Published in final edited form as:

Biol Trace Elem Res. 2018 July ; 184(1): 279–286. doi:10.1007/s12011-017-1163-3.

MRP2 and the Transport Kinetics of Cysteine Conjugates of Inorganic Mercury

Cláudia Oliveira^{*,†}, Lucy Joshee^{*}, and Christy C. Bridges^{*}

^{*}Mercer University School of Medicine, Division of Basic Medical Sciences, Macon, GA 31207

Abstract

Human exposure to mercuric species occurs regularly throughout the world. Mercuric ions may accumulate in target cells and subsequently lead to cellular intoxication and death. Therefore, it is important to have a thorough understanding of how transportable species of mercury are handled by specific membrane transporters. The purpose of the current study was to characterize the transport kinetics of cysteine (Cys)-*S*-conjugates of inorganic mercury (Cys-*S*-Hg-*S*-Cys) at the site of the multidrug resistance-associated transporter 2 (MRP2). In order to estimate the maximum velocity ($V_{\max}$) and Michaelis constant (K_m) for the uptake of Cys-*S*-Hg-*S*-Cys mediated by MRP2, *in vitro* studies were carried out using radioactive Cys-*S*-Hg-*S*-Cys (5 μ M) and inside-out membrane vesicles made from Sf9 cells transfected with MRP2. The $V_{\max}$ was estimated to be 74.3 ± 10.1 nmol/mg protein/30 s while the K_m was calculated to be 63.4 ± 27.3 μ M. In addition, *in vivo* studies were utilized to measure the disposition of inorganic mercury (administered dose: 0.5 μ mol kg⁻¹ in 2 mL normal saline) over time in Wistar and TR⁻ (Mrp2-deficient) rats. These studies measured the disposition of mercuric ions in kidney, liver, and blood. In general, the data suggest that the initial uptake of mercuric conjugates into select target cells is rapid followed by a period of slower uptake and accumulation. Overall, the data indicate that MRP2 transports Cys-*S*-Hg-*S*-Cys in a manner that is similar to that of other MRP2 substrates.

Keywords

mercury; multidrug resistance-associated protein 2; kidney; transport

INTRODUCTION

Humans may be exposed to various forms of mercury through environmental, occupational, and/or dietary sources. In general, the majority of human exposure to mercuric compounds occurs following the ingestion of fish contaminated with organic forms of mercury (e.g., methylmercury, MeHg) [1]. Individuals with diets high in fish and seafood often have blood levels of MeHg that are higher than the minimum level recommended by the EPA [2,3]. MeHg is absorbed readily by the gastrointestinal tract of humans [4,5], following which it may be biotransformed to inorganic mercury (Hg²⁺) [6–8]. The biotransformation process is

Corresponding author: Christy C. Bridges, Mercer University School of Medicine, Division of Basic Medical Sciences, 1550 College St., Macon, GA 31207, 478-301-2086, Bridges_cc@mercer.edu, ORCID: 0000-0003-1603-1445.

[†]Present address: Post-Graduate Course in Biological Science — Toxicological Biochemistry, Federal University of Santa Maria, Santa Maria, RS, Brazil

fairly rapid; approximately one-half of the absorbed MeHg is transformed to Hg^{2+} within 14 days after exposure [9]. Considering that cells are likely exposed to various species of Hg^{2+} , it is necessary to have a thorough understanding of the way in which Hg^{2+} is handled by target tissues, organs, and cells.

The kidney, specifically the proximal tubule, is the primary site of uptake and accumulation of Hg^{2+} [10]. Hg^{2+} appears to be taken up at the luminal and basolateral membranes of proximal tubular cells as a conjugate of one or more thiol-containing biomolecules, such as cysteine (Cys) [10]. At the luminal membrane of proximal tubular cells, the uptake of Cys-*S*-Hg-*S*-Cys has been shown to be mediated by the amino acid transporter, System $\text{b}^{0,+}$ [11]. At the basolateral membrane, organic anion transporters (OAT) 1 and 3 have been shown to play important roles in the uptake of Cys-*S*-Hg-*S*-Cys [12].

