₂-induced injury can be ameliorated by SOD or the antioxidants NAc and melatonin in some (3,7,8) but not all (2) studies. DMSO is an aprotic solvent with anti-ischemic, anti-inflammatory, and antioxidant properties [reviewed by Santos *et al.* (16)]. DMSO protects rats against I/R injury (13,17) and nephrotoxic injury when administered before HgCl₂ (2). Another hydroxyl radical scavenger, DMTU, protected the kidney when administered in a mixture with HgCl₂ (2). Late DMSO administration protects against chemically induced liver and kidney injury (18). Indeed, DMSO protected against chloroform-induced ARF when administered 10 h after the toxicant. However, the mechanism of action of DMSO has been questioned as it has the ability to form complexes with Hg (2). This DMSO-Hg complex formation may prevent Hg from binding to protein sulfhydryl groups. Also, several metal-ol agents, such as 2,3-dimercapto-1-propane sulfonate (DMPS), dimercaptosuccinic acid (DMSA), and the low molecular weight thiol NAc inhibit the renal tubular uptake of mercuric

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ion and enhance urinary excretion (19,20). Therefore, it has been suggested that DMSO may inhibit Hg-induced renal injury by reducing the intracellular availability of free Hg rather than by scavenging free radicals.

In this study, we determined the window of opportunity for DMSO protection of renal function in a mouse model of HgCl₂ injury and investigated potential mechanisms of protection. Because it has been suggested that DMSO may prevent uptake of HgCl₂ into cells by binding HgCl₂, the effect of DMSO on the uptake of Hg²⁺ into cultured renal cells was also studied.

Materials and Methods

Animals

Male BALB/c mice, aged 6 to 7 wk, were purchased from the National Institutes of Health (NIH). All animals had free access to water and chow (NIH-07 Rodent Chow; Zeigler Bros., Gardners, PA). Animal care followed the criteria of the National Institutes of Health for the care and use of laboratory animals in research.

Experimental Design

The animals were given HgCl₂ (5 mg/kg in normal saline) by subcutaneous injection, and DMSO (3.8 ml/kg, 40% in PBS) or vehicle (PBS) was administered by intraperitoneal injection at 0 and 24 h. Other groups of mice received DMSO treatment whereby the first dose was delayed for 3 and 5 h after HgCl₂ injection. Animals were killed at 12, 24, and 48 h after HgCl₂ injection. Blood was collected for measurement of serum creatinine, and both kidneys were harvested for histologic and other studies.

Histologic Examination

Four percent paraformaldehyde-fixed and paraffin-embedded kidney specimens were stained with periodic acid-Schiff reagent or Naphthol AS-D chloracetate esterase (kit no. 91-C; Sigma, St. Louis, MO). Histologic changes in the outer stripe of outer medulla were evaluated by semiquantitative measurement of tissue damage. Tubular damage was estimated semiquantitatively in eight high-power fields per section by using a scoring system based on the percentage of damaged tubules (*i.e.*, necrotic) per high-power field (1, < 25%; 2, 25 to 50%; 3, 50 to 75%; 4, > 75%). The mean score of each animal was compared. Leukocyte infiltration was evaluated by counting the number of esterase-positive cells in eight high-power fields in the outer stripe of outer medulla.

Measurement of Tissue GSH Level, GPx, SOD, and Catalase Activity

Renal cellular GSH levels were determined using a GSH colorimetric assay kit (Calbiochem, La Jolla, CA). Kidney tissue was homogenized in cold 5% (wt/vol) metaphosphoric acid (Aldrich, Milwaukee, WI), centrifuged at 3000 × *g* for 10 min at 4°C, and the supernatant was assayed for GSH according to instructions provided with the kit. The amount of GSH was expressed as μmol/g kidney (wet wt). The sensitivity of the assay was 0.25 μmol/g kidney.

Kidney GPx activity was measured indirectly by using a GPx assay kit (Calbiochem, La Jolla, CA). Briefly, kidney tissues were homogenized in ice-cold buffer that contained 50 mM Tris-HCl (pH 7.5), 5 mM EDTA, and 1 mM 2-mercaptoethanol and was centrifuged at 10,000 × *g* for 20 min at 4°C. The supernatant was added to a solution that contained 1 mM GSH, 4 U/ml GSH reductase, and 0.2 mM NADPH. The reaction was initiated by the addition of 0.22 mM tert-butyl-hydroperoxide. GPx activity was then quantified by mea-

suring the rate of change in the absorbance at 340 nm caused by the oxidation of NADPH. Enzyme activity was expressed as U/mg protein. The sensitivity of the assay was 90 U/mg protein.

