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Interaction of 2,3-Dimercapto-1-propane Sulfonate with the Human Organic Anion Transporter hOAT1

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ABSTRACT

The kidney is the primary target organ in which inorganic mercury (Hg^{2+}) accumulates and expresses its toxic effects. The chelating agent 2,3-dimercapto-1-propanesulfonic acid (DMPS) can rapidly reduce the renal burden of mercury and increase the urinary excretion of mercury. However, the cellular and molecular basis of its efficacy is still unknown. A number of previous studies implicated the "classical organic anion secretory pathway" in the secretion of DMPS and its chelation products. In this study we used the human ortholog of the organic anion transporter (hOAT1) expressed in the *Xenopus* oocyte expression system to study the interaction of DMPS and its mercury chelates with hOAT1. [^{3}H]PAH was used to show the transport activity of hOAT1 (K_m = 3.9 ±1.3 μM). Uptake of [^{3}H]*para*-aminohippuric acid (PAH) was inhibited by reduced DMPS (K_i = 22.4 ± 8.4 μM). We also investigated the interaction of oxidized DMPS with hOAT1 because it has been shown that at least 80% of DMPS in the blood is oxidized within 30 min. Oxidized DMPS also inhibited uptake of [^{3}H]PAH (K_i = 66 ±13.6 μM). In contrast, we found no interaction of the DMPS-Hg chelate with hOAT1. To determine whether DMPS and oxidized DMPS are transported by hOAT1 we examined the effect of inwardly directed gradients these two compounds on efflux of [^{3}H]PAH from HeLa cells transiently transfected with hOAT1. Gradients of both DMPS and oxidized DMPS significantly *trans*-stimulated efflux of [^{3}H]PAH. These data suggest that hOAT1 can transport DMPS and oxidized DMPS, whereas the DMPS-Hg chelate has no significant affinity for the transporter.

The proximal tubule of the kidney is a crucial target for accumulation and toxicity of inorganic mercury (Zalups, 1991; Zalups et al., 1993; Diamond and Zalups, 1998). Corresponding with this preferential accumulation of inorganic mercury in proximal tubules, the kidneys are also a critical site of action for various antidotes of mercury poisoning (Zalups, 1993; Zalups et al., 1998). Recent investigations of mercury antidotes have highlighted the advantages of using 2,3-dimercapto-1-propanesulfonic acid (DMPS) over those antidotes previously used, such as 2,3-mercaptopropanol (BAL) (Aposhian, 1998). DMPS does not demonstrate the toxicity of the more lipophilic BAL, can be taken orally, and is the most effective compound for removing mercury in both clinical tests (Gonzalez-Ramirez et al., 1998) and tests on isolated kidney tissues (Keith et al., 1997). In studies using a variety of mammals, administration of DMPS clearly reduces the renal mercury burden and at the same time increases mercury appearance in the urine, with a corresponding protection against renal injury (Diamond et al., 1988; Klotzbach and Diamond, 1988; Zalups, 1993).

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Although these characteristics of DMPS have made it the preferred antidote for mercury poisoning at the clinical level (Aposhian, 1998), the molecular and cellular basis of this efficacy is far from clear. Its effectiveness is undoubtedly due in part to the high stability constant for inorganic mercury (~10^{42}; Casas and Jones, 1980) conferred by its two sulfhydryl moieties. However, a high affinity constant is generally not considered sufficient for maximal effectiveness of an antidote. The effectiveness of DMPS as a chelator is presumed to arise not only from its great affinity for mercury but also from its ability to access the intracellular compartment, where chelation is presumed to take place and where mercury undoubtedly has some of its toxic effects (Zalups and Lash, 1994; Diamond and Zalups, 1998). Indeed, there is evidence that DMPS can access the intracellular compartment via the "classic" organic anion transport process. In vivo studies in chickens (Stewart and Diamond, 1987) and rats (Klotzbach and Diamond, 1988) show that urinary DMPS excretion is blocked by both the prototypical substrate *p*-aminohippurate (PAH) and prototypical inhibitor probenecid, of the organic anion secretory pathway. Furthermore, Zalups et al. (1998) recently reported that the DMPS-medi-

ABBREVIATIONS: DMPS, 2,3-dimercapto-1-propanesulfonic acid; BAL, 2,3-mercaptopropanol; PAH, *para*-aminohippuric acid; OAT, organic anion transporter; hOAT1, human ortholog of the organic anion transporter; BSP, sulfobromophthalein; BSA, bovine serum albumin; MRP2, multidrug resistance-associated protein 2.

ated removal of inorganic mercury from isolated, perfused proximal tubules can be blocked by PAH in the bathing medium, suggesting that this transporter is necessary for the antidotal action of DMPS.

Although these results are consistent with DMPS acting as a substrate for the organic anion transporter, there are no studies directly characterizing DMPS interaction with the molecular entity generally recognized as the active, peritubular element of the organic anion secretory process, i.e., OAT1 (Sekine et al., 1997; Sweet et al., 1997). Moreover, it is unclear whether the transport of DMPS might depend on the form of DMPS present (reduced or oxidized), and whether chelation of DMPS with inorganic mercury might alter its interaction with OAT1. This latter point is of particular interest, given recent suggestions that the DMPS-Hg chelate may not be handled by the organic anion transporter at all (Zalups et al., 1998). In the present study, we have characterized the interaction of the organic anion transporter in the renal handling of reduced DMPS, oxidized DMPS, and the DMPS-Hg chelate. For this purpose we used the human ortholog of the organic anion transporter (hOAT1/ROAT1). It mediates the transport of various organic anions, including PAH, dicarboxylates, β-lactam antibiotics, diuretics, and nonsteroidal anti-inflammatory drugs (Sekine et al., 1997; Sweet et al., 1997; Apiwattanakul et al., 1999; Jariyawat et al., 1999) across the basolateral membrane of the proximal tubule. Our results show that, whereas both reduced and oxidized DMPS bind to and are transported with comparatively high affinity by hOAT1, the chelate of DMPS and inorganic mercury has no interaction with the organic anion transporter.