Accumulation of mercuric ions within the intracellular compartment of cells may lead to oxidative stress, cellular intoxication, and eventual necrosis. Therefore, it is critical that cells possess mechanisms that can be utilized to eliminate mercuric ions. Previous studies have shown that the export of mercuric conjugates from within proximal tubular cells into the tubular lumen involves membrane transporters localized in the apical plasma membrane [13,14]. Two of these transporters have been identified as the multidrug resistance-associated transporter 2 (MRP2) and the breast cancer resistance protein (BCRP). Based on previous data, it appears that MRP2 is the predominant carrier involved in the proximal tubular export of mercuric conjugates. Despite the importance of this carrier in the proximal tubular export of Hg^{2+} , there is little information regarding the way in which mercuric conjugates are handled at the site of MRP2. Therefore, the purpose of the current study was to define and characterize the transport of a transportable form of Hg^{2+} (i.e., Cys-*S*-Hg-*S*-Cys) via MRP2.

MATERIALS AND METHODS

Radioactive Mercury

[^{203}Hg] ($6 - 12 \text{ mCi mg}^{-1}$) was generated according to a published method described previously [15,11]. Briefly, 3 mg of mercuric oxide (87% enriched ^{202}Hg ; Trace Sciences) was subjected to irradiation for 4 weeks at the University of Missouri Research Reactor (MURR). The irradiated mercuric oxide was dissolved in 1 N HCl and stored at -20°C .

Membrane Vesicles

Inside-out membrane vesicles made from Sf9 cells containing human MRP2 (catalog number SBVT05) were purchased from Sigma-Aldrich. These vesicles are produced by Solvo Biotechnology (Budapest, Hungary) and are distributed in the United States by Sigma-Aldrich. Each lot of vesicles produced by Solvo Biotechnology is tested for transporter functionality and is certified to be suitable for studies involving the designated transporter. Our laboratory has utilized vesicles from Solvo Biotechnology previously with success [16,17,13,18]. Vesicular transport assays were carried out using a rapid filtration method according to a published protocol [19,20,16]. Briefly, cysteine (Cys)-*S*-conjugates of Hg^{2+} (Cys-*S*-Hg-*S*-Cys) were formed by mixing 5 μM radioactive mercury ([^{203}Hg]) with

12.5 μM Cys in incubation buffer (250 mM sucrose, 10 mM Tris/HCl, pH 7.4) supplemented with 10 mM MgCl_2 , 10 mM creatine phosphate and 100 $\mu\text{g/ml}$ creatine phosphokinase in the presence of 4 mM ATP or AMP). Incubation buffer containing Cys-*S*-Hg-*S*-Cys was added to the vesicle mixture (7.5 μg protein) and incubated for 0.25, 0.5, 1, or 5 min at 37°C. For studies using [^3H]-*p*-aminohippurate (PAH), the uptake of 50 nM [^3H]-PAH was measured for 30 seconds at 37°C in the presence and absence of ATP and the presence or absence of 3 mM glutathione (GSH). Uptake of Cys-*S*-Hg-*S*-Cys in the presence and absence of GSH was measured under the same conditions. For the studies of saturation kinetics, 5 μM radioactive Cys-*S*-Hg-*S*-Cys was incubated with buffer or 50 μM , 100 μM or 500 μM unlabeled Cys-*S*-Hg-*S*-Cys for 30s. Following incubation, vesicles were washed in ice-cold buffer containing 1 mM 2,3-dimercapto propane-2-sulfonate (DMPS) to stop uptake and wash off any mercury bound to the outside of the vesicles. Each sample was filtered through a Multiscreen plate (0.45 μm ; Millipore, Billarica, MA). Filters were removed and the radioactivity contained on each filter was determined using gamma counting. In order to assess ATP-dependent transport, the amount of [^{203}Hg] associated with vesicles in the presence of AMP was subtracted from that in the presence of ATP.

