

# Glutathione Effects on Toxicity and Uptake of Mercuric Chloride and Sodium Arsenite in Rabbit Renal Cortical Slices

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The mechanism of renal uptake of nephrotoxic heavy metals such as HgCl<sub>2</sub> and NaAsO<sub>2</sub> is not clear. The metals are known to react with endogenous sulfhydryls such as glutathione (GSH), so metal-GSH conjugates may be delivered to the kidney. To study this possibility, renal cortical slices from male New Zealand white rabbits were incubated with 10<sup>-4</sup> M HgCl<sub>2</sub> or 10<sup>-3</sup> M NaAsO<sub>2</sub> ± stoichiometric amounts (1-3x) of GSH; or synthetic metal-GSH conjugates [10<sup>-4</sup> M Hg(SG)<sub>2</sub> or 10<sup>-3</sup> M As(SG)<sub>3</sub>]. Incubations were performed at 37°C in DME-F12 buffer (95/5 O<sub>2</sub>/CO<sub>2</sub>) for 8 hr. Hg(SG)<sub>2</sub> reduced slice K<sup>+</sup>/DNA content, as an indicator of viability, significantly less than HgCl<sub>2</sub>. As(SG)<sub>3</sub> exhibited a 2-hr delay in K<sup>+</sup>/DNA content reduction compared to NaAsO<sub>2</sub>. This delay in toxicity was not correlated to changes in uptake. Arsenic and mercury accumulation, determined by proton-induced X-ray emission, were also identical between the metal salts and the metal-GSH conjugates. Exogenous GSH decreased HgCl<sub>2</sub> cytotoxicity and was correlated to a decrease in Hg accumulation in the slice. Exogenous GSH had limited if any protective effects against cytotoxicity by NaAsO<sub>2</sub> and a decrease in As accumulation was not observed. Complex metal-GSH interactions appear to exist and impact on the uptake and toxicity of these metals. — Environ Health Perspect 103(Suppl 1):81-84 (1995)

Key words: nephrotoxicity, glutathione, mercuric chloride, arsenite, tissue slices, metals, metal toxicity, metal accumulation

## Introduction

Heavy metals have become one of many contaminants found in our environment. Many of these metals, including HgCl<sub>2</sub> and NaAsO<sub>2</sub>, are known nephrotoxicants. Several studies have shown that HgCl<sub>2</sub> and NaAsO<sub>2</sub> accumulate in specific zones of the kidney cortex. HgCl<sub>2</sub> first accumulates in the straight proximal tubule (1), while NaAsO<sub>2</sub> initially affects the convoluted proximal tubule (2). A number of mechanisms of uptake have been postulated; however, the actual process is as yet unknown.

Hg(II) and As(III) interact with the various sulfhydryls that exist in the body. Many studies have implicated glutathione (GSH) as one of the sulfhydryls. The degradation of GSH and reabsorption of its amino acid constituents occur along the entire proximal tubule (3). One *in vivo* study demonstrated that depleted hepatic GSH leads to decreased nephrotoxicity and

uptake of HgCl<sub>2</sub> (4,5). In contrast, another study using NaAsO<sub>2</sub> found toxicity and uptake of As was increased with depletion of hepatic GSH (2). Co-administration of GSH with HgCl<sub>2</sub> was found to increase renal Hg content compared to mice treated with HgCl<sub>2</sub> alone (4). The formation of the inorganic mercury-GSH complex has been observed in rats (6). Indications have also been found for the transport of As(III) as a GSH complex (7).

These studies indicate GSH-metal conjugates may be involved in the mechanism of renal uptake and toxicity for HgCl<sub>2</sub> and NaAsO<sub>2</sub> but with different results. To study this possibility, rabbit renal cortical slices were used, since precision-cut renal slices retain the cellular identity of the kidney but allow several concentration and/or time treatments to be studied with one animal (8). The slices were incubated with HgCl<sub>2</sub> or NaAsO<sub>2</sub> ± stoichiometric amounts (1-3x) of GSH or synthetic metal-GSH conjugates.

