Multidrug Resistance Proteins and the Renal Elimination of Inorganic Mercury Mediated by 2,3-Dimercaptopropane-1-Sulfonic Acid and Meso-2,3-dimercaptosuccinic Acid

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Abstract

Current therapies for inorganic mercury (Hg^{2+}) intoxication include administration of a metal chelator, either 2,3-dimercaptopropane-1-sulfonic acid (DMPS) or meso-2,3-dimercaptosuccinic acid (DMSA). After exposure to either chelator, Hg^{2+} is rapidly eliminated from the kidneys and excreted in the urine, presumably as an *S*-conjugate of DMPS or DMSA. The multidrug resistance protein 2 (Mrp2) has been implicated in this process. We hypothesize that Mrp2 mediates the secretion of DMPS- or DMSA-*S*-conjugates of Hg^{2+} from proximal tubular cells. To test this hypothesis, the disposition of Hg^{2+} was examined in control and Mrp2-deficient TR⁻ rats. Rats were injected i.v. with 0.5 μmol/kg $HgCl_2$ containing $^{203}Hg^{2+}$. Twenty-four and 28 h later, rats were injected with saline, DMPS, or DMSA. Tissues were harvested 48 h after $HgCl_2$ exposure. The renal and hepatic burden of Hg^{2+} in the saline-injected TR⁻ rats was greater than that of controls. In contrast, the amount of Hg^{2+} excreted in urine and feces of TR⁻ rats was less than that of controls. DMPS, but not DMSA, significantly reduced the renal and hepatic content of Hg^{2+} in both groups of rats, with the greatest reduction in controls. A significant increase in urinary and fecal excretion of Hg^{2+}, which was greater in the controls, was also observed following DMPS treatment. Experiments utilizing inside-out membrane vesicles expressing MRP2 support these observations by demonstrating that DMPS- and DMSA-*S*-conjugates of Hg^{2+} are transportable substrates of MRP2. Collectively, these data support a role for Mrp2 in the DMPS- and DMSA-mediated elimination of Hg^{2+} from the kidney.

2,3-Dimercaptopropane-1-sulfonic acid (DMPS) and meso-2,3-dimercaptosuccinic acid (DMSA) are dithiol metal chelators that have been demonstrated to reduce significantly and efficiently the body burden of mercury (Planas-Bohne, 1981; Aposhian, 1983; Aposhian et al., 1992; Zalups et al., 1992; Ruprecht, 1997). These chelators act mainly by promoting the unidirectional extraction of mercuric ions from within renal proximal tubular epithelial cells into the luminal compartment of the nephron. This extraction provides an effective route for the elimination of mercuric ions in the urine. Unlike other chelators, DMPS and DMSA are highly water soluble and are relatively nontoxic, which has contributed to their use as antidotes for mercury poisoning throughout the world. Surprisingly, only DMSA has been approved for use in humans by the Food and Drug Administration of the United States. Because of structural and chemical differences between DMPS and DMSA and their varied use throughout the world, it is important to understand the effect of each of these chelators on the disposition and handling of the various species of mercury.

After exposure to inorganic mercury (Hg^{2+}), mercuric ions accumulate primarily in the renal proximal tubule (Zalups, 2000). Hg^{2+} gains access to proximal tubular cells primarily via amino

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acid transporters in the luminal plasma membrane and the organic anion transporter 1 in the basolateral plasma membrane (Zalups, 2000; Bridges and Zalups, 2005). The extraction of Hg^{2+} from proximal tubular cells via DMPS or DMSA appears to be unidirectional, where mercuric ions are moved from the intracellular compartment into the tubular lumen. Indeed, treatment of humans and rats with DMPS or DMSA has been shown to reduce the amount of Hg^{2+} within the kidneys and increase the urinary excretion of Hg^{2+} (Planas-Bohne, 1981; Aposhian, 1983; Aposhian and Aposhian, 1990; Aposhian et al., 1992; Zalups, 1993; Zalups et al., 1998). The extraction of Hg^{2+} from renal tubular cells does not appear to involve glomerular filtration but rather a direct secretory process whereby Hg^{2+} moves from peritubular blood into the tubular lumen (Diamond et al., 1988; Zalups et al., 1998). It is well documented that reduced and oxidized forms of DMPS (but not DMPS *S*-conjugates of Hg^{2+}) are taken up by the organic anion transporter 1 in the basolateral membrane of proximal tubular cells (Zalups et al., 1998; Islinger et al., 2001; Bahn et al., 2002). DMSA, however, appears to be taken up by the sodium-dependent dicarboxylate transporter, which is also localized in the basolateral membrane of these cells (Burckhardt et al., 2002).

Given the current body of scientific knowledge, we hypothesize that after being taken up into cells, DMPS and DMSA bond to intracellular Hg^{2+}, creating DMPS *S*- or DMSA *S*-conjugates of Hg^{2+}. These conjugates are then exported across the luminal plasma membrane into the tubular lumen for elimination in the urine. Although it is well known that treatment with DMPS and/or DMSA facilitates a profound decrease in the renal burden of Hg^{2+} (Aposhian, 1983), the cellular mechanisms involved in this process have not been defined fully.

Because of data from rats implicating the multidrug resistance protein (Mrp) in the hepatobiliary transport of Hg^{2+} (Sugawara et al., 1998), we suggest that this carrier may also be involved in the export of DMPS *S*- and DMSA *S*-conjugates of Hg^{2+} from proximal tubular cells. Mrp2 is a member of the ATP-binding cassette transporter superfamily and is localized in the luminal membrane of proximal tubular cells (Schaub et al., 1997). This transporter has been hypothesized to play a role in the excretion of a number of metabolic wastes and xenobiotics (Leslie et al., 2005). The activity of human MRP2 appears to be dependent upon the cotransport of glutathione for the secretion of certain compounds (Leslie et al., 2005). Interestingly, Tanaka-Kagawa et al. (1993) suggest that the secretion of Hg^{2+} from proximal tubular cells also appears to require the cotransport of glutathione. These data suggest that Mrp2 may be responsible for at least part of the proximal tubular elimination of Hg^{2+}.