Experimental Procedures

Materials. The cDNA encoding hOAT1 was a generous gift from Drs. T. Cihlar and D. Sweet (Cihlar et al., 1999). [^{3}H]PAH (5 Ci/mmol) was purchased from PerkinElmer Life Science Products (Boston, MA). DMPS and Ellman's Reagent [5,5′-dithiobis(2-nitrobenzoic acid)] were purchased from Sigma Chemical (St. Louis, MO). All other chemical were purchased from standard sources and were generally the highest purity available.

hOAT1 Expression and Uptake Studies in Oocytes. hOAT1/pcDNA3.1 was linearized at the 3′ end by digestion with *Not*I. cRNA synthesis was performed using T7 RNA polymerase and the Cap analog.

After surgical removal of oocytes from *Xenopus laevis*, collagenase treatment was used to remove the follicular coats. Stage 5 and 6 oocytes were selected using a dissecting microscope and transferred to 60-mm culture dishes filled with ND96 (96 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM HEPES, 2.5 mM pyruvate, 6 mg of penicillin/100 ml of media, and 10 mg of streptomycin/100 ml of media, pH 7.6). After 24 h each oocyte was injected with 20 ng of hOAT1-cRNA in 50 nl of diethyl pyrocarbonate-treated water and incubated in ND96 at 18°C for 2 to 3 days. For uptake studies oocytes were transferred to 24-well plates filled with sodium-containing uptake solution (100 mM NaCl, 2 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, and 10 mM HEPES, pH 7.4) containing 0.4 μM [^{3}H]PAH (2 μCi/ml). At prescribed intervals uptake was stopped by aspirating the uptake solution followed by four sequential rinses with ice-cold uptake solution. Radioactivity accumulated by individual oocytes was assessed using liquid scintillation spectrometry to determine [^{3}H]PAH content. Generally, the results of representative experiments are shown in the figures. The various experimental observations were typically confirmed in two or three separate experiments with different batches of oocytes. When indicated, sample size (n) refers to the number of separate batches of oocytes. Unless indicated otherwise, data are mean uptakes (expressed per oocyte) ± S.E. Surgeries performed on intact *Xenopus* were conducted in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health.

Transient Expression in HeLa Cells and Efflux Experiments. hOAT1, cloned into the pcDNA3.1(+) vector with the coding strand under the control of the cytomegalovirus promotor, was transiently expressed in HeLa cells following the instructions of the QIAGEN Effectene Transfection Reagent Handbook (Valencia, CA). After 12 h of incubation at 37°C, the transfection mixture was removed and replaced by fresh medium. After another 24 h of incubation, transport experiments were performed using [^{3}H]PAH (0.2 μM) in 1 ml of Waymouth buffer (Lu et al., 1999; 135 mM NaCl, 13 mM HEPES, 2.5 mM $CaCl_2$, 1.2 mM $MgCl_2$, 0.8 mM $MgSO_4$, 5 mM KCl, and 28 mM D-glucose, pH 7.4). To determine efflux, cells were preloaded with [^{3}H]PAH for 1 h at 37°C. Preloading was stopped by aspirating the solution and washing the cells with Waymouth buffer. After 3 min of efflux, cells were rinsed with ice-cold Waymouth buffer, lysed with 1 ml of NaOH, and transferred to vials for scintillation counting.

Handling of DMPS. Solutions with reduced DMPS were prepared immediately before experiments, and oxidation of the free sulfhydryls within DMPS occurred at a rate of less than 6% per 60 min (the typical time course of a transport experiment) under the conditions used in these studies. Solutions of oxidized DMPS were obtained by bubbling a DMPS-containing solution in sodium uptake solution with 95% O_2, 5% CO_2 at 37°C for at least 24 h. No free thiol groups were detected after this period. By mass spectrometry (Finnigan LCQ ion trap mass spectrometer equipped with an electrospray ionization source in negative ion mode), >90% of the material was distributed as cyclic dimers. The remaining material consisted of higher molecular weight cyclic oligomers.

Measurement of Free Thiol Groups. Ellman's Reagent was used to determine free thiol groups (Ellman, 1959). Briefly, dissolved Ellman's Reagent, pH 9.0, and the DMPS solution were combined, pH adjusted to 8.0 with phosphate buffer, and the absorption measured at 412 nm after 5 min. Concentrations were calculated by comparison with standards containing known concentrations of reduced glutathione.

Results

Characterization of Organic Anion Transporter hOAT1. To confirm the transport activity of hOAT1 expressed in *Xenopus* oocytes, we determined the accumulation of [^{3}H]PAH in oocytes over 1 h. Figure 1A shows the linear uptake of [^{3}H]PAH in hOAT1-injected oocytes (the rate of uptake of [^{3}H]PAH into water-injected control oocytes was <1% of that into the hOAT1-injected oocytes). The hOAT1-mediated transport of PAH followed Michaelis-Menten kinetics (Fig. 1B) with a calculated K_m of 3.9 ± 1.3 μM (mean ± S.E., n = 3 experiments). This result was in close agreement with values for hOAT1-mediated PAH uptake in oocytes (4–9 μM; Cihlar et al., 1999; Hosoyamada et al., 1999) and cultured mammalian cells (5 μM; Lu et al., 1999).