## Methods and Materials

Renal slices were prepared from male New Zealand white rabbits (1.5-2.0 kg, Myrtle Rabbitry, Myrtle, TN). The kidneys were removed, decapsulated, and cylindrical cores taken along the cortical-papillary axis. The cortex was trimmed from the medullary tissue and was cut with a Krumdieck tissue slicer (Alabama Research

and Development Corp., Munford, AL) (9). The slices obtained were approximately 250 to 275 μm thick. Slices were collected and stored in ice-cold Krebs-Hepes buffer prior to use.

The renal slices were incubated at 37°C in an incubation vessel consisting of 16 teflon cups through which 95/5% O<sub>2</sub>/CO<sub>2</sub> was bubbled (8). Each cup was filled with 20 ml of Dulbecco's modified Eagles/Ham's nutrient mix F-12 media (Sigma Chemical Co., St. Louis, MO) containing 2 mM heptanoic acid (Sigma). Four slices were placed on the stainless steel screen located near the base of the cup. Test solutions were added at the desired concentrations for 8-hr time periods. All buffers, media, and test solutions were gassed with O<sub>2</sub>/CO<sub>2</sub> and pH-adjusted to 7.4.

NaAsO<sub>2</sub>, HgCl<sub>2</sub>, and reduced GSH were obtained from Sigma. As(SG)<sub>3</sub> was synthesized using a procedure by Scott (10). NaAsO<sub>2</sub> (0.085 g, 6.5 × 10<sup>-4</sup> M) and GSH (0.6 g, 1.96 × 10<sup>-3</sup> M) were dissolved in distilled, degassed water and mixed for 5 to 6 hr at room temperature under an N<sub>2</sub> atmosphere. Methanol was added to precipitate the product. The mixture was filtered and the residual water removed under reduced pressure. Hg(SG)<sub>2</sub> was synthesized by a similar method.

As a general indicator of cell viability, slice K<sup>+</sup> was evaluated (11) and normalized

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to DNA content (12). Slices were placed in 500  $\mu$ l deionized water and homogenized. A 400- $\mu$ l aliquot was transferred to a 12  $\times$  75 mm culture tube. Bovine serum albumin (Sigma) was added to help pellet the DNA (50  $\mu$ l, 5 mg/ml). Perchloric acid (30  $\mu$ l, 70%) was added to precipitate the samples. Samples were vortexed and centrifuged (1400g, 4°C, 10 min) before assaying the supernatant for K<sup>+</sup> with a flame photometer (Model CA-51, Perkin-Elmer, Danbury, CT). Acid alcohol (3.65 ml 100% ethanol, 0.10 ml HCl) was added to the pellet. The tubes were capped and shaken horizontally for 30 min, then placed in 4°C cooler for 2 to 4 hr. Samples were centrifuged, the supernatant was discarded, and the pellet allowed to dry overnight. Diaminobenzoic acid (100  $\mu$ l, 30% w/v) was added to each pellet. The tubes were capped and incubated in a water bath (75°C, 35 min). After cooling, 3.50 ml of filtered 1 N HCl was added and fluorescence was measured at 410 nm excitation, 500 nm emission (Hitachi F-2000 Fluorescence Spectrophotometer).

Metal content in the slices was analyzed by proton-induced X-ray emission (PIXE) (13). The renal slices were blotted and cen-

tered on mylar film (6.0  $\mu$ m thick) stretched across XRF sample cups. The slice was held in place by a second mylar film (3.5  $\mu$ m). The mylar was punctured twice so slices could dehydrate prior to analysis. The functional equation of the GUPIX program used for PIXE analysis determined the number of atoms of an element being irradiated in a sample that was converted to nanograms. The value was normalized to square centimeters, as the beam may or may not pass through the sample.

Student's *t*-test was used to determine the significance of any observed differences.

### Results

When the slices were exposed to 10<sup>-4</sup> M HgCl<sub>2</sub>, a marked decrease was noted in K<sup>+</sup> content by 2 hr (Figure 1A). When the slices were co-incubated with GSH, the loss in K<sup>+</sup> content decreased with increasing GSH concentrations. With a 3 $\times$  stoichiometric GSH concentration, K<sup>+</sup> content was not significantly different from controls (Figure 1A). The 3 $\times$  GSH concentration also reduced Hg accumulation to approximately half the accumulation observed with HgCl<sub>2</sub> alone (Figure 1B).