Given the aforementioned data, we hypothesize that Mrp2 (and possibly additional members of the superfamily of ABC transporters) plays a role in the secretion of DMPS *S*- or DMSA *S*-conjugates of Hg^{2+} from within proximal tubular cells into the tubular lumen. We tested this hypothesis by examining the disposition of Hg^{2+} in Mrp2-deficient rats (TR⁻), before and after treatment with DMPS or DMSA. To our knowledge, the results of the current study provide the first substantive line of evidence indicating that DMPS *S*-conjugates of Hg^{2+} can be transported into the tubular lumen by Mrp2. Perhaps more importantly, our data also provide evidence suggesting that Mrp2 may be the primary transporter involved in the secretion of DMSA *S*-conjugates of Hg^{2+}.

Materials and Methods

Chemicals

DMPS (sodium salt), DMSA, sodium pentobarbital, and $HgCl_2$ were all obtained from Sigma-Aldrich (St. Louis, MO). Radioactive $HgCl_2$ ($^{203}Hg^{2+}$) was produced by irradiation of mercuric oxide at the Missouri University Research Reactor facility as described previously (Belanger et al., 2001; Bridges et al., 2004). The specific activity of the $^{203}Hg^{2+}$ ranged from 6 to 12 mCi/mmol.

Animals

Transport-deficient (TR$^-$) rats possess a mutation in the *mrp2* gene (Mayer et al., 1995; Paulusma et al., 1996); consequently, the Mrp2 protein is absent in the tissues and organs of these animals (Mayer et al., 1995). These rats represent a reliable model that has been used to study the hepatic and renal secretion of various Mrp2 substrates (de Vries et al., 1989; Masereeuw et al., 2003; Smeets et al., 2004).

Male TR$^-$ and normal (control) Wistar rats weighing 200 to 225 g were purchased from Harlan Laboratories (Indianapolis, IN). There were no significant differences between body weights of the animals used for these studies. All animals were provided a commercial laboratory diet (Tekland 6% rat diet; Harlan Laboratories) and water ad libitum throughout all aspects of animal experimentation. Animals were handled in accordance with the Institute of Laboratory Animal Resources (1996).

Intravenous Injections

Control and TR$^-$ rats were injected i.v. according to a previously published protocol (Zalups et al., 1992; Zalups, 1993). Each animal was anesthetized lightly with ether, and a small incision was made in the skin in the midventral region of the thigh to expose the femoral vein and artery. The fascia around the femoral vein was trimmed and a non-nephrotoxic dose of HgCl$_2$ (0.5 μmol/kg in 2 ml of normal saline containing 1 μCi of ^{203}Hg^{2+} per rat) was administered into the vein. The wound was closed using two 9-mm stainless steel wound clips.

Experimental Design

Eight control and eight TR$^-$ rats were injected i.v. with a 0.5 μmol/kg dose of HgCl$_2$ as detailed above. Following the administration of HgCl$_2$, control rats were divided randomly into two groups, with each group consisting of four rats. TR$^-$ rats were also divided randomly into two groups of four rats each. Animals were then placed in individual plastic metabolic cages, in which water and food were provided ad libitum.

Twenty-four hours after the injection of HgCl$_2$, four control rats and four TR$^-$ rats were injected i.p. with a 100 mg/kg (0.39 μmol/kg) dose of DMPS (in 2 ml/kg normal saline). At the same time, another group of four control rats and a group of four TR$^-$ rats were injected i.p. with normal saline (2 ml/kg). Four hours later (28 h after injection of HgCl$_2$), rats were administered a second i.p. dose of DMPS (100 mg/kg) or saline (2 ml/kg). Twenty-four hours after the initial dose of DMPS or saline (48 h after injection with HgCl$_2$), rats were sacrificed by exsanguination, and blood, liver, kidneys, urine, and feces were collected for analysis of Hg^{2+} content. In a separate set of experiments, rats were treated with 100 mg/kg (0.55 μmol/kg) DMSA instead of DMPS. All other experimental details remained the same.

Collection of Tissues, Organs, Urine, and Feces

Forty-eight hours after the initial injection of Hg^{2+}, rats were anesthetized with an i.p. overdose of sodium pentobarbital (100 mg/kg in 2 ml of saline). Two 1-ml samples of blood were obtained from the inferior vena cava with a 3-ml syringe and a 20-gauge needle. One of the samples was placed in a polystyrene tube for determination of ^{203}Hg^{2+} content, whereas the other sample was placed in a Microtainer tube (Becton Dickinson and Co., Franklin Lakes, NJ), which was centrifuged at 21,000g for 90 s. Subsequently, the cellular and plasma fractions were removed and placed in separate polystyrene tubes for estimation of Hg content. Total blood volume was estimated to be 6% of body weight.

The right and left kidneys were also removed from each animal. Each kidney was weighed and cut in half along a transverse plain. A 3-mm transverse slice of the left kidney was utilized for separation of cortex, outer stripe of outer medulla, inner stripe of outer medulla, and inner

medulla. Each zone of the kidney was weighed and placed in a polystyrene tube for estimation of $^{203}Hg^{2+}$ content. The liver was then excised carefully, weighed, and a 1-g section was removed for determination of $^{203}Hg^{2+}$ content.

Urine and feces were collected throughout the duration of each experiment. The urine excreted by each animal was collected 24 and 48 h after injection with $HgCl_2$. Subsequently, the urine from each animal was mixed by vortexing, and a 1-ml sample was weighed and placed in a polystyrene tube for estimation of $^{203}Hg^{2+}$ content. All of the feces excreted by each animal during each 24-h period were counted to determine accurately the total fecal content of $^{203}Hg^{2+}$. The content of $^{203}Hg^{2+}$ in each sample was determined by counting the samples in a Wallac Wizard 3 automatic gamma counter (PerkinElmer Life and Analytical Sciences, Boston, MA).

Membrane Vesicle Transport Assays

Membrane vesicle transport assays were performed using a rapid filtration method as described previously (Cui et al., 1999; Chancy et al., 2000). Inside-out membrane vesicles prepared from either Sf9 cells were transfected with human MRP2 or control SF9 cells obtained from Xenotech (Lenexa, KS). Before use, vesicles were centrifuged at 100,000g for 40 min at 4°C to remove the storage buffer. Vesicles were resuspended in ice-cold incubation buffer (250 mM sucrose, 10 mM Tris/HCl, 10 mM $MgCl_2$, pH 7.4) by passing the suspension through a 27-gauge needle 25 times. Mercuric conjugates were formed by mixing 5 μM $^{203}Hg^{2+}$ with 12.5 μM DMPS or DMSA in incubation buffer supplemented with 10 mM creatine phosphate and 100 μg/ml creatine phosphokinase in the presence or absence of 5 mM ATP. At the time of the experiment, membrane vesicles and mercuric conjugates were warmed to 37°C. Transport was initiated by the addition of 40 μl of membrane vesicle solution (20 μg) to 160 μl of DMPS-*S*-Hg-*S*-DMPS or DMSA-*S*-Hg-*S*-DMSA. Transport was allowed to proceed for 15 s, after which vesicles were collected on prewet HNWP nylon filter discs (pore size, 0.2 μm; Millipore, Billerica, MA). Filters were washed with two changes (8 ml each) of ice-cold incubation buffer containing 1 mM DMPS. Filters were placed in scintillation vials and the radioactivity present was determined by gamma counting in a Wallac gamma counter (PerkinElmer, Waltham, MA).