Animals

Male Wistar and Transport deficient (TR^-) rats weighing 200–225 g were used for these studies. A genetic mutation in TR^- rats leads to an early stop codon in the mRNA sequence of the *mrp2* gene, resulting in a non-functional Mrp2 protein [21,22]. These rats represent a reliable model that has been used to study the hepatic and renal secretion of various Mrp2 substrates [23–25]. 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 Guide for the Care and Use of Laboratory Animals as adopted by the National Institutes of Health. The animal protocol utilized in these studies was approved by the Mercer University Animal Care and Use Committee.

Intravenous Injections

Twelve Wistar (control) and twelve TR^- rats were injected intravenously according to a previously published protocol [17,13]. Each animal was anesthetized with isoflurane and a small incision was made in the skin in the mid-ventral 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 $\mu\text{mol kg}^{-1}$ in 2 mL normal saline containing 1 μCi of [^{203}Hg] per rat) was administered into the vein. The wound was closed using two 9-mm stainless steel wound clips. Following the administration of HgCl_2 , Wistar and TR^- rats were divided randomly into three groups, with each group consisting of four rats. Animals were placed in individual plastic metabolic cages, in which water and food were provided *ad libitum*. Rats were euthanized 15 min, 1 h, or 24 h after administration of HgCl_2 .

Collection of Tissues, Organs, Urine and Feces

At the time of euthanasia, rats were anesthetized with an intraperitoneal injection of ketamine (70 mg kg^{-1}) and xylazine (30 mg kg^{-1}) in 2 mL/kg saline. Two 1-mL samples of blood were obtained from the inferior vena cava. One of the samples was placed in a

polystyrene tube for determination of [^{203}Hg] content, while the other sample was placed in a Microtainer tube (Becton Dickinson), which was centrifuged at 21,000 x g for 90 seconds. 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 [26].

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] content. The liver was then excised carefully, weighed, and a 1-g section was removed for determination of [^{203}Hg] content. Urine and feces were unable to be collected during these experiments due to the short time periods used in the study. The content of [^{203}Hg] in each sample was determined by counting the samples in a Wallac Wizard 3 automatic gamma counter (Perkin Elmer, Boston, MA).

Data Analyses

Data for each 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 two-way analysis of variance (ANOVA) to assess differences among the means. When statistically significant *F*-values were obtained with ANOVA, the data were analyzed using Tukey's *post hoc* multiple comparison test. A *p*-value of < 0.05 was considered statistically significant.

RESULTS

Analyses of Hg Transport in Membrane Vesicles

The viability of inside-out membrane vesicles containing hMRP2 as a system to study the activity of MRP2 was assessed by measuring the uptake of PAH in the presence and absence of 3 mM GSH (Figure 1A). The ATP-dependent uptake of PAH was inhibited significantly by the presence of excess GSH, which is a competitive substrate of MRP2. In addition, the uptake of Cys-*S*-Hg-*S*-Cys into hMRP2 vesicles was measured in the presence and absence of 3 mM GSH (Figure 1B). Like PAH, the presence of excess GSH significantly inhibited the uptake of Cys-*S*-Hg-*S*-Cys. Collectively, these data indicate that the inside-out membrane vesicles containing hMRP2, purchased from Solvo, are appropriate models of the study of MRP2-mediated transport processes.

The time-course of uptake of Cys-*S*-Hg-*S*-Cys into inside-out membrane vesicles containing hMRP2 is shown in Figure 2A. The uptake of Cys-*S*-Hg-*S*-Cys into MRP2 vesicles increased significantly over time. Uptake was linear between 15 s and 1 min; therefore, 30 s was chosen as a time point for subsequent studies. The Michaelis-Menten kinetics of Cys-*S*-Hg-*S*-Cys transport by MRP2 was assessed in inside-out membrane vesicles containing hMRP2 (Figure 2B). The V_{max} was estimated to be $74.3 \pm 10.1 \text{ nmol mg protein}^{-1} 30 \text{ s}^{-1}$ while the K_m was calculated to be $63.4 \pm 27.3 \text{ }\mu\text{M}$. The Eadie-Hofstee plot (inset) shows that a single transport process is involved in this transport.