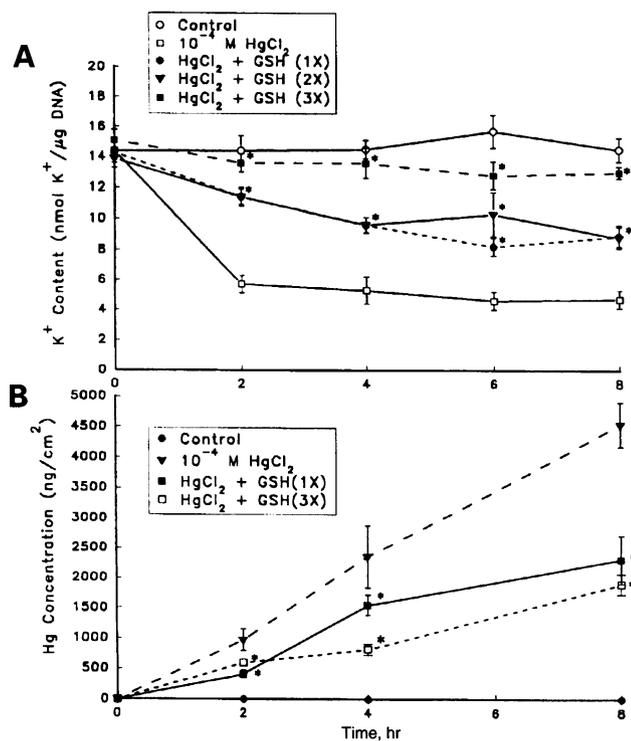
Similar to results obtained using exogenous GSH, the synthetic Hg(SG)<sub>2</sub> did not decrease K<sup>+</sup> content to the extent of that in slices exposed to the same concentration of HgCl<sub>2</sub> (Figure 2A). However, mercury accumulation was found to be similar for HgCl<sub>2</sub> and Hg(SG)<sub>2</sub> (Figure 2B).

Slices exposed to 10<sup>-3</sup> M NaAsO<sub>2</sub> demonstrated a noticeable decline in K<sup>+</sup> content by 2 hr, and continued to decrease with further exposure (Figure 3A). Co-incubation with increasing concentrations of GSH slowed the loss of K<sup>+</sup> from the slices for approximately 2 hr, but by 6 hr there was no difference in K<sup>+</sup> content (Figure 3A). Co-incubation with 1 $\times$  and 3 $\times$  stoichiometric GSH concentrations did not significantly affect As accumulation (Figure 3B).

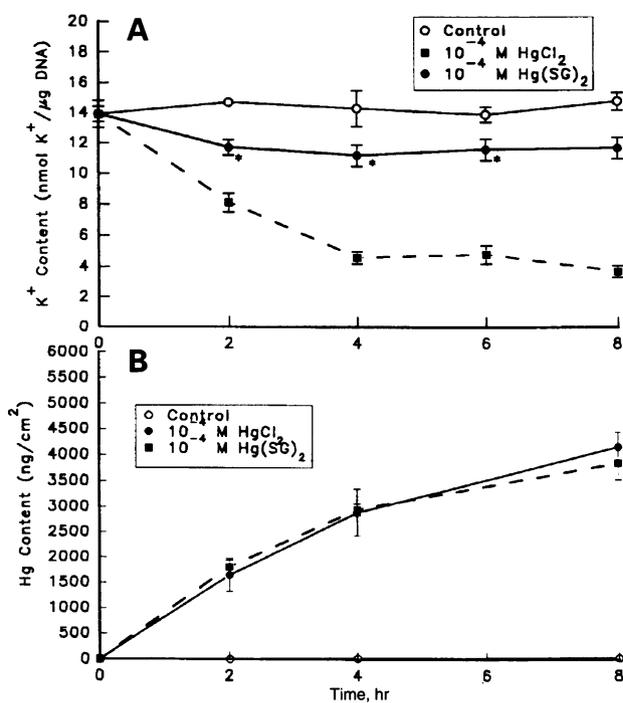
When the slices were incubated with synthetic As(SG)<sub>3</sub>, there was a 2-hr delay before K<sup>+</sup> loss was observed compared to NaAsO<sub>2</sub> (Figure 4A). However, there was no difference in As accumulation between slices exposed to either As compound (Figure 4B).