Data Analyses

Data for each rat experiment were analyzed first with the Kolmogorov-Smirnov test for normality and then with Levene's test for homogeneity of variances. Data were then analyzed using a 2 × 2 two-way analysis of variance to assess differences among the means. When statistically significant *F* values were obtained with analysis of variance, the data were analyzed using Tukey's post hoc multiple comparison test. A *p* value < 0.05 was considered statistically significant.

Data from membrane vesicle assays were analyzed by first calculating ATP-dependent transport. This calculation was performed by subtracting the amount of Hg^{2+} associated with the membrane vesicles in the absence of ATP from that associated with vesicles in the presence of ATP. The resulting data were analyzed by the Student's *t* test. A *p* value < 0.05 was considered statistically significant.

Results

Effect of DMPS on the Renal Burden of Hg^{2+} after Exposure to $HgCl_2$

Figure 1 shows the renal burden of Hg^{2+} in control rats and TR⁻ rats treated with either saline or DMPS 24 and 28 h after the injection of a 0.5 μmol/kg dose of Hg^{2+}. The content of Hg^{2+} in the left kidney of TR⁻ rats injected with saline (approximately 31% of dose) was significantly

greater in than that of corresponding saline-injected controls (approximately 25% of dose). The total renal burden of Hg^{2+} was also greater in saline-injected TR^- rats (approximately 61% of dose) than in matched controls (approximately 48% of dose). Treatment with DMPS caused a significant reduction in the renal content of Hg^{2+} in control and TR^- rats. In controls, treatment with DMPS lowered the content of Hg^{2+} in the total renal mass by approximately 87%, whereas in the TR^- rats, treatment with DMPS lowered the content of Hg^{2+} in the total renal mass by only approximately 38%. Even with these decreases in the renal burden of Hg^{2+}, the content of Hg^{2+} in the total renal mass of TR^- rats remained 6-fold greater than that of corresponding DMPS-treated controls.

The effect of DMPS on the disposition of Hg^{2+} in the four zones of the kidney is shown in Fig. 2. The concentration of Hg^{2+} in the renal cortex was significantly greater in the saline-treated TR^- rats than in the corresponding saline-treated control rats. However, the concentration of mercury in the outer stripe of the outer medulla was significantly greater in the saline-treated control rats than in the saline-treated TR^- rats. In the DMPS-treated control rats, the concentration of Hg^{2+} was profoundly decreased in both the cortex and outer stripe of the outer medulla. Although treatment with DMPS induced significant reductions in the concentration of Hg^{2+} in the renal cortex and outer stripe of the outer medulla in the TR^- rats, the reductions were not as great as that detected in the DMPS-treated control rats.

Nominal amounts of Hg^{2+} were detected in the inner stripe of the outer medulla and inner medulla. Although some differences in the disposition of Hg^{2+} were detected in these two zones among the four groups of rats studied, the differences are viewed as being due to slight differences in the separation of zones at the time of sacrifice.

Effect of DMPS on the Burden of Hg^{2+} in Liver and Blood after Exposure to $HgCl_2$

The total amount of Hg^{2+} in liver of saline-injected and DMPS-treated TR^- rats (approximately 9% of the dose in both groups) was significantly greater than that of corresponding saline-treated and DMPS-treated control rats (approximately 3% of dose in both groups) (Fig. 3). Interestingly, treatment with DMPS had no significant effect on the hepatic burden of Hg^{2+} in both control and TR^- rats.

Hg^{2+} levels in blood were greater in saline-treated and DMPS-treated TR^- rats than in corresponding control rats treated in the same manner. Moreover, administration of DMPS reduced the amount of Hg^{2+} in blood by approximately 30% in both control and TR^- rats (Fig. 3).

Effect of DMPS on the Urinary and Fecal Excretion of Hg^{2+} after Exposure to $HgCl_2$

The amount of Hg^{2+} excreted in the urine after treatment with saline was significantly greater in the control rats than in the corresponding TR^- rats (Fig. 4A). Treatment with DMPS increased the urinary excretion of Hg^{2+} in control rats by approximately 13-fold and in TR^- rats by nearly 40-fold. Nonetheless, the urinary excretion of Hg^{2+} by the TR^- rats, following treatment with DMPS, remained significantly less than that of control rats.

The fecal elimination of Hg^{2+} was also compared in control and TR^- rats (Fig. 4B). The amount of Hg^{2+} excreted into the feces by the control rats treated with saline was approximately 25% greater than that excreted in the feces by the corresponding saline-injected TR^- rats. In control rats, DMPS enhanced the fecal elimination of Hg^{2+} by approximately 2-fold. In contrast, treatment with DMPS did not alter significantly the fecal elimination of Hg^{2+} by TR^- rats.

Effect of DMSA on the Renal Burden of Hg^{2+} after Exposure to $HgCl_2$

Separate groups of control and TR^- rats were treated with saline or DMSA 24 and 28 h after the i.v. injection of $HgCl_2$. The disposition of Hg^{2+} in these animals was examined 48 h after the initial injection of Hg^{2+}. The content of Hg^{2+} in the left kidney was significantly greater in TR^- rats injected with saline (approximately 32% of dose) than in corresponding saline-treated controls (approximately 24% of dose) (Fig. 5). Likewise, the percentage of administered dose of Hg^{2+} in the total renal mass was greater in saline-injected TR^- rats (approximately 66% of dose) than in corresponding saline-injected controls (approximately 48% of dose) (Fig. 5). Treatment with DMSA reduced the total renal content of Hg^{2+} in control rats by approximately 80%. Interestingly, DMSA did not alter the renal burden of Hg^{2+} in the TR^- rats.