Analysis of the Disposition of Hg in Rat Organs

The burden of Hg^{2+} in the total renal mass of rats following exposure to $0.5 \mu\text{mol kg}^{-1}$ HgCl_2 is shown in Figure 3. The accumulation of Hg^{2+} was greater in kidneys of TR^- rats than in kidneys of Wistar rats at each time measured.

When the concentration of Hg^{2+} was assessed in the zones of the kidney, the cortex was found to be the primary site of accumulation of mercuric ions (Figure 4A). The cortical concentration of Hg^{2+} was greater in TR^- rats than Wistar rats at each time studied. The amount of Hg^{2+} also increased significantly over time. In the outer stripe of the outer medulla (OSOM), the concentration of Hg^{2+} was greater in TR^- rats than in Wistar animals following the 15-min and 1-h exposures (Figure 4B). The concentration of Hg^{2+} in the OSOM of Wistar rats following the 24-h exposure to HgCl_2 was not significantly different from that in TR^- rats. The amount of Hg^{2+} detected in the inner stripe of the outer medulla and in the inner medulla was minimal (data not shown).

The amount of Hg^{2+} in the liver was also measured 15 min, 1 h, and 24 h after exposure to HgCl_2 (Figure 5). In Wistar rats, the hepatic burden of Hg^{2+} decreased over time. The amount of Hg^{2+} in liver of Wistar rats after a 1-h exposure to HgCl_2 was significantly lower than that following a 15-min exposure. Similarly, the hepatic burden of Hg^{2+} following a 24-h exposure was significantly lower than that of the 1-h and 15-min exposures. Interestingly, there was no significant difference in the hepatic burden of Hg^{2+} among the three groups of TR^- rats. The hepatic burden of Hg^{2+} was, however, significantly greater TR^- rats exposed for 1 h and 24 h than in the corresponding groups of Wistar rats.

The hematologic burden of Hg^{2+} (Figure 6) was similar in Wistar and TR^- rats. In each strain of rats, the amount of Hg^{2+} in blood was greatest after the 15-min exposure to HgCl_2 . The hematologic burden of Hg^{2+} after a 1-h exposure to HgCl_2 was significantly lower than that of the 15-min exposure. Similarly, the amount of Hg^{2+} in blood after a 24-h exposure was significantly lower than that observed after the 15-min and 1-h exposures. There was no significant difference in hematologic burden of Hg^{2+} between Wistar and TR^- rats at any time point.

DISCUSSION

Understanding the transport kinetics of mercuric conjugates at the site of a specific transport protein is an important step in understanding how mercuric ions are handled within a specific cell-type and organ. There are few data characterizing the transport kinetics of any mercuric conjugate at the site of a specific transporter. Yet, a thorough understanding of the way mercuric conjugates are eliminated from the body is important in that the development of therapeutic regimes for mercury intoxication relies on data related to the transport properties of Hg^{2+} . Since proximal tubular cells are the primary sites of Hg^{2+} accumulation and intoxication, and because they appear to play a major role in the excretion of Hg^{2+} , the current study was designed to characterize the export of mercuric ions from within proximal tubular cells. Furthermore, since MRP2 has been shown to play an important role in the export of mercuric conjugates from within proximal tubular cells [17,13,16], this study focused on characterizing the transport of Hg^{2+} at the site of MRP2.

Following Hg^{2+} intoxication, 2,3-dimercapto-1-propanesulfonic acid (DMPS) may be administered to patients as a means to extract mercuric ions from within target cells [27]. DMPS-*S*-conjugates of Hg^{2+} have been shown to be transported readily by MRP2 [17,13] but the formation of these conjugates is dependent upon intravenous administration of DMPS by a physician and subsequent uptake of DMPS into affected cells. In the current study, we chose to focus on the transport of a species of Hg^{2+} that may form in the initial moments after exposure to mercuric compounds (e.g., Cys-*S*-Hg-*S*-Cys). Since Cys-*S*-Hg-*S*-Cys may be formed in blood and target cells immediately after exposure to Hg^{2+} [28], this study mimics conditions immediately following exposure Hg^{2+} (but prior to administration of a chelator compound) [18].