### Discussion

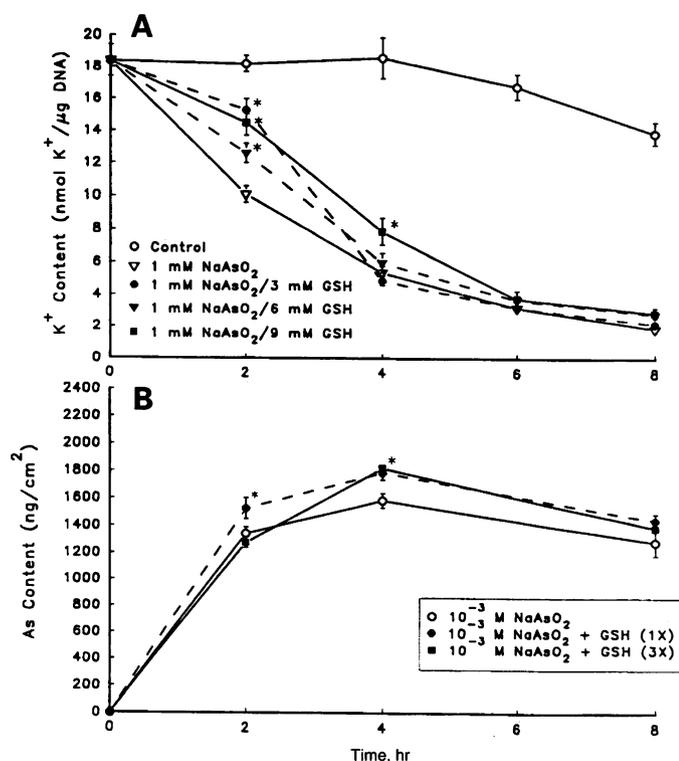
GSH appears to affect HgCl<sub>2</sub> toxicity and uptake. Extracellular GSH was shown to have protective effects against HgCl<sub>2</sub> toxic-



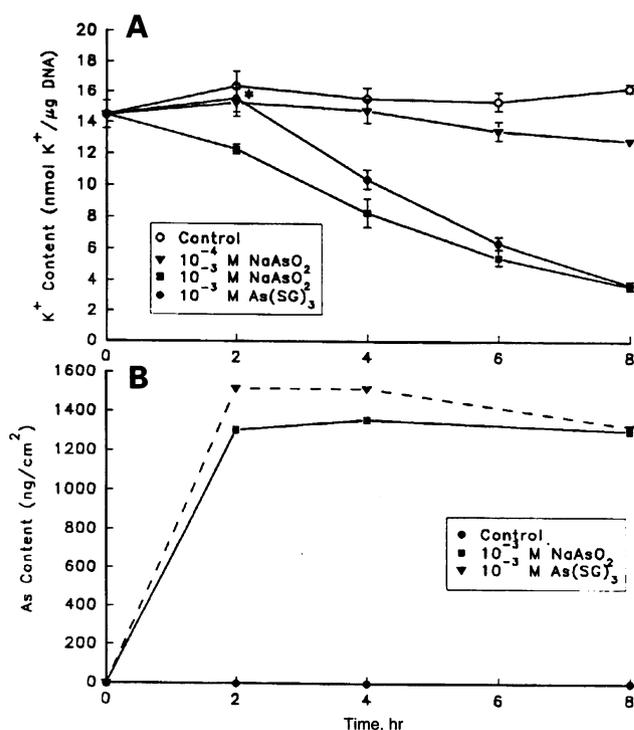
**Figure 1.** Effects of exogenous GSH on HgCl<sub>2</sub> toxicity and uptake in renal cortical slices. Results are shown as mean  $\pm$  SEM, four slices/animal for each treatment and time point, for K<sup>+</sup> content *n*=3, for Hg accumulation *n*=2. Asterisk indicates values that differ from HgCl<sub>2</sub> controls (*p*<0.05).