The effect of DMSA on the concentration of Hg^{2+} in the various zones of the kidney was also examined (Fig. 6). Significant differences in the concentration of Hg^{2+} were detected among the four groups of rats mainly in the cortex and outer stripe of the outer medulla. The concentration of Hg^{2+} in the renal cortex was significantly greater in both groups of TR^- rats than in the corresponding groups of control animals. In contrast, the concentration of Hg^{2+} in the outer stripe of the outer medulla was significantly less in both groups of TR^- rats than in the corresponding groups of control animals. One of the most striking findings in the renal zones is that treatment with DMSA effectively reduced the concentration of Hg^{2+} in each of the zones of control rats yet had no effect on the concentration of Hg^{2+} in the TR^- rats.

Effect of DMSA on the Burden of Hg^{2+} in Liver and Blood after Exposure to $HgCl_2$

The hepatic burden of Hg^{2+} in the TR^- rats treated with DMSA (approximately 8% of dose) was over 2-fold greater than that of control animals (approximately 3% of dose) (Fig. 7). Interestingly, treatment of animals with DMSA had little effect on the content of Hg^{2+} in the liver of either strain of rat.

When the content of Hg^{2+} in blood was measured, only approximately 1% of the dose was detected in blood of saline-injected TR^- rats. A similar amount of Hg^{2+} was detected in the blood of corresponding saline-treated controls. Treatment with DMSA significantly reduced the amount of Hg^{2+} in blood of control animals but had no affect on that of TR^- rats (Fig. 7).

Effect of DMSA on the Urinary and Fecal Excretion of Hg^{2+} Exposure to $HgCl_2$

Urinary excretion of Hg^{2+} was over 12-fold greater in the control rats treated with DMSA than in the control rats treated with saline (Fig. 8A). Interestingly, there was little difference in the urinary excretion of Hg^{2+} between the two groups of TR^- rats. Moreover, the urinary excretion of Hg^{2+} in the two groups of TR^- rats was significantly less than that in the corresponding group of control rats.

Fecal excretion of Hg^{2+} in the saline-injected control rats (approximately 3.5% of the dose) was greater than that in the corresponding TR^- rats (approximately 1% of the dose) (Fig. 8B). DMSA increased significantly the fecal excretion of Hg^{2+} in control animals to approximately 5% of the dose. In TR^- rats, DMSA did not alter significantly the amount of Hg^{2+} excreted in the feces.

Transport of Hg^{2+} in Inside-out Membrane Vesicles Expressing MRP2

ATP-dependent transport of DMPS-S-Hg-S-DMPS was significantly greater in inside-out membrane vesicles expressing MRP2 than in corresponding control vesicles (Fig. 9). In addition, the ATP-dependent transport of DMSA-S-Hg-S-DMSA was also significantly greater in membrane vesicles expressing MRP2 than in control vesicles (Fig. 9).

Discussion

The purpose of the current study was to test the hypothesis that Mrp2 is involved in the export of DMPS and/or DMSA conjugates of Hg^{2+} from the intracellular compartment of proximal tubular cells into the tubular lumen. More specifically, we hypothesize that following administration of DMPS or DMSA, these chelators are taken up at the basolateral membrane of proximal tubular epithelial cells and form complexes with intracellular Hg^{2+}, which are then exported into the tubular lumen via Mrp2. TR⁻ rats were chosen as a model in which to test this hypothesis because of their lack of a functional Mrp2 (Jansen et al., 1985).

The current data clearly show differences in the disposition of Hg^{2+} between control and TR⁻ rats that were not treated with DMPS or DMSA. In TR⁻ rats injected only with saline, the levels of Hg^{2+} in the total renal mass, liver, and blood were greater than those in saline-treated control rats. Within the kidneys, the greatest amounts of Hg^{2+} were found in the cortex, although significant amounts of Hg^{2+} were also present in the outer stripe of the outer medulla. This pattern of renal disposition of Hg^{2+} is consistent with the zonal localization of the three segments of the proximal tubule in the kidneys. Moreover, this distribution parallels that of Mrp2 in the kidney (Peng et al., 1999; Schaub et al., 1997).

In addition, the amount of Hg^{2+} excreted in the urine and feces was significantly less in TR⁻ rats than in controls. These differences in the handling of Hg^{2+} between saline-injected TR⁻ rats and corresponding control rats suggest that Mrp2 and/or additional transporters altered in the TR⁻ rats are involved in the proximal tubular elimination of Hg^{2+}.

Numerous studies in humans have demonstrated that DMPS and DMSA reduce the body burden of Hg^{2+} rapidly and effectively (Aposhian, 1983). The results of the current study are consistent with these previous findings. DMPS and DMSA each reduced significantly the renal content of Hg^{2+} in control rats. Interestingly, in TR⁻ rats, only DMPS was capable of reducing the renal burden of Hg^{2+}. The percentage by which DMPS reduced the amount of Hg^{2+} in the kidneys of TR⁻ rats (approximately a 38% reduction) was far less than that detected in control rats also treated with DMPS (approximately a 87% reduction). Since DMPS appears to be capable of reducing the renal burden of Hg^{2+} to a limited degree in TR⁻ rats, we suggest that Mrp2 and at least one additional transporter (such as Mrp4, which is also localized in the luminal plasma membrane of proximal tubular epithelial cells; Van Aubel et al., 2002) are involved in the secretion of DMPS S-conjugates of Hg^{2+} into the lumen of the proximal tubule. Our conclusion about the involvement of Mrp2 in the luminal secretion of DMPS S-conjugates of Hg^{2+} is based primarily on the large body of data obtained from TR⁻ rats that have implicated this carrier protein in the luminal secretion of other toxicants and metabolites.

Unlike DMPS, DMSA reduced effectively the renal burden of Hg^{2+} only in control animals (by approximately 82%). It had no effect on the renal disposition of Hg^{2+} in the TR⁻ rats. These data indicate strongly that Mrp2 is the primary mechanism involved in the proximal tubular elimination of Hg^{2+} following treatment with DMSA. However, since a number of other transport proteins have been shown to be altered in TR⁻ rats, we cannot conclude definitively that Mrp2 is the sole mechanism for the secretion of DMSA S-conjugates of Hg^{2+} into the lumen of the proximal tubule. Additional studies are clearly necessary to elucidate fully all the potential mechanisms responsible for the elimination of Hg^{2+} following chelation therapy.