Time course studies using inside-out membrane vesicles containing human MRP2 indicate that MRP2-mediated transport of Cys-*S*-Hg-*S*-Cys is linear and rapid up to one minute, at which point, accumulation within the vesicles begins to decline. Based on this finding, we suggest that the most rapid transport of Cys-*S*-Hg-*S*-Cys by MRP2 may occur in the initial moments after this mercuric conjugate is taken up into proximal tubular cells. Michaelis-Menten transport kinetics were assessed for Cys-*S*-Hg-*S*-Cys at the site of MRP2 and a K_m of $63.4 \pm 27.3 \mu\text{M}$ was calculated. MRP2 has been shown to transport numerous substrates with a variety of different affinities. Leukotriene C_4 is considered to be a high affinity substrate of MRP2 with a K_m of 250 nM [29] while the K_m for glutathione disulfide (GSSG), which is also considered to be an excellent substrate of MRP2, has been shown to be $93 \pm 26 \mu\text{M}$ [30]. The K_m for Cys-*S*-Hg-*S*-Cys is similar to that of two well-known substrates of MRP2, β -estradiol 17-(β -D-glucuronide) ($\text{E}_2\text{17G}$; $75 \pm 7.0 \mu\text{M}$) and β -estradiol 3-(β -D-glucuronide) ($\text{E}_2\text{3G}$; $122 \pm 23 \mu\text{M}$) [31]. Since the K_m values of $\text{E}_2\text{17G}$ and $\text{E}_2\text{3G}$ are similar to that of Cys-*S*-Hg-*S*-Cys, we propose that Cys-*S*-Hg-*S*-Cys is transported by MRP2 in a way similar to that of $\text{E}_2\text{17G}$ and $\text{E}_2\text{3G}$. Considering that $\text{E}_2\text{17G}$ and $\text{E}_2\text{3G}$ are often utilized as model substrates that are transported readily by MRP2, we suggest that Cys-*S*-Hg-*S*-Cys may also be transported readily by this carrier. Recently, the MRP2-mediated transport of oxaliplatin, a platinum-based chemotherapeutic agent, was characterized in MRP2-containing vesicles. The K_m of oxaliplatin was calculated to be 301 μM [32], which is significantly higher than that calculated for Cys-*S*-Hg-*S*-Cys. This finding provides some perspective on the transportability of Cys-*S*-Hg-*S*-Cys by MRP2 in that the affinity of MRP2 for Cys-*S*-Hg-*S*-Cys appears to fall in the middle of the range of substrate affinities exhibited by MRP2.

In vivo studies were utilized to assess the transport of Hg^{2+} in various organs of Wistar and TR^- rats exposed to a non-nephrotoxic dose of HgCl_2 for 15 min, 1h, or 24h. Following exposure, the disposition of Hg^{2+} was assessed in kidneys, liver, and blood. The findings from these studies suggest that the initial uptake of mercuric ions into proximal tubular cells is rapid. In the absence of MRP2, the amount of Hg^{2+} taken up after 15 min was approximately one-half of the amount of Hg^{2+} that accumulated after 1 h and about one-third of the total Hg^{2+} that accumulated after 24 h. These findings suggest that the most rapid period of Hg^{2+} uptake into target cells is in the initial minutes after exposure. Interestingly, the amount of Hg^{2+} in the cortex of TR^- rats after 15 minutes of exposure was 3.2 times greater than that of corresponding Wistar rats. In contrast, after 1 h and 24 h of exposure, the amount of Hg^{2+} in the cortex of TR^- rats was only 1.3 and 1.4 times greater,

respectively, than that of corresponding Wistar rats. These data suggest that the ability of Mrp2 to remove mercuric ions from proximal tubular cells is the greatest during the initial period of exposure. It is important to note that the breast cancer resistance protein (BCRP) has also been shown to mediate the proximal tubular export of Hg^{2+} [14] and thus, it may also play an important, albeit more minor, role in the export of mercuric ions in both Wistar and TR^- rats. It should also be considered that P-glycoprotein (multidrug-resistance protein, MDR1; *ABCB1*) has also been localized in the apical membrane of proximal tubular cells [33] and although it has not been shown to mediate the transport of mercuric ions, it may indeed play a role in this process. The protein levels of Bcrp and MDR1 have been shown to be similar in Wistar and TR^- rats [34], therefore, we conclude that differences in the accumulation of mercuric ions between these two strains of rats is not due to alterations in the ability of these proteins to transport mercuric ions.