**Figure 2.** Effect of Hg(SG)<sub>2</sub> on Hg(II) toxicity in renal cortical slices. Results are shown as mean  $\pm$  SEM, four slices/animal for each treatment and time point, *n*=2. Asterisk indicates values that differ from HgCl<sub>2</sub> controls (*p*<0.05).



**Figure 3.** Effect of exogenous GSH on  $\text{NaAsO}_2$  toxicity and uptake in renal cortical slices. Results are shown as mean  $\pm$  SEM, four slices/animal for each treatment and time point,  $n = 2$ . Asterisk indicates values that differ significantly from  $\text{NaAsO}_2$  controls ( $p < 0.05$ ).



**Figure 4.** Effect of  $\text{As(SG)}_3$  on toxicity and uptake of  $\text{As(III)}$  in renal cortical slices. Results are shown as mean  $\pm$  SEM, four slices/animal for each treatment and time point,  $n = 2$ . Asterisk indicates values that differ significantly from  $\text{NaAsO}_2$  controls ( $p < 0.05$ ).

city. As the concentration of GSH in the media was increased, the toxicity of  $\text{HgCl}_2$  was decreased, as demonstrated by the lack of slice  $\text{K}^+$  depletion (Figure 1). The lack of toxicity may be due to inhibition of Hg uptake as a significant decrease in Hg accumulation was observed (Figure 1). This decrease in toxicity and uptake by exogenous GSH has been demonstrated for  $\text{CdCl}_2$ , another divalent metal, in two different cell cultures (14,15) as well as for  $\text{HgCl}_2$  in proximal tubule suspensions (16).

It was postulated that Cd-GSH complexes were the mechanism of uptake inhibition and decreased toxicity (14,15). This study partially supports this postulate. Synthetic  $\text{Hg(SG)}_2$  appears to decrease the toxicity of HG, as observed by the decrease in  $\text{K}^+$  loss (Figure 2). However,  $\text{Hg(SG)}_2$  does not appear to inhibit HG uptake, as metal accumulation in the slice was equivalent whether Hg was introduced as a salt or as the conjugate (Figure 2). This suggests Hg may be taken up by the cell as part of a GSH moiety, specifically the amino acid cysteine. As  $\text{Hg(SG)}_2$  has a high constant of formation (17),  $\text{Hg(SG)}_2$  could be formed in the media. Thus, the excess GSH or cysteine present in the media, not

complexed to Hg, could produce a competitive inhibition for Hg uptake. This mechanism of uptake is supported by *in vivo* studies done by Tanaka et al. (4), Berndt et al. (18), and Ballatori and Clarkson (6). Ballatori and Clarkson showed that Hg-GSH complexes were excreted by the liver, so  $\text{Hg(SG)}_2$  may be one form of Hg(II) delivered to the kidney. Tanaka et al. that demonstrated pretreatment with 1,2-dichloro-4-nitrobenzene depletes hepatic GSH, hence fewer Hg-GSH complexes formed and renal Hg accumulation decreased. Both Tanaka and Berndt demonstrated inhibition of GSH degradation in the proximal tubule, which decreased renal GSH uptake and also decreased renal Hg content.

Unlike for  $\text{HgCl}_2$ , exogenous GSH did not exhibit protective effects against  $\text{NaAsO}_2$  (Figure 3). This is further supported by little change in As accumulation (Figure 3), which suggests extracellular GSH does not affect the uptake mechanism for  $\text{NaAsO}_2$ .

When As is introduced as  $\text{As(SG)}_3$ , there is again no significant change in As accumulation; however, there is approximately a 2-hr delay before  $\text{K}^+$  depletion is

observed (Figure 4). Since  $\text{As(SG)}_3$  is fairly stable at  $\text{pH} < 9.0$  (19), this implies As can be taken up in different species but that  $\text{As(SG)}_3$  needs to go through additional changes to manifest its toxic effects. This change could simply be dissociation from the GSH moiety or a more complex metabolism process.

While GSH seems to be highly involved in  $\text{HgCl}_2$  uptake and toxicity, its specific role is still unclear. In  $\text{As(III)}$  toxicity and uptake, GSH involvement is uncertain. While GSH appears to be necessary for As methylation to prevent toxicity once  $\text{As(III)}$  is in the cell, its role, if any, in uptake has yet to be determined (2).

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