Total SOD activities were determined by a SOD assay kit (Calbiochem) that is based on the SOD-mediated increase in the rate of auto-oxidation of 5,6, 6a,11b-tetrahydro-3,9,10-trihydroxybenzofluorene in aqueous alkaline solution. The activity of total SOD was determined from the ratio of the auto-oxidation rates measured in the presence and in the absence of SOD. The activities were expressed as U/mg protein. The sensitivity of the assay was 0.2 U/mg protein.

Catalase activity was determined by a catalase assay kit (Calbiochem). Briefly, supernatant from a tissue homogenate was incubated with 10 mM H₂O₂ for 1 min, and the amount of H₂O₂ that remained in the reaction mixture was determined by an oxidative coupling reaction of chromagen in the presence of H₂O₂. The change in absorbance was read at 520 nm. Catalase activities were expressed as U/mg protein. The sensitivity of the assay was 2 U/mg protein.

In Vitro ²⁰³Hg Uptake Study

To examine the effect of DMSO on the uptake of Hg²⁺ into tubular cells, ²⁰³Hg uptake in the presence of 1% DMSO, or low molecular thiol, we examined NAc (20 μM) in hOAT1-transfected MDCK cells. hOAT1-transfected MDCK cells were generated as described previously (21,22). ²⁰³Hg was a gift from Dr. Delon Barfuss (Georgia State University) and Dr. Rudolfs K. Zalups (Mercer University) and was produced as described previously (21,23). Cells were plated in 24-well culture plates (5 × 10⁵ cells/well) in EMEM supplemented with 10% FBS and 1 mM pyruvate. After 2 d of growth in a humidified incubator (5% CO₂/95% air, 37°C), ²⁰³Hg²⁺ transport was assessed. Briefly, cells were washed three times with Hanks' buffered saline solution with 10 mM HEPES (pH 7.4; transport buffer), and then transport buffer that contained 5 microM ²⁰³Hg²⁺ with or without 1% DMSO or 20 μM NAc was added to each well. After 1 h, wells were rinsed with cold (4°C) stop buffer (transport buffer that contained 1 mM DMPS and 200 μM probenecid). One milliliter of 1 N NaOH was then added to each well to lyse the cells. A total of 700 μl of cell lysate was then removed from each well and neutralized with 700 μl of 1 N HCl. This lysate mixture was then added to 15 ml of EcoLume (ICN Biomedical, Cleveland, OH) scintillation fluid, and sample radioactivity was determined by scintillation counting with a Tri-Carb 2900 TR Liquid Scintillation Analyzer (Packard, Meriden, CT). Fifty microliters of the remaining cell lysate was processed for Bradford protein assay, and data were normalized to the protein concentration.

Statistical Analyses

All data were expressed as means ± SEM. Differences between experimental groups were examined by *t* test (SigmaStat), and *P* < 0.05 was considered statistically significant.

Results

Effect of DMSO in HgCl₂-Induced Renal Injury

To evaluate the effect of DMSO in HgCl₂-induced renal injury, we examined serum creatinine and histologic changes at 48 h after injection. As expected, HgCl₂ administration increased the serum creatinine (normal, 0.3 ± 0.0, *versus* HgCl₂ + vehicle, 2.1 ± 0.2 mg/dl). DMSO, when injected simultaneously with HgCl₂, significantly decreased serum creatinine compared with vehicle-treated mice (DMSO, 0.7 ± 0.2 mg/dl; *P* < 0.05; Figure 1). Similarly, extensive tubular damage and necrosis in vehicle-treated mice significantly decreased in

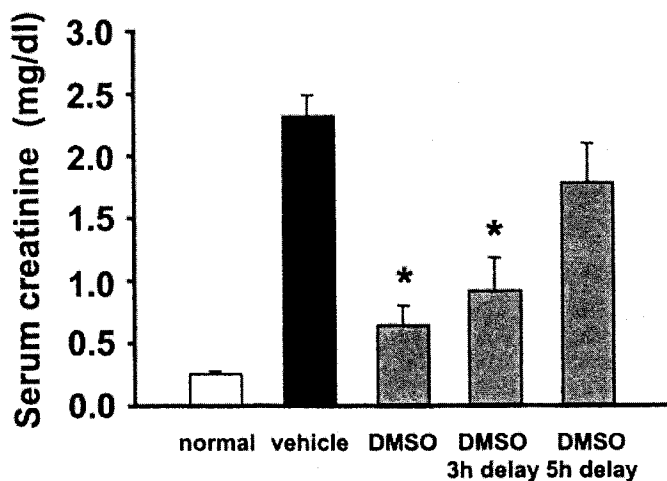


Figure 1. Effect of DMSO on serum creatinine level at 48 h after HgCl₂ injection. Mice received a subcutaneous injection of 5 mg/kg HgCl₂ and vehicle (PBS; ■) or DMSO (3.8 ml/kg, 40% in PBS; ▨) by intraperitoneal injection at 0, 3, and 5 h after HgCl₂, and an additional dose of DMSO was given 24 h later. □, normal mice; n = 10 animals/group.