The pattern of Hg^{2+} accumulation in the OSOM was similar to that in the cortex. Following 15 min and 1 h of exposure to Hg^{2+} , the amount of Hg^{2+} in the OSOM was greater in TR^- rats than in Wistar rats. Like in the cortex, it appears that Mrp2 may play a role in the initial export of mercuric ions from proximal tubules in the OSOM. Interestingly, the accumulation of Hg^{2+} after 24 h of exposure was similar in Wistar and TR^- rats. This finding is similar to that of previous studies that measured 24-h exposure of Wistar and TR^- rats to Hg^{2+} [13,18]. One possible explanation for this finding may relate to the distribution of Mrp2 in the kidney. The amount of Mrp2 protein has been shown to be lower in the OSOM than in the cortex [35] suggesting that Mrp2 may play less of a role in the export of mercuric ions from within proximal tubules located in the OSOM than in those located in the cortex. Interestingly, protein levels of Bcrp appear to be greater in the OSOM than in the cortex [14] and thus Bcrp may play a more significant role in the export of mercuric species from tubules in the OSOM, which may account for the lack of difference in the accumulation of Hg^{2+} after 24 h between Wistar and TR^- rats.

When rats were exposed to HgCl_2 for 1 h or 24 h, the hepatic burden of Hg^{2+} was significantly greater in TR^- rats than in that of Wistar rats. This pattern of accumulation suggests that hepatobiliary excretion may account for the decrease in hepatic accumulation of Hg^{2+} in Wistar rats, while in TR^- rats the absence of Mrp2 prevents a large fraction of Hg^{2+} from being exported at the canalicular membrane and consequently leads to accumulation of Hg^{2+} in hepatocytes. Interestingly, the hepatic burden of Hg^{2+} was not significantly different between Wistar and TR^- rats after 15 min of exposure. A possible explanation for this observation is that the accumulation of Hg^{2+} within hepatocytes must meet a minimum capacity before hepatobiliary export begins to occur. Under these circumstances, export of mercuric ions may not occur or may be minimal during the initial minutes after exposure. This pattern of accumulation could lead to similar levels of Hg^{2+} in hepatocytes of Wistar and TR^- rats.

The amount of Hg^{2+} in blood of Wistar and TR^- rats decreased significantly over time with less than 5% of the administered dose remaining in the blood after 24 h. The decrease in hematologic content of Hg^{2+} may be due to accumulation in target cells of organs and/or urinary and fecal excretion. Glomerular filtration and exposure of the basolateral plasma membrane of proximal tubular cells to mercuric species may lead to luminal and basolateral

absorption, respectively, by proximal tubular cells. Mercuric ions may be retained in proximal tubular cells or excreted subsequently in urine. In addition, Hg^{2+} in the blood is delivered to the liver where it can be taken by hepatocytes and eliminated subsequently in feces.

In summary, the current data suggest that MRP2 transports Cys-*S*-conjugates of Hg^{2+} in a manner similar to that of other substrates for which it has a moderate affinity. Furthermore, MRP2 serves as an important mechanism for the export of mercuric species out of renal tubular cells and hepatocytes. A thorough understanding of the way in which MRP2 mediates that transport of specific species of Hg^{2+} will serve as the foundation for the development of effective therapies for extraction of mercuric ions from target cells.

Acknowledgments

FUNDING INFORMATION

This work was supported by grants awarded by the National Institutes of Health (National Institute of Environmental Health Sciences) [ES019991 to CCB] and Navicent Health Foundation (awarded to CCB).