It is clear that following administration of DMPS or DMSA to humans or animals exposed to Hg^{2+}, the urinary excretion of mercuric ions is enhanced. Following treatment with either chelator, Hg^{2+} and the respective chelator have been detected in urine (Maiorino et al., 1991; Ruprecht, 1997). Moreover, data from isolated perfused S2 segments of the rabbit proximal tubule indicate that DMPS can extract mercuric ions that have accumulated in proximal tubular epithelial cells (Zalups et al., 1998). Although it is not clear from the present study as to what

extent the amount of mercuric ions excreted in the urine were bound to DMPS or DMSA molecules, there are data from studies in humans showing that the rate of urinary excretion of Hg^{2+} is similar to that of DMPS (Maiorino et al., 1991). All of these data are consistent with our hypothesis that treatment with either DMPS or DMSA leads to mercuric ions being extracted from proximal tubular epithelial cells as conjugates of DMPS or DMSA, respectively.

DMPS and DMSA have been shown to be present in blood primarily as disulfides, bound to another DMPS or DMSA molecule, respectively, or to another sulfhydryl-containing molecule such as cysteine (Aposhian and Aposhian, 1990; Maiorino et al., 1991). Interestingly, studies in Sperber chickens have shown that DMPS-disulfides are taken up by and reduced within renal tubular cells. Once reduced, these chelators appear to form *S*-conjugates of Hg^{2+} by removing mercuric ions from intracellular ligands by a mechanism involving thiol competition (Stewart and Diamond, 1987, 1988; Klotzbach and Diamond, 1988; Diamond et al., 1988). These findings form an additional line of support for the hypothesis that, in humans, DMPS or DMSA taken up from the blood can form complexes with intracellular Hg^{2+} in renal proximal tubular epithelial cells, which are probable substrates of MRP2. Indeed, the current in vitro data from membrane vesicles demonstrate that DMPS *S*- and DMSA *S*-complexes of Hg^{2+} are transportable substrates of MRP2.

The actual structures of the DMPS *S*- or DMSA *S*-complexes of Hg^{2+} formed in proximal tubular cells are unclear presently. However, several studies have shown that stable DMPS *S*- and DMSA *S*-conjugates of Hg^{2+} are formed by a single mercuric ion bonding to the vicinal thiol groups on a single molecule of DMPS and DMSA, respectively (Rivera et al., 1989; Aposhian and Aposhian, 1990; Maiorino et al., 1991; Aposhian et al., 1995; Ruprecht, 1997). In contrast, George et al. (2004) argue that at least two molecules of DMPS or DMSA and two atoms of Hg^{2+} are required to form respective DMPS *S*- or DMSA *S*-conjugates of Hg^{2+}. Regardless of the exact species of the complexes formed intracellularly, the findings from the current study and those of other studies show clearly that DMPS and DMSA are each capable of effectively reducing the renal and body burden of Hg^{2+} (Planas-Bohne, 1981; Aposhian, 1983; Aposhian et al., 1992; Zalups et al., 1992; Ruprecht, 1997).

In summary, the results of the present study apparently represent the first line of substantive evidence defining a role for Mrp2 in the renal cellular elimination and secretion of Hg^{2+} in rats treated with or without DMPS or DMSA. It should be reiterated that although Mrp2 is a likely participant in the secretion of DMPS *S*- or DMSA *S*-conjugates of Hg^{2+}, one cannot rule out the participation of other mechanisms in this process. Only additional studies can resolve this possibility. Overall, however, the findings from the present study provide significant insights into the mechanisms by which DMPS or DMSA reduce the renal and body burden of Hg^{2+}.

Acknowledgments

ABBREVIATIONS

DMPS, 2,3-dimercaptopropane-1-sulfonic acid; DMSA, meso-2,3-dimercaptosuccinic acid; Mrp, multidrug resistance protein..