To determine the substrate selectivity of hOAT1 expressed in oocytes, we measured [^{3}H]PAH uptake in the presence of a variety of different compounds. Figure 2 shows that the organic anions PAH, probenecid, fluorescein, and sulfobromophthalein (BSP) had strong inhibitory effects on hOAT1-mediated [^{3}H]PAH transport, whereas penicillin G and the bile acid derivative taurocholate had relatively weak effects. The cationic substrate tetraethylammonium did not inhibit [^{3}H]PAH transport.

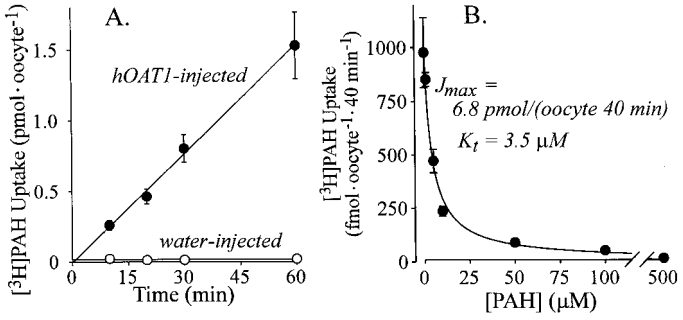

Fig. 1. A, time course of 0.4 μM [^{3}H]PAH uptake into *Xenopus* oocytes injected with 50 ng of hOAT1 cRNA (●) or an equal volume (20 nl) of water (○). Each point is the mean (±S.E.) of uptakes measured in 7 to 10 individual oocytes from a single animal. B, effect of increasing concentrations of unlabeled PAH on the 40-min uptake of 0.4 μM [^{3}H]PAH into hOAT1-expressing oocytes. Each point is the mean (±S.E.) of uptakes measured in 7 to 10 oocytes from a single animal. The line was fit to the data by using a nonlinear regression algorithm (SigmaPlot 3.0), according to the Michaelis-Menten equation for the competitive interaction of the labeled and unlabeled species of PAH (Malo and Berteloot, 1991).

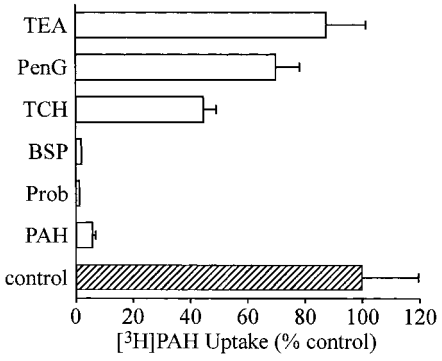

Fig. 2. Effect of an array of anionic and cationic compounds on the rate of PAH transport in hOAT1-expressing *Xenopus* oocytes. The 40-min uptakes of 0.4 μM [^{3}H]PAH were measured in the absence and presence of 1 mM concentrations of each test agent (with the exception of BSP, which was 100 μM). The length of each horizontal column represents the mean (±S.E.) of uptakes measured in seven to nine oocytes from a single animal, with uptake presented as the percentage of radiolabel accumulation measured in the absence of a test inhibitor. PenG, penicillin G; Prob, probenecid; TCH, taurocholate; TEA, tetraethylammonium.

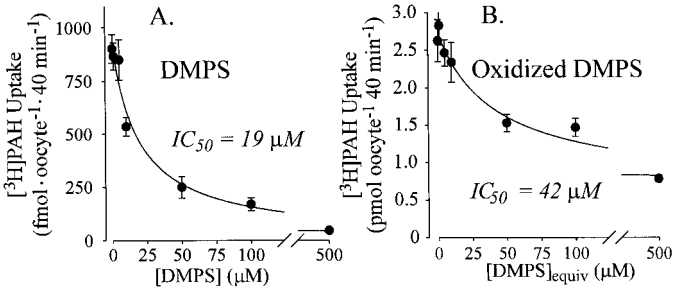

Fig. 3. Kinetics of inhibition of PAH uptake into hOAT1-expressing *Xenopus* oocytes produced by reduced DMPS (A) or oxidized DMPS (B). The 40-min uptakes of 0.4 μM [^{3}H]PAH were measured in individual oocytes injected with hOAT1 cRNA in the presence of increasing concentrations of test substrate. Each point indicates the mean (±S.E.) of uptakes measured in 7 to 10 individual oocytes from a single animal. The lines were fit to the data by using a nonlinear regression algorithm according the Michaelis-Menten equation assuming the interaction between PAH and the test inhibitors was competitive (Groves et al., 1994), although the results of this analysis are presented as "inhibitor constants" (IC_{50} values). The abscissas of Fig. 3, A and B, are expressed in terms of the concentration of reduced DMPS added to the uptake solutions. For Fig. 3A the concentration of reduced DMPS in the experimental solution (in terms of the corresponding concentration of reactive thiols) was verified in independent measurements. For Fig. 3B, the concentration of DMPS before complete oxidation is indicated (DMPS equivalent concentration; $[DMPS]_{equiv}$; see text). This material was quantitatively oxidized before the experiment, although the chemical identity of the resulting products of oxidation was not known with certainty. It was likely, however, that cyclic dimers of DMPS predominated (see text) and, consequently, that the actual concentration of these species is somewhat less than one-half of the starting concentration of DMPS.