DMSO-treated mice (vehicle, 3.7 ± 0.0 , versus DMSO, 2.1 ± 0.1 ; $P < 0.05$; Figure 2).

Delayed DMSO Administration

To investigate the therapeutic window of DMSO treatment for HgCl₂-induced renal injury, we examined the effect of delayed DMSO treatment 3 or 5 h after HgCl₂ administration. DMSO given 3 h after HgCl₂ attenuated renal damage (creatinine, 0.7 ± 0.2 mg/dl; tubular damage score, 2.2 ± 0.1 ; both $P < 0.05$ versus vehicle), but 5-h delayed DMSO treatment had no significant effect (creatinine, 2.0 ± 0.1 mg/dl; tubular damage score, 2.9 ± 0.2 ; Figures 1 through 3). In a separate experiment, we found that lower doses of DMSO (1.9 and 0.95 ml/kg) administered with a 3-h delay also had significantly lower serum creatinine values (vehicle, 3.3 ± 0.2 , 1.9 ml/kg, 1.4 ± 0.3 , $P < 0.05$ versus vehicle; 0.95 ml/kg, 1.2 ± 0.2 mg/dl, $P < 0.05$ versus vehicle).

Effect of DMSO on Oxidative Stress

Previous studies have shown that oxidative stress plays a key role in HgCl₂-induced renal injury (8). Therefore, the effect of DMSO on various parameters of oxidative stress was investigated. HgCl₂ induced depletion of renal GSH content at 24 and 48 h after injection (Figure 4). In contrast, DMSO treatment significantly increased the intracellular GSH level at both time points (normal, 0.46 ± 0.07 , versus HgCl₂ + vehicle 0.28 ± 0.02 , versus HgCl₂ + DMSO 0.83 ± 0.02 at 24 h; HgCl₂ + vehicle 0.29 ± 0.01 , versus HgCl₂ + DMSO 0.73 ± 0.02 $\mu\text{mol/g}$ tissue at 48 h).

GPx activity also decreased at 48 h after HgCl₂ injection, and this decrease was partially reversed in DMSO-treated animals (Figure 5). The difference between groups was not significantly different at 24 h (normal, 700 ± 154 , versus

HgCl₂ + vehicle, 232 ± 19 , versus HgCl₂ + DMSO, 379 ± 59 U/mg protein) but was significantly different at 48 h (HgCl₂ + vehicle, 179 ± 29 , versus HgCl₂ + DMSO, 357 ± 7 U/mg protein).

Total SOD activity was also markedly decreased at 24 h after mercuric chloride injection, and DMSO treatment restored SOD activity at 24 h (normal, 0.67 ± 0.02 , versus HgCl₂ + vehicle, 0.3 ± 0.06 , versus HgCl₂ + DMSO, 0.72 ± 0.07 U/mg protein at 24 h). At 48 h, SOD activity was not significantly different from normal (Figure 6).

Renal catalase activity was not different at any time point after HgCl₂ injection, and DMSO had no effect on its level (normal, 50.2 ± 3.6 , versus HgCl₂ + vehicle, 47.0 ± 4.1 , versus HgCl₂ + DMSO, 60.3 ± 10.0 at 24 h; HgCl₂ + vehicle, 47.9 ± 8.5 , versus HgCl₂ + DMSO, 61.2 ± 8.1 U/mg protein; Figure 7).

²⁰³Hg Uptake in MDCK Cells

A previous study by Paller (2) suggested that the beneficial effect of DMSO in mercuric chloride-induced ARF could be mediated through its ability to complex with Hg²⁺, thus protecting protein sulfhydryl groups. Several metal complexing agents, such as DMPS, DMSA, and the low molecular weight thiol NAC, inhibit injury and are thought to additionally inhibit the cellular uptake of mercuric ion and enhance urinary excretion (19,20). To determine whether DMSO complexes with Hg²⁺ and thus prevents its uptake into tubular cells, we measured the uptake of ²⁰³Hg²⁺ *in vitro* using hOAT1-transfected MDCK cells. Co-incubation of ²⁰³Hg²⁺ with 20 μM NAC markedly decreased Hg²⁺ uptake to 5.8% of control (Figure 8). In contrast, co-incubation with 1% DMSO did not affect significantly Hg²⁺ uptake, indicating that DMSO did not have an effect on the uptake of Hg²⁺ into tubular cells. (Hg²⁺, 100, versus Hg²⁺ + DMSO, 87 ± 5 , versus Hg²⁺ + NAC, $5.8 \pm 0.02\%$).

Discussion

This study demonstrates that late administration of DMSO inhibits nephrotoxic injury caused by HgCl₂ even when DMSO is given 3 h after HgCl₂. Whereas HgCl₂ decreases the levels of GSH, GPx, and SOD, DMSO restores the level of GPx and SOD toward normal and results in supranormal levels of GSH. DMSO has no effect on Hg entry into cultured renal tubule cells.