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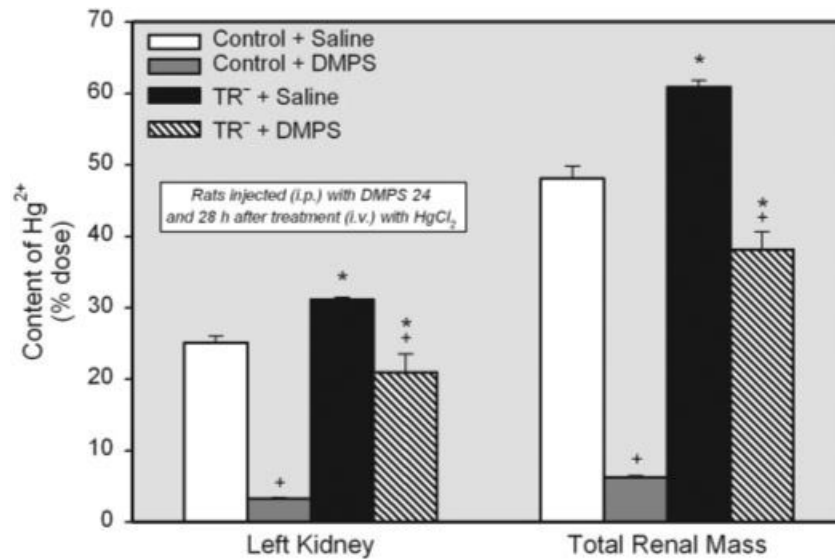
Fig. 1.
Content of Hg^{2+} in the left kidney and total renal mass (percentage of administered dose) of control and TR^- rats injected i.v. with 0.5 μmol/kg $HgCl_2$. Rats were injected i.p. with 100 mg/kg DMPS or 2 ml/kg saline 24 and 28 h after injection of $HgCl_2$. Kidneys were harvested for determination of Hg^{2+} content after 48-h exposure to $HgCl_2$. Data represent mean ± S.E. of four rats. *, significantly different ($p < 0.05$) from the corresponding mean for control rats treated in the same manner.
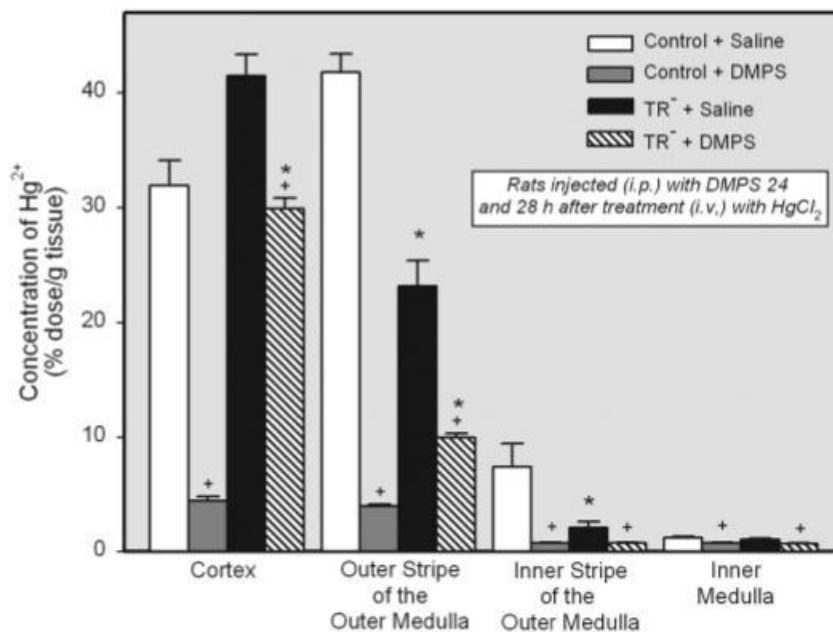
Fig. 2.
Concentration of Hg^{2+} (percentage of administered dose per gram of tissue) in the various zones of kidney of control and TR^- rats injected i.v. with 0.5 μmol/kg $HgCl_2$. Rats were injected i.p. with 100 mg/kg DMPS or 2 ml/kg saline 24 and 28 h after injection of $HgCl_2$. Kidneys were harvested for determination of Hg^{2+} content