References

1. ATSDR Agency for Toxic Substance and Disease Registry. Toxicological Profile for Mercury. Centers for Disease Control; Atlanta, GA: 2008.
2. Buchanan S, Anglen J, Turyk M. Methyl mercury exposure in populations at risk: Analysis of NHANES 2011–2012. *Environ Res.* 2015; 140:56–64. DOI: 10.1016/j.envres.2015.03.005 [PubMed: 25825131]
3. Mortensen ME, Caudill SP, Caldwell KL, Ward CD, Jones RL. Total and methyl mercury in whole blood measured for the first time in the U.S. population: NHANES 2011–2012. *Environ Res.* 2014; 134:257–264. DOI: 10.1016/j.envres.2014.07.019 [PubMed: 25173092]
4. Hirayama K. Transport mechanism of methyl mercury. Intestinal absorption, biliary excretion and distribution of methyl mercury. *Kumamoto Med J.* 1975; 28(4):151–163. [PubMed: 1223551]
5. Vazquez M, Calatayud M, Velez D, Devesa V. Intestinal transport of methylmercury and inorganic mercury in various models of Caco-2 and HT29-MTX cells. *Toxicology.* 2013; 311(3):147–153. DOI: 10.1016/j.tox.2013.06.002 [PubMed: 23793072]
6. Daniel JW. The biotransformation of organomercury compounds. *Biochem J.* 1972; 130(2):64P–65P.
7. Norseth T, Clarkson TW. Studies on the biotransformation of ^{203}Hg -labeled methyl mercury chloride in rats. *Arch Environ Health.* 1970; 21(6):717–727. [PubMed: 5478557]
8. Norseth T, Clarkson TW. Biotransformation of methylmercury salts in the rat studied by specific determination of inorganic mercury. *Biochem Pharmacol.* 1970; 19(10):2775–2783. 0006-2952(70)90104-8 [pii]. [PubMed: 5478586]
9. Omata S, Sato M, Sakimura K, Sugano H. Time-dependent accumulation of inorganic mercury in subcellular fractions of kidney, liver, and brain of rats exposed to methylmercury. *Arch Toxicol.* 1980; 44(4):231–241. [PubMed: 7396710]
10. Bridges CC, Zalups RK. Mechanisms involved in the transport of mercuric ions in target tissues. *Arch Toxicol.* 2017; 91(1):63–81. DOI: 10.1007/s00204-016-1803-y [PubMed: 27422290]
11. Bridges CC, Bauch C, Verrey F, Zalups RK. Mercuric conjugates of cysteine are transported by the amino acid transporter system b(0,+): implications of molecular mimicry. *J Am Soc Nephrol.* 2004; 15(3):663–673. [PubMed: 14978168]
12. Aslamkhan AG, Han YH, Yang XP, Zalups RK, Pritchard JB. Human renal organic anion transporter 1-dependent uptake and toxicity of mercuric-thiol conjugates in Madin-Darby canine kidney cells. *Mol Pharmacol.* 2003; 63(3):590–596. [PubMed: 12606766]