Protection and Window of Opportunity

Renal damage was assessed by both changes in creatinine and renal histology. DMSO dramatically decreases the rise in serum creatinine and also decreases the histologic damage (Figures 1 through 3). This protection is more effective than seen in previous studies. Paller (2) saw only a 60% protection in serum creatinine, despite giving DMSO 10 min before HgCl₂. As all previous studies had given DMSO either before or at the same time as HgCl₂, it was surprising to find a 3-h window of opportunity for DMSO to attenuate renal injury (Figures 1 through 3). Another antioxidant, melatonin, works only if administered 30 min before HgCl₂; administration at the

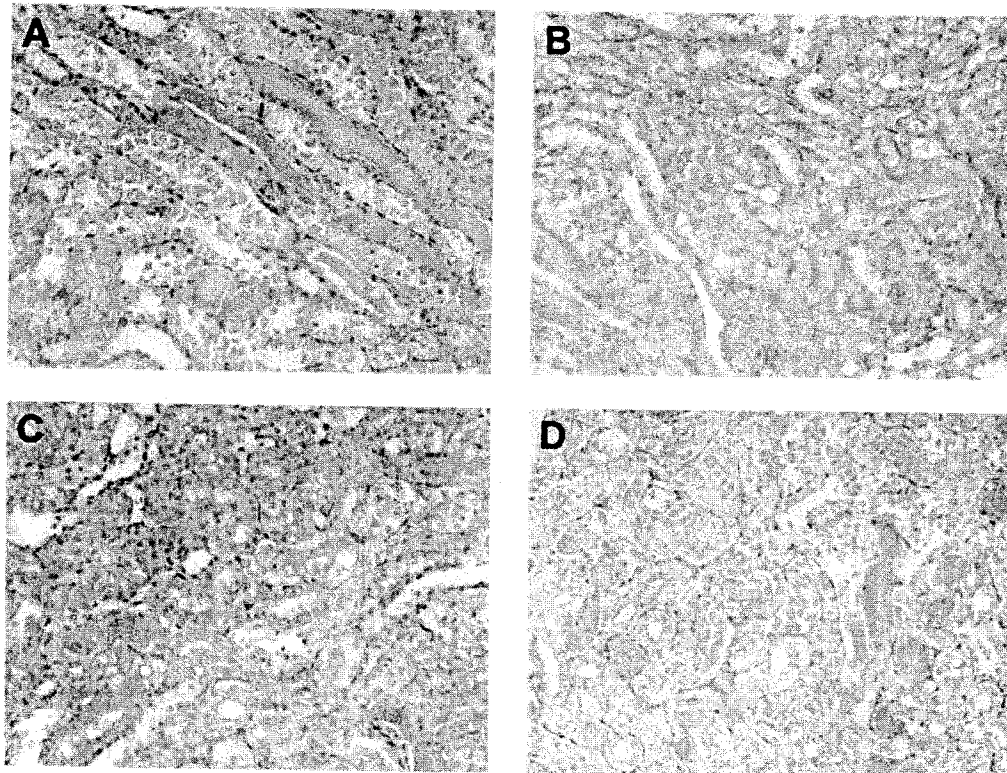


Figure 2. Renal histology 48 h after HgCl₂ administration. Mice received a subcutaneous injection of 5 mg/kg HgCl₂ and an intraperitoneal injection of vehicle (A) or DMSO at 0 (B), 3 (C), or 5 h (D) after HgCl₂ injection. An additional dose of DMSO was given at 24 h.

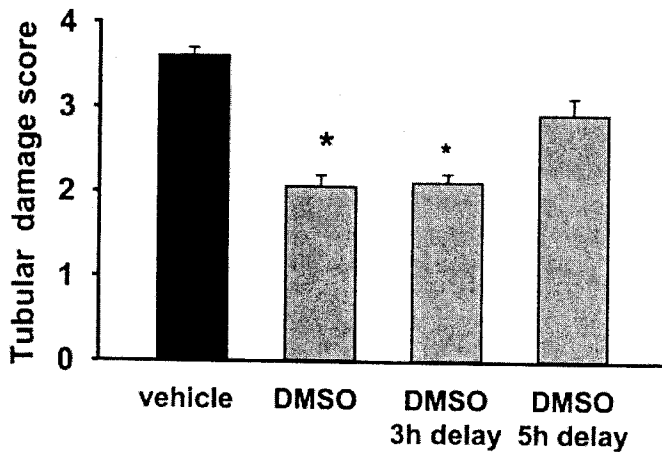


Figure 3. Effect of DMSO on semiquantitative renal histology at 48 h after HgCl₂ injection. Mice received a subcutaneous injection of 5 mg/kg HgCl₂ and vehicle (■) or DMSO (▨). Data are mean scores that correspond to the percentage of damaged tubules in eight high-power fields per section (see Figure 2; Materials and Methods). **P* < 0.05 versus vehicle; *n* = 7 to 8 animals/group.

time or 30 min after Hg is ineffective (8). The results with DMSO are particularly striking because mercury enters cells rapidly after administration (24) and renal damage can be detected early. Therefore, when given 3 h after HgCl₂ injection, DMSO may be acting after the initiation of renal cellular injury.