after 48-h exposure to $HgCl_2$. Data represent mean ± S.E. of four rats. *, significantly different ($p < 0.05$) from the corresponding mean for control rats treated in the same manner. +, significantly different ($p < 0.05$) from the corresponding mean for the same strain of rats treated with saline.
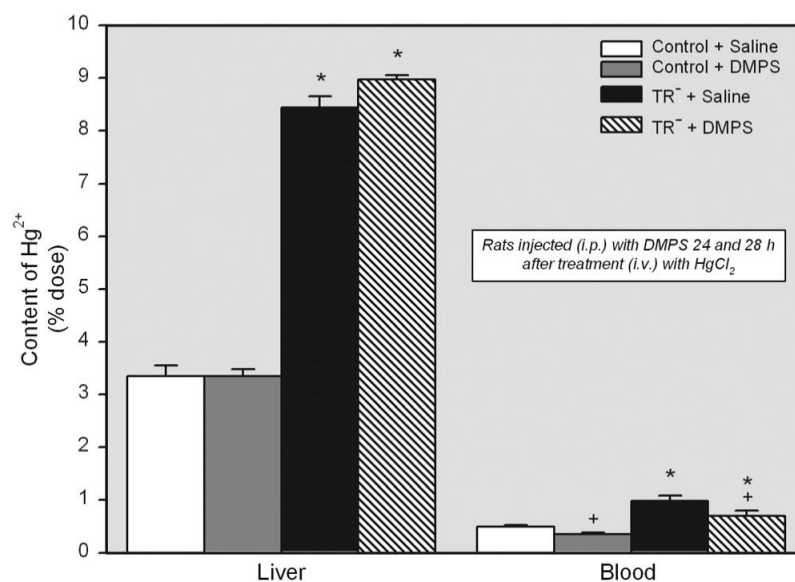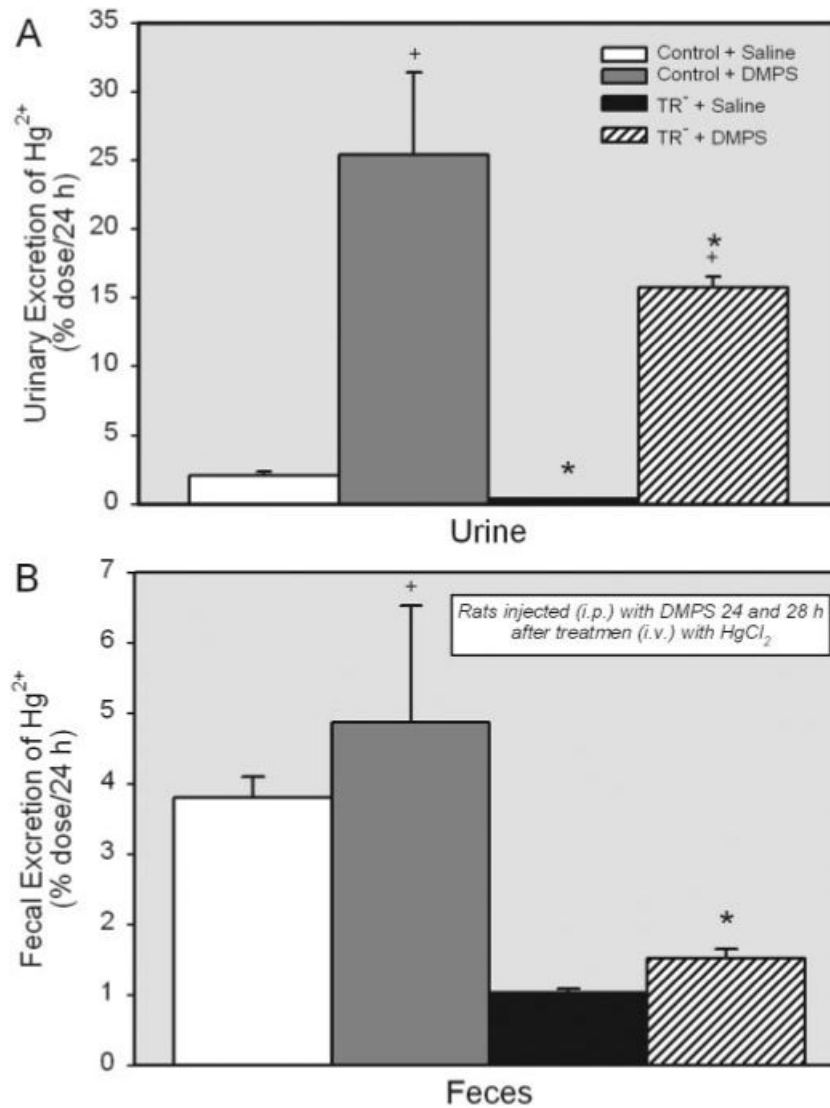
Fig. 3.
Content of Hg^{2+} in liver and blood (percentage of administered dose) of control and TR^- rats injected i.v. with 0.5 μmol/kg $HgCl_2$ followed 24 and 28 h later by treatment i.p. with 100 mg/kg DMPS or 2 ml/kg saline. Livers and blood were harvested 48 h after exposure to $HgCl_2$. Data represent mean ± S.E. of four rats. *, significantly different ($p < 0.05$) from the cor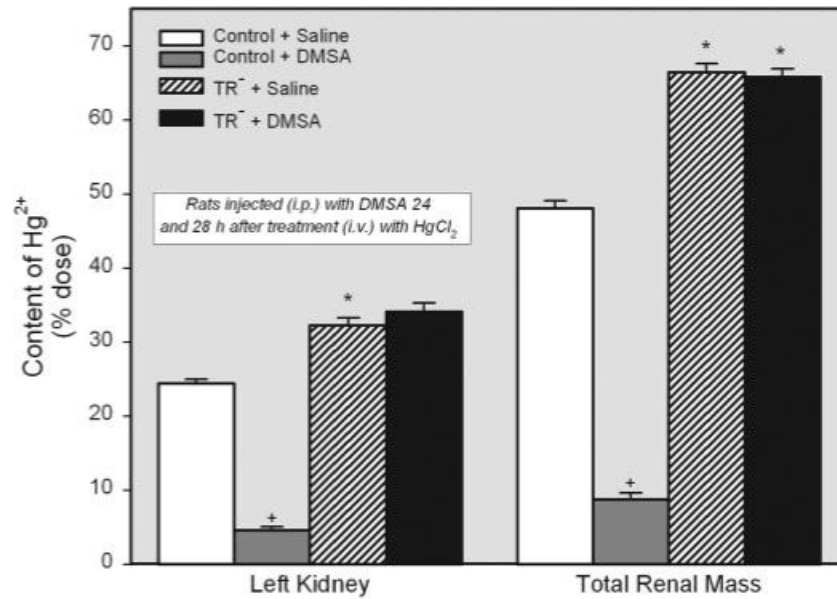responding mean for control rats treated in the same manner. +, significantly different ($p < 0.05$) from the corresponding mean for the same strain of rats treated with saline.