Inhibition of [^{3}H]PAH Uptake by Reduced and Oxidized DMPS. As shown in Fig. 3A, DMPS inhibited [^{3}H]PAH uptake in oocytes with a calculated K_i of 22.4 ± 8.4 μM (mean ± S.E., n = 3), indicating that DMPS has a comparatively high affinity for hOAT1. Because previous studies (Maiorino et al., 1991; Hurlbut et al., 1994) indicated that DMPS probably exists primarily in the oxidized form in the blood, we also investigated the ability of oxidized DMPS to inhibit [^{3}H]PAH uptake. This analysis was complicated by the observation that in saline solution oxidized DMPS exists as a mixture of species. Although we confirmed by mass spectrometry that over 90% of the oxidized DMPS was in a cyclic dimeric form, the test solutions presumably included both cis- and *trans*-configurations (Maiorino et al., 1988). As a result, the molecular form (and therefore molar concentration) of oxidized DMPS in our solutions was not precisely defined. In view of this, we expressed the concentration of oxidized DMPS as the concentration of DMPS present before oxidation (DMPS equivalent concentration). Although there was ambiguity concerning the exact molar concentration of oxidized DMPS in our solutions, it was clear that oxidized DMPS had a potent inhibitory effect on [^{3}H]PAH uptake in *Xenopus* oocytes (Fig. 3B). We calculated a K_i of 66.0 ± 13.6 μM (DMPS equivalents; n = 3). Oxidized DMPS therefore effectively interacted with (i.e., bound to) hOAT1 despite its presence as (primarily) cyclic dimers of DMPS.

Effect of DMPS on [^{3}H]PAH Uptake in the Presence of Albumin. In humans, most (>80%) of the DMPS is found bound to albumin (Maiorino et al., 1996). Therefore, we measured [^{3}H]PAH uptake in oocytes in the presence of 40 μM DMPS (reduced and oxidized forms) and 0.1 mM BSA. Albumin per se did not inhibit [^{3}H]PAH uptake, and both DMPS and oxidized DMPS, in the absence of albumin, produced the expected inhibition (Fig. 4). However, coexposure of DMPS and oxidized DMPS with albumin completely eliminated the inhibitory effect of the chelator on OAT1-mediated [^{3}H]PAH transport (Fig. 4) suggesting that, under the conditions of this experiment, both oxidized and reduced DMPS were bound to albumin.

Interestingly, the free sulfhydryl groups of DMPS may not play a role in the binding to albumin. Although reduced DMPS had free sulfhydryl groups and therefore could form disulfide bonds (with albumin), oxidized DMPS mainly consisted of the cyclic form of the dimer (as described above), and therefore had no free sulfhydryl groups. However, in both cases the presence of albumin prevented DMPS from inhibiting [^{3}H]PAH uptake, suggesting that DMPS can bind to albumin independently of the formation of disulfide bonds.

Effect of the DMPS-Hg Chelate on [^{3}H]PAH Uptake. Because the interest in DMPS arises mainly from its ability to act as an antidote to poisoning with mercury (and other metals; Aposhian et al., 1995), we also investigated the ability of the DMPS-Hg chelate to inhibit [^{3}H]PAH uptake. To establish the toxic effect of inorganic (mercuric) mercury on

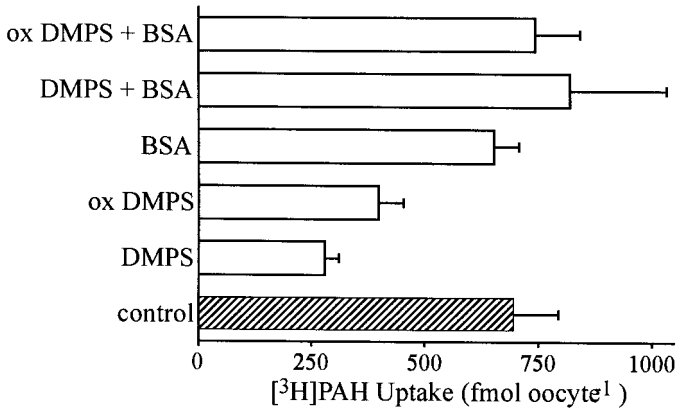

Fig. 4. Effect of albumin on the inhibitory effect of DMPS and oxidized DMPS on PAH transport into hOAT1-expressing oocytes. The 40-min uptakes of 0.4 μM [^{3}H]PAH were measured in the absence (control) or presence of DMPS or oxidized DMPS (40 μM, expressed as the DMPS-equivalent concentration; see legend of Fig. 3); or in the absence or presence of 100 μM BSA (mol. wt. 70 kDa). The length of each horizontal column represents the mean (±S.E.) of uptakes measured in 7 to 10 oocytes from a single animal.

anion transport, we investigated the influence of $HgCl_2$ on [^{3}H]PAH uptake in oocytes: 1 μM mercuric mercury inhibited [^{3}H]PAH uptake ~50% (data not shown). Similarly, 40 μM DMPS was shown to exert its predicted inhibition of [^{3}H]PAH uptake (~60%; Fig. 5). We then titrated 40 μM DMPS against increasing concentrations of mercuric mercury until a 1:1 ratio (40 μM $HgCl_2$ + 40 μM DMPS) was reached. The dashed line in Fig. 5 shows the predicted level of inhibition expected if the addition of Hg^{2+} stoichiometrically converted DMPS to a Hg-DMPS chelate that did not inhibit OAT1. The bars in Fig. 5 show that the addition of Hg^{2+} did, in fact, decrease the inhibitory effectiveness of DMPS to approximately the level predicted if the chelate is noninhibitory. This result also confirmed the formation of very stable DMPS-Hg chelates, because very low concentrations of mercuric mercury would have been enough to decrease [^{3}H]PAH uptake.