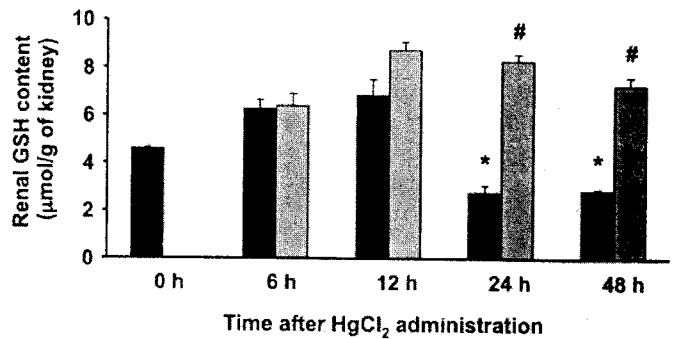


Figure 4. Effect of DMSO on renal GSH content. Mice received a subcutaneous injection of 5 mg/kg HgCl₂ and vehicle (■) or DMSO (▨) intraperitoneally. Mice were killed at 0, 6, 24, or 48 h. Data are mean ± SEM. **P* < 0.05 versus normal; #*P* < 0.05 versus vehicle; *n* = 4 animals/group.

Binding and Sequestration of HgCl₂

Some therapeutics, such as spironolactone, have been shown to bind HgCl₂ in the circulation. The resulting stable complex is secreted by the liver into the bile, thus bypassing the kidney (4). Liver secretion of Hg is further enhanced by the ability of spironolactone to induce drug-metabolizing enzymes. Other agents, such as NAc and DMSA, also bind Hg and increase urinary excretion. However, this study demonstrates that delayed DMSO is still effective (Figures 1 through 3) and that DMSO had no effect on Hg uptake into renal tubule cells

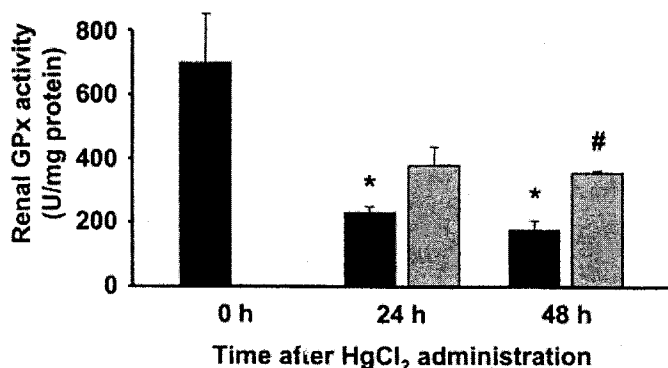


Figure 5. Effect of DMSO on renal glutathione peroxidase (GPx) activity. Mice received a subcutaneous injection of 5 mg/kg HgCl₂ and vehicle (■) or DMSO (▨). Mice were killed at 24 and 48 h. Data are displayed as in Figure 4; *n* = 4 animals/group.

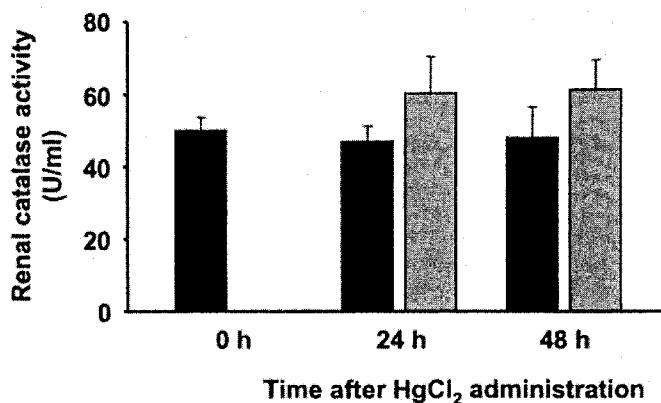


Figure 7. Effect of DMSO on renal catalase activity. Mice received a subcutaneous injection of 5 mg/kg HgCl₂ and vehicle (■) or DMSO (▨). Mice were killed at 24 and 48 h. Data are displayed as in Figure 4; *n* = 3 animals/group.