Fig. 4.
Amount of Hg^{2+} excreted in urine and feces (percentage of administered dose) of control and TR^- rats injected i.v. with 0.5 μmol/kg $HgCl_2$, followed 24 and 28 h later by injection with 100 mg/kg DMPS or saline (2 ml/kg). Data represent the amount of Hg^{2+} in urine and feces collected during the 24 h following treatment with DMPS. Data represent mean ± S.E. of four rats. *, significantly different ($p < 0.05$) from the corresponding mean for control rats treated in the same manner.

Fig. 5.
Content of Hg^{2+} (percentage of administered dose) in the left kidney and total renal mass of
control and TR^- rats injected i.v. with 0.5 μmol/kg $HgCl_2$. Rats were injected i.p. with 100
mg/kg DMSA or 2 ml/kg saline 24 and 28 h after injection of $HgCl_2$. Kidneys were harvested
for determination of Hg^{2+} content after 48-h exposure to $HgCl_2$. *, significantly different ($p <$
0.05) from the corresponding mean for control rats treated in the same manner. +, significantly
different ($p < 0.05$) from the corresponding mean for the same strain of rats treated with saline.
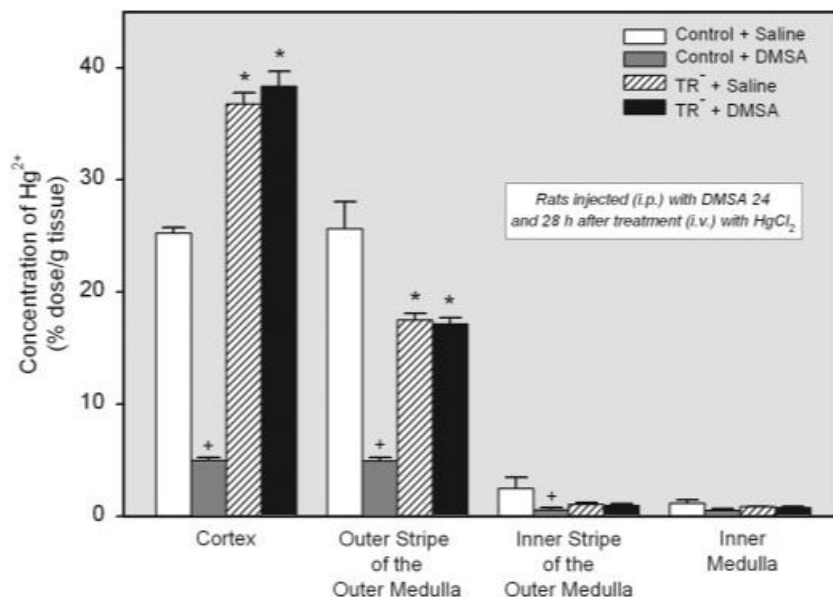
Fig. 6.
Concentration of Hg^{2+} (percent administered dose per gram of tissue) in the various zones of kidneys removed from the same animals. Rats were injected i.p. with 100 mg/kg DMSA or 2 ml/kg saline 24 and 28 h after injection of $HgCl_2$. Kidneys were harvested for determination of Hg^{2+} content after 48-h exposure to $HgCl_2$. *, significantly different ($p < 0.05$) from the corresponding mean for control rats treated in the same manner. +, significantly different ($p < 0.05$) from the corresponding mean for the same strain of rats treated with saline.
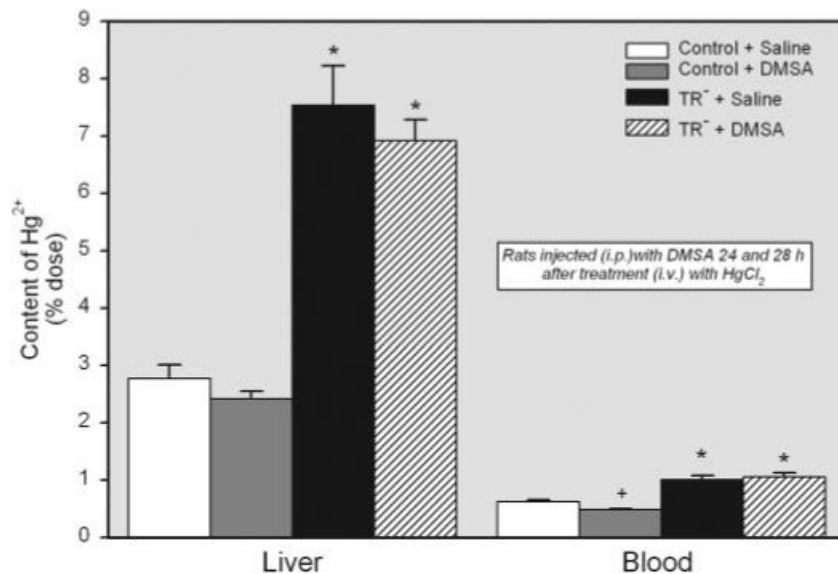
Fig. 7.
Content of Hg^{2+} in the liver and blood (percentage of administered dose) of control and TR^- rats injected i.v. with 0.5 μmol/kg $HgCl_2$ followed 24 and 28 h later by injection i.p. with 100 mg/kg DMSA. Livers and blood were harvested for determination of Hg^{2+} content 48 h after injection with $HgCl_2$. Data represent mean ± S.E. of four rats. *, significantly different ($p < 0.05$) from the corresponding mean for control rats treated in the same manner. +, significantly different ($p < 0.05$) from the corresponding mean for the same strain of rats treated with saline.
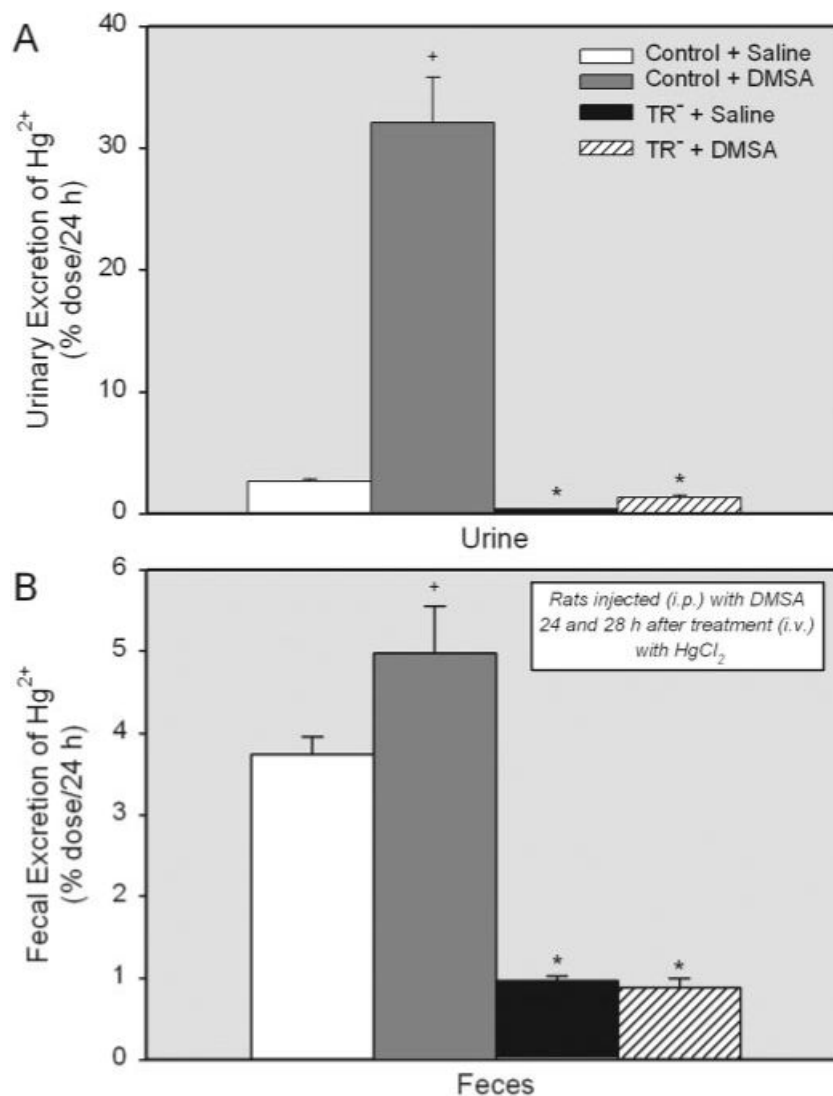
Fig. 8.
Content of Hg^{2+} excreted in urine (A) and feces (B) (percentage of administered dose) from control and TR^- rats injected i.v. with 0.5 µmol/kg $HgCl_2$ followed 24 and 28 h later by injection i.p. with 100 mg/kg DMSA. Data represent the amount of Hg^{2+} in urine and feces collected during the 24 h following treatment with DMSA. Data represent mean ± S.E. of four rats. *, significantly different ($p < 0.05$) from the corresponding mean for control rats treated in the same manner. +, significantly different ($p < 0.05$) from the corresponding mean for the same strain of rats treated with saline.
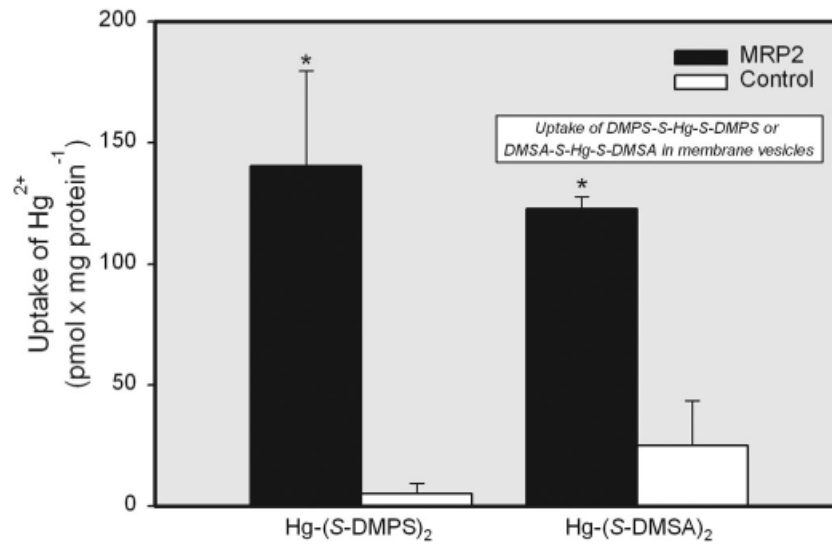
Fig. 9.
Transport of Hg^{2+} into inside-out membrane vesicles expressing MRP2. The transport of 5 μM Hg^{2+}, conjugated to either 12.5 μM DMPS or DMSA, was measured at 37°C for 15 s in control and MRP2-expressing membrane vesicles. Data represent mean ± S.E. of two experiments, performed in triplicate. *, significantly different ($p < 0.05$) from the corresponding mean for control vesicles treated with the same mercuric conjugate.