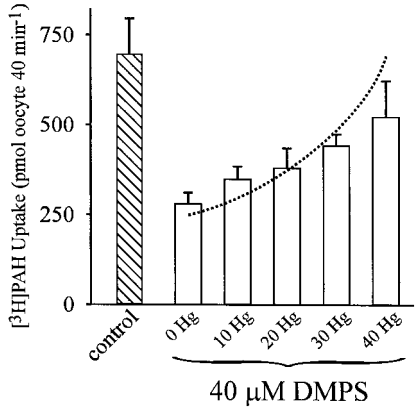

Fig. 5. Effect of mercury chelation on the inhibitory interaction of DMPS with hOAT1. The 40-min uptakes of 0.4 μM [^{3}H]PAH into hOAT1-expressing oocytes were measured in the absence (▨) or presence (□) of 40 μM DMPS. Uptakes in the presence of DMPS were measured in the presence of 0 to 40 μM Hg^{2+} (supplied as $HgCl_2$). The height of each column represents the mean (±S.E.) of uptakes in 7 to 10 oocytes from a single animal. The dotted line shows the amount of [^{3}H]PAH uptake predicted to occur, assuming that the amount of DMPS in the test solution was titrated quantitatively by Hg^{2+}, and that the DMPS-Hg chelate so-formed had no inhibitory interaction with hOAT1.

Stimulation of [^{3}H]PAH Efflux by Reduced and Oxidized DMPS. Inhibition of [^{3}H]PAH transport by DMPS and oxidized DMPS does not necessitate translocation of the inhibitory molecules across the cell membrane. To determine whether the interactions of DMPS and oxidized DMPS with hOAT1 included translocation of these molecules, we measured the extent of *trans*-stimulation of PAH efflux that inwardly directed solute gradients produced in HeLa cells preloaded with [^{3}H]PAH. The presence of 100 μM PAH in the extracellular solution, a positive control, stimulated efflux by 10.5% ($P < 0.05$) as shown in Fig. 6. The presence of 400 μM DMPS or 1 mM oxidized DMPS in the extracellular solution also stimulated efflux (by 11.2 and 8.7%, respectively, $P < 0.05$). These results suggest that both reduced and oxidized DMPS were translocated across the cell membrane by hOAT1.

Discussion

DMPS is the preferred antidote for inorganic mercury poisoning. It can reduce rapidly the renal burden of mercury and increase the urinary excretion of mercury (Aposhian et al., 1995). There is evidence from various investigations that the classical organic anion secretory pathway is involved in this process, but there are still no data confirming these findings on a molecular level. Consequently, we investigated the role of the cloned human organic anion transporter hOAT1 in the antidotal activity of DMPS by analyzing the ability of various forms of DMPS to interact with this transporter.

After confirming the transport activity of hOAT1 in the *Xenopus* oocyte expression system under our experimental conditions (Figs. 1 and 2), we observed that reduced DMPS

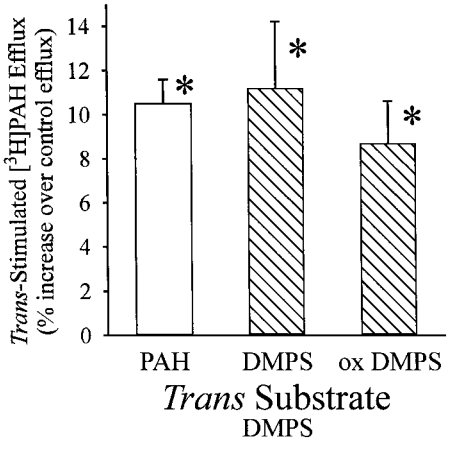

Fig. 6. Effect of inwardly directed gradients of PAH, DMPS, and oxidized DMPS on the efflux of [^{3}H]PAH from preloaded, transiently transfected hOAT1-expressing HeLa cells. Cells were preloaded for 60 min by incubation in uptake solution containing 0.2 μM [^{3}H]PAH. Efflux was initiated by washing the cells two times with 37°C, substrate-free uptake solution. The cells were then incubated in efflux solutions containing either no test substrate (control) or a test agent [100 μM PAH, 400 μM DMPS, 1 mM oxidized DMPS (as the DMPS-equivalent concentration]. The rate of efflux of [^{3}H]PAH from the cells under these conditions was determined by measuring the amount of radioactivity left in the cells after 3 min, compared with the amount present at time 0 (i.e., after the 60-min preload). The height of the columns indicates the rate of efflux under the three test conditions, expressed as the percent increase over the efflux measured under the control condition (±S.E.; $n = 3$ test wells). The results are from a single representative experiment.

inhibited the uptake of [^{3}H]PAH in a concentration-dependent manner, inhibiting hOAT1 activity with a K_i of 22 μM (Fig. 3). Furthermore, DMPS not only blocked the transport activity of hOAT1 but also *trans*-stimulated efflux of [^{3}H]PAH (Fig. 6), suggesting that hOAT1 supports DMPS transport across the peritubular membrane of renal proximal cells. Previous studies showed that DMPS is rapidly oxidized in both saline and blood: in humans, at least 80% of DMPS in blood is oxidized within 30 min (Maiorino et al., 1991). For this reason, it was of physiological relevance to investigate the interaction of oxidized DMPS with hOAT1, as well. Although it is difficult, given the complexity of DMPS oxidation, to define with precision the concentration of DMPS oxidation products under the conditions associated with physiological experiments, we can say with confidence that our oxidized DMPS-containing solutions consisted predominantly of cyclic dimers of DMPS, and did not contain monomeric DMPS. These oxidation products of DMPS inhibited [^{3}H]PAH uptake in a concentration-dependent manner, with a calculated K_i of 66 μM (expressed as DMPS equivalent concentration). This K_i is almost 3 times the K_i calculated for DMPS. However, because the oxidized DMPS solution consisted primarily of dimers and, in a lower percentage, trimers, the true oxidized DMPS concentration can be expected to have been less than one-half of the concentration expressed in DMPS equivalents. This suggests that hOAT1 has a comparable affinity for oxidized DMPS and DMPS. Furthermore, oxidized DMPS seems to be transported by hOAT1, because it also stimulated efflux of [^{3}H]PAH from HeLa cells (Fig. 6). Although hOAT1 is frequently viewed as a transporter of monovalent anions of widely diverse structure, its ability to exchange monovalent substrates for α-ketoglutarate (and a very restricted set of divalent anions) exemplifies its capacity to act as a transporter of divalent substrates. Presumably, the interaction of oxidized DMPS with hOAT1 arises because of structural similarities between the dimeric species of oxidized DMPS, with their -2 valences, and the dicarboxylate substrates that interact effectively with OAT1. In fact, the distances between the anionic sulfonyl residues of the cyclic dimers of oxidized DMPS (about 7 or 8.5 Å, for the cis- and *trans*-isomers, respectively), are very similar to the distances between the carboxyl residues of adipate (6.3 Å) and suberate (8.9 Å), both of which are known to serve as substrates of the renal organic anion transporter (Shimada et al., 1987; Sullivan and Grantham, 1992).