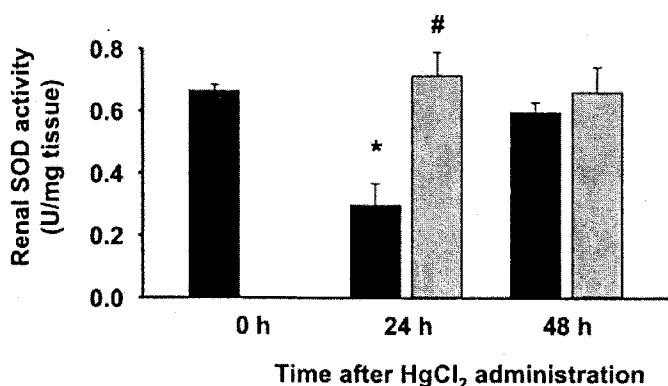


Figure 6. Effect of DMSO on renal superoxide dismutase (SOD) activity. Mice received a subcutaneous injection of 5 mg/kg HgCl₂ and vehicle (■) or DMSO (▨). Mice were killed at 24 and 48 h. Data are displayed as in Figure 4; *n* = 3 animals/group.

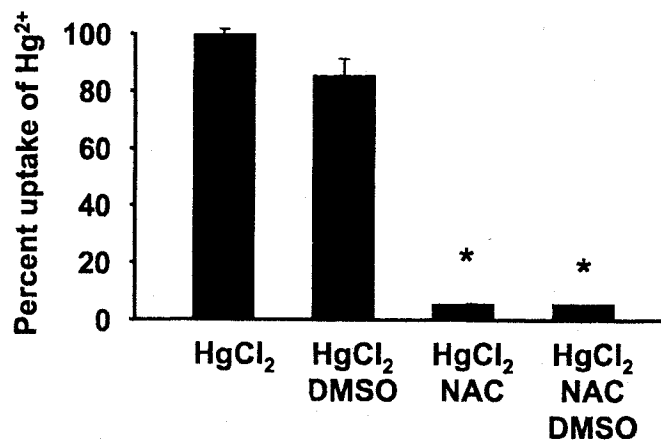


Figure 8. Effect of DMSO and N-acetylcysteine (NAC) on the uptake of ²⁰³Hg²⁺ in hOAT1-transfected MDCK cells. Cells were treated in transport buffer that contained 5 μM ²⁰³Hg²⁺ alone (control), with 1% DMSO or 20 μM NAC. After 1 h, cells were washed and radioactivity was measured. Data were expressed as the percentage of ²⁰³Hg²⁺ uptake compared with control. **P* < 0.01 versus normal; *n* = 3 replicates/group.

(Figure 8). In contrast, NAc reduced the Hg uptake into renal tubule cells, as described previously (21). These data suggest that DMSO does not act *via* blocking HgCl₂ entry into cells, although we cannot eliminate the possibility that HgCl₂ could enter proximal tubule cells *in vivo* through a non-OAT1 transporter. Once HgCl₂ has entered cells, it is still possible that DMSO could bind to unreacted HgCl₂ intracellularly.

Mechanism of Action of DMSO

DMSO has a potentially complex mechanism of action as it has anti-ischemic, anti-inflammatory, and antioxidant properties (16). For example, DMSO prevents induction of inflammatory cascades (*e.g.*, activation of NF-κB), adhesion molecules (*e.g.*, intracellular adhesion molecule-1), and cytokines (*e.g.*, IL-8). Previous studies of anti-inflammatory drugs (*e.g.*, α-MSH, IL-10) failed to confer renal protection in animal models of mercury injury. This suggests that the mechanism of action of DMSO may be due to its antioxidant properties.

GSH represents an important cellular defense system against oxidative stress. Gstraunthaler *et al.* (25) showed that mercury caused oxidative damage in renal proximal tubule cells, which

they demonstrated by a decrease in GSH content. The pathophysiologic importance of reductions in GSH was demonstrated by Girardi and Elias (26), who showed that modulation of tissue nonprotein sulfhydryl levels and GSH paralleled renal injury. Thus, reduction of GSH levels with diethylmaleate increased renal injury, whereas increasing GSH levels with NAc decreased renal injury. The data presented in this article confirm that HgCl₂ decreases GSH levels and demonstrate that DMSO prevents the decrease in GSH levels (Figure 4).

The effect of DMSO on GPx was also studied, because this enzyme consumes free peroxides in the cell by oxidizing GSH. Some studies have demonstrated that HgCl₂ inhibits the activity of renal GPx *in vivo* in a selenium-dependent manner (27,28). We found that HgCl₂ decreased GPx levels, although the decrease was statistically significant only at 48 h (Figure 5). DMSO administration restored GPx levels toward normal.

Reactive oxygen species can be destroyed by SOD and catalase. Twenty-four hours after administration, HgCl₂ administration significantly decreased (~50%) kidney SOD activity, which was completely restored to normal by DMSO (Figure 6). Thus, DMSO reverses the harmful effects of HgCl₂ on SOD, one of the major pathways for ROS destruction. Because approximately three fourths of the SOD activity in the mouse kidney is Cu,Zn SOD (29), this isoform accounts for at least part of the inhibition by HgCl₂. Mn SOD activity is also inhibited in mice that receive an injection of HgCl₂, which may act directly on the enzyme (30). Although HgCl₂ decreases SOD activity after 24 h, by 48 h the SOD activity returns to normal levels. This restoration of SOD activity may be a result of induction of Mn SOD, which is increased in response to HgCl₂ in the brain by a posttranscriptional and dexamethasone-sensitive mechanism (31). The importance of SOD in HgCl₂-induced renal injury is supported by exogenous SOD treatment, which results in protection from HgCl₂. In contrast, HgCl₂ had no effect on catalase activity (Figure 7), in agreement with the findings of previous studies (32).

The ability of HgCl₂ to impair antioxidant defense, as reflected in decreased GSH, GPx, and SOD, suggests that it causes renal injury *via* free radical damage. It has been suggested that HgCl₂ binds to protein sulfhydryl groups and inhibits enzyme function, leading to oxidative stress in the renal cells (2). A recent study suggested a role for nitric oxide in HgCl₂ nephrotoxicity in rats. The level of mRNA for inducible nitric oxide synthase (iNOS) was increased in the renal cortex and proximal tubule epithelium after HgCl₂ exposure. The selective iNOS inhibitor aminoguanidine modestly reduced proximal tubule injury and the rise in creatinine induced by HgCl₂ (33). Therefore, the oxidative stress induced by HgCl₂ may be due, at least in part, to excess nitric oxide production from iNOS. That the antioxidant DMSO antagonizes the nephrotoxicity induced by HgCl₂ further supports the hypothesis that HgCl₂ is nephrotoxic as a result of free radical generation. DMSO does not affect entry of HgCl₂ into cells *in vitro*, but the possibility that DMSO forms a complex with Hg intracellularly still exists. The formation of the complex may inhibit the ability of Hg to produce cellular toxicity.

In summary, treatment or, more critically, delayed treatment with DMSO markedly decreased inorganic mercury–induced nephrotoxicity with a 3-h window of opportunity, which is much wider than any other reported agent. Because of effects on GSH, GPx, and SOD, the mechanism of action of DMSO could be related to its antioxidant properties.

Acknowledgments

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References

- Star RA: Treatment of acute renal failure. *Kidney Int* 54: 1817–1831, 1998
- Paller MS: Free radical scavengers in mercuric chloride-induced acute renal failure in the rat. *J Lab Clin Med* 105: 459–463, 1985
- Girardi G, Elias MM: Effectiveness of ²⁰³Hg²⁺ alone-acetylcysteine in protecting against mercuric chloride-induced nephrotoxicity. *Toxicology* 67: 155–164, 1991
- Kourounakis PN, Rekkas E: Effect of spironolactone on dimethyl mercury toxicity. A possible molecular mechanism. *Arzneimittelforschung* 44: 1150–1153, 1994
- Kawaida K, Matsumoto K, Shimazu H, Nakamura T: Hepatocyte growth factor prevents acute renal failure and accelerates renal regeneration in mice. *Proc Natl Acad Sci U S A* 91: 4357–4361, 1994
- Yamasaki N, Nagano T, Mori-Kudo I, Tsuchida A, Kawamura T, Seki H, Taiji M, Noguchi H: Hepatocyte growth factor protects functional and histological disorders of HgCl₂(2)-induced acute renal failure mice. *Nephron* 90: 195–205, 2002
- Girardi G, Elias MM: Evidence for renal ischaemia as a cause of mercuric chloride nephrotoxicity. *Arch Toxicol* 69: 603–607, 1995
- Nava M, Romero F, Quiroz Y, Parra G, Bonet L, Rodriguez-Iturbe B: Melatonin attenuates acute renal failure and oxidative stress induced by mercuric chloride in rats. *Am J Physiol Renal Physiol* 279: F910–F918, 2000
- Miyaji T, Hu X, Star RA: Alpha-melanocyte-stimulating hormone and interleukin-10 do not protect the kidney against mercuric chloride-induced injury. *Am J Physiol Renal Physiol* 282: F795–F801, 2002
- Ghielli M, Verstrepen WA, De Greef KE, Helbert MH, Ysebaert DK, Nouwen EJ, De Broe ME: Antibodies to both ICAM-1 and LFA-1 do not protect the kidney against toxic (HgCl₂) injury. *Kidney Int* 58: 1121–1134, 2000
- Paller MS, Hoidal JR, Ferris TF: Oxygen free radicals in ischemic acute renal failure in the rat. *J Clin Invest* 74: 1156–1164, 1984
- Linas SL, Shanley PF, White CW, Parker NP, Repine JE: O₂ metabolite-mediated injury in perfused kidneys is reflected by consumption of DMTU and glutathione. *Am J Physiol* 253: F692–F701, 1987
- Fleck C, Heller J: Age-dependent differences in the effect of ischaemia on the rat kidney: Prevention of the postischaemic damage by different drugs. *Exp Toxicol Pathol* 45: 381–387, 1993
- Miller DM, Lund BO, Woods JS: Reactivity of Hg(II) with superoxide: Evidence for the catalytic dismutation of superoxide by Hg(II). *J Biochem Toxicol* 6: 293–298, 1991
- Mahboob M, Shireen KF, Atkinson A, Khan AT: Lipid peroxidation and antioxidant enzyme activity in different organs of mice exposed to low level of mercury. *J Environ Sci Health B* 36: 687–697, 2001
- Santos NC, Figueira-Coelho J, Martins-Silva J, Saldanha C: Multidisciplinary utilization of dimethyl sulfoxide: Pharmacological, cellular, and molecular aspects. *Biochem Pharmacol* 65: 1035–1041, 2003
- Kedar I, Jacob ET, Bar-Natan N, Ravid M: Dimethyl sulfoxide in acute ischemia of the kidney. *Ann N Y Acad Sci* 411: 131–134, 1983
- Lind RC, Gandolfi AJ: Late dimethyl sulfoxide administration provides a protective action against chemically induced injury in both the liver and the kidney. *Toxicol Appl Pharmacol* 142: 201–207, 1997