In blood most DMPS is probably bound to plasma constituents such as cysteine (Maiorino et al., 1996) or proteins. In humans, for example, >80% of the DMPS is bound to albumin (Maiorino et al., 1996). Our results show that 0.1 mM BSA neutralized the inhibitory effects of DMPS and oxidized DMPS on [^{3}H]PAH uptake, whereas 0.1 mM BSA alone had no effect on hOAT1 activity. These findings suggest that most DMPS was bound to albumin and therefore did not have access to hOAT1. The fact that oxidized DMPS also seemed to be bound to albumin, although it has no free thiol groups, confirms the results of previous studies showing that binding of DMPS to serum proteins does not include substantial formation of disulfide bonds (Ruprecht, 1997). The fact that administration of DMPS is effective in clearing mercury from renal cells and excreting it in the urine, despite being largely bound to plasma proteins, suggests that the secretory processes are sufficiently rapid that they establish a steady-state gradient between the interstitial space and the plasma that supports continuous dissociation of the weakly bound substrate.

The high stability constant of the DMPS-Hg chelate is another reason for the effectiveness of DMPS as antidote for mercury poisoning. Therefore, we investigated whether the DMPS-Hg chelate interacts with hOAT1. When DMPS was titrated against increasing concentrations of $HgCl_2$, inhibition of [^{3}H]PAH uptake was relieved, presumably through the formation of the DMPS-Hg chelate and a concomitant decrease in the concentration of free DMPS available for competition with [^{3}H]PAH for hOAT1. This indicates that hOAT1 has little or no affinity for the DMPS-Hg chelate. Therefore, hOAT1 does not provide an effective way for any DMPS-mediated translocation of inorganic mercury across cell membranes. This observation is consistent with the observation of Zalups et al. (1998), who showed that coexposure of perfused rabbit renal proximal tubules to a perfusate containing both ^{203}Hg and DMPS eliminates tubular accumulation of mercury. It also provides another basis for the effectiveness of DMPS as an antidote for mercury poisoning. First, the formation of the DMPS-Hg chelate in the blood plasma prevents more mercury from accumulating in the kidney; the DMPS-Hg chelate in the blood should be excreted from the body by glomerular filtration. Second, once the DMPS-Hg chelate has formed inside the tubule cell, no back leak to the blood via hOAT1 is possible; excretion of the chelate in the urine across the apical membrane is the only way for accumulated chelate to leave the cell.

It is not known how DMPS or the DMPS-Hg chelate is secreted in the urine across the apical membrane. A likely candidate is the multidrug resistance-associated protein MRP2, which has been localized to the apical membrane of renal proximal tubule cells (Schaub et al., 1997, 1999). MRP2 has been shown to handle anionic conjugates, including glutathione conjugates (Keppler et al., 1998). The Eisai hyperbilirubinemic rat, which lacks MRP2 in the canalicular membrane of liver cells (Büchler et al., 1996), displays a reduced biliary excretion of mercury (Sugawara et al., 1998), suggesting that MRP2 could also play an important role as an apical transport protein in the renal secretion of mercury. In this context, it is important to mention that, as a primary active ATPase, MRP2 is a unidirectional efflux pump: it can only move substrates from the inside to the outside of the cell. This would prevent the DMPS-Hg chelate in the lumen of the tubule (after glomerular filtration or luminal excretion by MRP2) from being reabsorbed into tubule cells. Together with the observation that the DMPS-Hg chelate cannot be transported by hOAT1, these characteristics could be important for the effectiveness of DMPS in clearing mercury from the kidney.

The observations reported here support the following model for the transport of DMPS in renal proximal cells (Fig. 7). DMPS enters renal proximal cells across the basolateral membrane, from the blood, via OAT1. Although both reduced and oxidized species of DMPS interact with OAT1, the principal chemical form entering renal cells is probably an oxidized species, owing to their larger concentration in the blood. Although not shown in the model, we cannot exclude the possibility that DMPS enters proximal cells by one or more other transport processes, as well. Within renal cells oxidized DMPS is reduced back to DMPS in the cytoplasm by

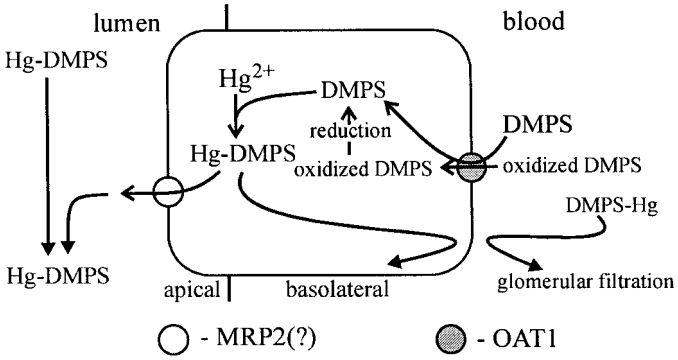

Fig. 7. Model of the proposed organization of transporters associated with the movement of DMPS and DMPS-Hg chelates across proximal tubule cell membranes. See text for discussion.

a glutathione-dependent thiol-disulfide exchange reaction in the proximal tubule cell (Stewart and Diamond, 1988). DMPS can subsequently chelate accumulated mercury within the intracellular compartment. The DMPS-Hg formed within proximal cells cannot exit across the basolateral membrane via OAT1, and is secreted to the tubule lumen, presumably via an export pump (e.g., MRP2). Hg-DMPS in the lumen is refractory to reabsorption and is excreted in the urine.

The potential role of OAT1 on the excretory flux of DMPS may also include limiting this chelator's access to extrarenal tissues, particularly the brain. DMPS is not effective at clearing metals from brain tissue, compared with the more lipophilic BAL (Muckter et al., 1997). OAT1 appears to be expressed in the apical membrane of choroid plexus where it presumably plays a role in clearing the cerebrospinal fluid of organic anions (Pritchard et al., 1999). Although it is likely that the extreme hydrophilicity of DMPS is the most important factor in limiting its passage across the blood-brain barrier (Aposhian et al., 1995), to the extent that it does enter the brain, excretion by OAT1 can be expected to further reduce its potential therapeutic impact.

In conclusion, the present study suggests that the organic anion transporter hOAT1 plays a fundamental role in the antidotal action of DMPS, even if this action does not include uptake or efflux of the DMPS-Hg chelate itself. The importance of other mechanisms, particularly transport of DMPS and its chelates across the apical membrane of proximal tubule cells, are at present not known.

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References

Apiwattanakul N, Sekine T, Chairoungdua A, Kanai Y, Nakajima N, Sophasan S, and Endou H (1999) Transport properties of nonsteroidal anti-inflammatory drugs by organic anion transporter 1 expressed in Xenopus laevis oocytes. *Mol Pharmacol* **55:**847–854.

Aposhian HV (1998) Mobilization of mercury and arsenic in humans by sodium 2,3-dimercapto-1-propane sulfonate (DMPS). *Environ Health Perspect* **106 (Suppl 4):**1017–1025.

Aposhian HV, Maiorino RM, Gonzalez-Ramirez D, Zuniga-Charles M, Xu Z, Hurlbut KM, Junco MP, Dart RC, and Aposhian MM (1995) Moblization of heavy metals by newer, therapeutically useful chelating agents. *Toxicology* **97:**23–38.

Büchler M, König J, Brom M, Kartenbeck J, Spring H, Hories T and Keppler D (1996) cDNA cloning of the hepatocyte canalicular isoform of the multidrug resistance protein, cMrp, reveals a novel conjugate export pump deficient in hyperbilirubinemic mutant rats. *J Biol Chem* **271:**15091–15098.

Casas JS and Jones MM (1980) Mercury(II) complexes with sulhydryl containing chelating agents; stability constant inconsistencies and their resolutions. *J Inorg Nucl Chem* **42:**99–102.

Cihlar T, Lin DC, Pritchard JB, Fuller MD, Mendel DB, and Sweet DH (1999) The antiviral nucleotide analogs cidofovir and adefovir are novel substrates for human and rat renal organic anion transporter 1. *Mol Pharmacol* **56:**570–580.

Diamond GL, Klotzbach JM, and Stewart JR (1988) Complexing activity of 2,3-dimercapto-1-propanesulfonate and its disulfide auto-oxidation product in rat kidney. *J Pharmacol Exp Ther* **246:**270–274.

Diamond GL and Zalups RK (1998) Understanding renal toxicity of heavy metals. *Toxicol Pathol* **26:**92–103.

Ellman GL (1959) Tissue sulfhydryl groups. *Arch Biochem Biophys* **82:**70–77.

Gonzalez-Ramirez D, Zuniga-Charles M, Narro-Juarez A, Molina-Recio Y, Hurlbut KM, Dart RC, and Aposhian HV (1998) DMPS (2,3-dimercaptopropane-1-sulfonate, dimaval) decreases the body burden of mercury in humans exposed to mercurous chloride. *J Pharmacol Exp Ther* **287:**8–12.

Groves CE, Evans K, Dantzler WH, and Wright SH (1994) Peritubular organic cation transport in isolated rabbit proximal tubules. *Am J Physiol* **266:**F450–F458.

Hosoyamada M, Sekine T, Kanai Y, and Endou H (1999) Molecular cloning and functional expression of a multispecific organic anion transporter from human kidney. *Am J Physiol* **276:**F122–F128.

Hurlbut KM, Maiorino RM, Mayersohn M, Dart RC, Bruce DC, and Aposhian HV (1994) Determination and metabolism of dithiol chelating agents. XVI: Pharmacokinetics of 2,3-dimercapto-1-propanesulfonate after intravenous administration in human volunteers. *J Pharmacol Exp Ther* **268:**662–668.

Jariyawat S, Sekine T, Takeda M, Apiwattanakul N, Kanai Y, Sophasan S, and Endou H (1999) The interaction and transport of beta-lactam antibiotics with the cloned rat renal organic anion transporter 1. *J Pharmacol Exp Ther* **290:**672–677.

Keith RL, Setiarahardjo I, Fernando Q, Aposhian HV, and Gandolfi AJ (1997) Utilization of renal slices to evaluate the efficacy of chelating agents for removing mercury from the kidney. *Toxicology* **116:**67–75.