19. Aaseth J, Alexander J, Raknerud N: Treatment of mercuric chloride poisoning with dimercaptosuccinic acid and diuretics: Preliminary studies. *J Toxicol Clin Toxicol* 19: 173–186, 1982
20. Zalups RK, Barfuss DW: Participation of mercuric conjugates of cysteine, homocysteine, and N-acetylcysteine in mechanisms involved in the renal tubular uptake of inorganic mercury. *J Am Soc Nephrol* 9: 551–561, 1998
21. Aslamkhan AG, Han YH, Yang XP, Zalups RK, Pritchard JB: Human renal organic anion transporter 1-dependent uptake and toxicity of mercuric-thiol conjugates in Madin-Darby canine kidney cells. *Mol Pharmacol* 63: 590–596, 2003
22. Aslamkhan A, Han YH, Walden R, Sweet DH, Pritchard JB: Stoichiometry of organic anion/dicarboxylate exchange in membrane vesicles from rat renal cortex and hoat1-expressing cells. *Am J Physiol Renal Physiol* 285: F775–F783, 2003
23. Zalups RK, Aslamkhan AG, Ahmad S: Human organic anion transporter 1 mediates cellular uptake of cysteine-S conjugates of inorganic mercury. *Kidney Int* 66: 251–261, 2004
24. Hultman P, Enestrom S: Localization of mercury in the kidney during experimental acute tubular necrosis studied by the cytochemical silver amplification method. *Br J Exp Pathol* 67: 493–503, 1986
25. Gstraunthaler G, Pfaller W, Kotanko P: Glutathione depletion and in vitro lipid peroxidation in mercury or maleate induced acute renal failure. *Biochem Pharmacol* 32: 2969–2972, 1983
26. Girardi G, Elias MM: Effect of different renal glutathione levels on renal mercury disposition and excretion in the rat. *Toxicology* 81: 57–67, 1993
27. Wada O, Yamaguchi N, Ono T, Morimura T, Yokoo Y: [Decrease of activity of glutathione peroxidase in the kidney of mice treated with methyl mercury and the effect of simultaneous administration of selenite]. *Nippon Eiseigaku Zasshi* 30: 60, 1975
28. Nielsen JB, Andersen HR, Andersen O, Starklint H: Dose and time relations in Hg(++)-induced tubular necrosis and regeneration. *Environ Health Perspect* 102[Suppl 3]: 317–320, 1994
29. Tanaka-Kagawa T, Suzuki M, Naganuma A, Yamanaka N, Imura N: Strain difference in sensitivity of mice to renal toxicity of inorganic mercury. *J Pharmacol Exp Ther* 285: 335–341, 1998
30. Shimojo N, Kumagai Y, Nagafune J: Difference between kidney and liver in decreased manganese superoxide dismutase activity caused by exposure of mice to mercuric chloride. *Arch Toxicol* 76: 383–387, 2002
31. Kumagai Y, Mizukado S, Nagafune J, Shinyashiki M, Homma-Takeda S, Shimojo N: Post-transcriptional elevation of mouse brain Mn-SOD protein by mercuric chloride. *Brain Res* 769: 178–182, 1997
32. Nath KA, Croatt AJ, Likely S, Behrens TW, Warden D: Renal oxidant injury and oxidant response induced by mercury. *Kidney Int* 50: 1032–1043, 1996
33. Yanagisawa H, Nodera M, Wada O: Inducible nitric oxide synthase expression in mercury chloride-induced acute tubular necrosis. *Ind Health* 36: 324–330, 1998

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