Keppler D, Leier I, Jedlitschky G, and König J (1998) ATP-dependent transport of glutathione *S*-conjugates by the multidrug resistance protein MRP1 and its apical isoform MRP2. *Chem-Biol Interact* **111–112:**153–161.

Klotzbach JM and Diamond GL (1988) Complexing activity and excretion of 2,3-dimercapto-1-propane sulfonate in rat kidney. *Am J Physiol* **254:**F871–F878.

Lu R, Chan BS, and Schuster VL (1999) Cloning of the human kidney PAH transporter: narrow substrate specificity and regulation by protein kinase C. *Am J Physiol* **276:**F295–F303.

Maiorino RM, Dart RC, Carter DE, and Aposhian HV (1991) Determination and metabolism of dithiol chelating agents. XII. Metabolism and pharmacokinetics of sodium 2,3-dimercaptopropane-1-sulfonate in humans. *J Pharmacol Exp Ther* **259:**808–814.

Maiorino RM, Weber GL, and Aposhian HV (1988) Determination and metabolism of dithiol chelating agents. III. Formation of oxidized metabolites of 2,3-dimercaptopropane-1-sulfonic acid in rabbits. *Drug Metab Dispos* **16:**455–463.

Maiorino RM, Xu ZF, and Aposhian HV (1996) Determination and metabolism of dithiol chelating agents. XVII. In humans, sodium 2,3-dimercapto-1-propanesulfonate is bound to plasma albumin via mixed disulfide formation and is found in the urine as cyclic polymeric disulfides. *J Pharmacol Exp Ther* **277:**375–384.

Malo C and Berteloot A (1991) Analysis of kinetic data in transport studies: new insights from kinetic studies of Na^+-D-glucose cotransport in human intestinal brush-border membrane vesicles using a fast sampling, rapid filtration apparatus. *J Membr Biol* **122:**127–141.

Muckter H, Liebl B, Reichl FX, Hunder G, Walther U, and Fichtl B (1997) Are we ready to replace dimercaprol (BAL) as an arsenic antidote? *Hum Exp Toxicol* **16:**460–465.

Pritchard JB, Sweet DH, Miller DS, and Walden R (1999) Mechanism of organic anion transport across the apical membrane of choroid plexus. *J Biol Chem* **274:**33382–33387.

Ruprecht J (1997) *Dimaval (DMPS)*. Heyltex Corporation, Houston, TX.

Schaub TP, Kartenbeck J, Konig J, Spring H, Dorsam J, Staehler G, Storkel S, Thon WF, and Keppler D (1999) Expression of the MRP2 gene-encoded conjugate export pump in human kidney proximal tubules and in renal cell carcinoma. *J Am Soc Nephrol* **10:**1159–1169.

Schaub TP, Kartenbeck J, König J, Vogel O, Witzgall R, Kriz W, and Keppler D (1997) Expression of the conjugate export pump encoded by the *mrp2* gene in the apical membrane of kidney proximal tubules. *J Am Soc Nephrol* **8:**1213–1221.

Sekine T, Watanabe N, Hosoyamada M, Kanaim Y, and Endou H (1997) Expression cloning and characterization of a novel multispecific organic anion transporter. *J Biol Chem* **272:**18526–18529.

Shimada H, Moewes B, and Burckhardt G (1987) Indirect coupling to Na^+ of *p*-aminohippuric acid uptake into rat renal basolateral membrane vesicles. *Am J Physiol* **253:**F795–F801.

Stewart JR and Diamond GL (1987) Renal tubular secretion of the alkanesulfonate 2,3-dimercapto-1-propanesulfonate. *Am J Physiol* **252:**F800–F810.

Stewart JR and Diamond GL (1988) In vivo renal tubular secretion and metabolism of the disulfide of 2,3-dimercaptopropane-1-sulfonate. *Drug Metab Dispos* **16:**189–195.

Sugawara N, Lai Y-R, Saguware C, and Arizono K (1998) Decreased hepatobiliary secretion of inorganic mercury, its deposition and toxicity in the Esai hyperbilirubinemic rat with no hepatic canalicular organic anion transporter. *Toxicology* **126:**23–31.

Sullivan LP and Grantham JJ (1992) Specificity of basolateral organic anion exchanger in proximal tubule for cellular and extracellular solutes. *J Am Soc Nephrol* **2:**1192–1200.

Sweet DH, Wolff NA, and Pritchard JB (1997) Expression cloning and characteriza-

tion of ROAT1: the renal basolateral organic anion transporter in rat kidney. *J Biol Chem*, **272:**30088–30095.
Zalups RK (1991) Autometallographic localization of inorganic mercury in the kidneys of rats: effect of unilateral nephrectomy and compensatory renal growth. *Exp Mol Pathol* **54:**10–21.
Zalups RK (1993) Influence of 2,3-dimercaptopropane-1-sulfonate (DMPS) and meso-2,3-dimercaptosuccinic acid (DMSA) on the renal disposition of mercury in normal and uninephrectomized rats exposed to inorganic mercury. *J Pharmacol Exp Ther* **267:**791–800.
Zalups RK, Knutson KL, and Schnellmann RG (1993) In vitro analysis of the accumulation and toxicity of inorganic mercury in segments of the proximal tubule isolated from the rabbit kidney. *Toxicol Appl Pharmacol* **119:**221–227.
Zalups RK and Lash LH (1994) Advances in understanding the renal transport and toxicity of mercury. *J Toxicol Environ Health* **42:**1–44.
Zalups RK, Parks LD, Cannon VT, and Barfuss DW (1998) Mechanisms of action of 2,3-dimercaptopropane-1-sulfonate and the transport, disposition, and toxicity of inorganic mercury in isolated perfused segments of rabbit proximal tubules. *Mol Pharmacol* **54:**353–363.

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