13. Bridges CC, Joshee L, Zalups RK. Multidrug resistance proteins and the renal elimination of inorganic mercury mediated by 2,3-dimercaptopropane-1-sulfonic acid and meso-2,3-dimercaptosuccinic acid. *J Pharmacol Exp Ther*. 2008; 324(1):383–390. doi:jpet.107.130708 [pii]10.1124/jpet.107.130708. [PubMed: 17940195]
14. Bridges CC, Zalups RK, Joshee L. Toxicological significance of renal Bcrp: Another potential transporter in the elimination of mercuric ions from proximal tubular cells. *Toxicol Appl Pharmacol*. 2015; 285(2):110–117. DOI: 10.1016/j.taap.2015.03.027 [PubMed: 25868844]
15. Belanger M, Westin A, Barfuss DW. Some health physics aspects of working with 203Hg in university research. *Health Phys*. 2001; 80(2 Suppl):S28–30. [PubMed: 11197511]
16. Bridges CC, Joshee L, van den Heuvel JJ, Russel FG, Zalups RK. Glutathione status and the renal elimination of inorganic mercury in the Mrp2(–/–) mouse. *PloS one*. 2013; 8(9):e73559.doi: 10.1371/journal.pone.0073559 [PubMed: 24039982]
17. Bridges CC, Joshee L, Zalups RK. MRP2 and the DMPS- and DMSA-mediated elimination of mercury in TR(–) and control rats exposed to thiol S-conjugates of inorganic mercury. *Toxicol Sci*. 2008; 105(1):211–220. doi:kfn107 [pii]10.1093/toxsci/kfn107. [PubMed: 18511429]
18. Bridges CC, Joshee L, Zalups RK. MRP2 and the handling of mercuric ions in rats exposed acutely to inorganic and organic species of mercury. *Toxicol Appl Pharmacol*. 2011; 251(1):50–58. DOI: 10.1016/j.taap.2010.11.015 [PubMed: 21134393]
19. El-Sheikh AA, van den Heuvel JJ, Koenderink JB, Russel FG. Interaction of nonsteroidal anti-inflammatory drugs with multidrug resistance protein (MRP) 2/ABCC2- and MRP4/ABCC4-mediated methotrexate transport. *J Pharmacol Exp Ther*. 2007; 320(1):229–235. DOI: 10.1124/jpet.106.110379 [PubMed: 17005917]
20. Van Aubel RA, Koenderink JB, Peters JG, Van Os CH, Russel FG. Mechanisms and interaction of vinblastine and reduced glutathione transport in membrane vesicles by the rabbit multidrug resistance protein Mrp2 expressed in insect cells. *Mol Pharmacol*. 1999; 56(4):714–719. [PubMed: 10496953]
21. Mayer R, Kartenbeck J, Buchler M, Jedlitschky G, Leier I, Keppler D. Expression of the MRP gene-encoded conjugate export pump in liver and its selective absence from the canalicular membrane in transport-deficient mutant hepatocytes. *J Cell Biol*. 1995; 131(1):137–150. [PubMed: 7559771]
22. Paulusma CC, Bosma PJ, Zaman GJ, Bakker CT, Otter M, Scheffer GL, Scheper RJ, Borst P, Oude Elferink RP. Congenital jaundice in rats with a mutation in a multidrug resistance-associated protein gene. *Science*. 1996; 271(5252):1126–1128. [PubMed: 8599091]
23. de Vries MH, Redegeld FA, Koster AS, Noordhoek J, de Haan JG, Oude Elferink RP, Jansen PL. Hepatic, intestinal and renal transport of 1-naphthol-beta-D-glucuronide in mutant rats with hereditary-conjugated hyperbilirubinemia. *Naunyn Schmiedebergs Arch Pharmacol*. 1989; 340(5): 588–592. [PubMed: 2615850]
24. Masereeuw R, Notenboom S, Smeets PH, Wouterse AC, Russel FG. Impaired renal secretion of substrates for the multidrug resistance protein 2 in mutant transport-deficient (TR–) rats. *J Am Soc Nephrol*. 2003; 14(11):2741–2749. [PubMed: 14569083]
25. Smeets PH, van Aubel RA, Wouterse AC, van den Heuvel JJ, Russel FG. Contribution of multidrug resistance protein 2 (MRP2/ABCC2) to the renal excretion of p-aminohippurate (PAH) and identification of MRP4 (ABCC4) as a novel PAH transporter. *J Am Soc Nephrol*. 2004; 15(11):2828–2835. doi:15/11/2828 [pii]10.1097/01.ASN.0000143473.64430.AC. [PubMed: 15504935]
26. Lee HB, Blaufox MD. Blood volume in the rat. *J Nucl Med*. 1985; 26(1):72–76. [PubMed: 3965655]
27. Aposhian HV, Maiorino RM, Rivera M, Bruce DC, Dart RC, Hurlbut KM, Levine DJ, Zheng W, Fernando Q, Carter D, et al. Human studies with the chelating agents, DMPS and DMSA. *J Toxicol Clin Toxicol*. 1992; 30(4):505–528. [PubMed: 1331491]
28. Zalups RK. Molecular interactions with mercury in the kidney. *Pharmacol Rev*. 2000; 52(1):113–143. [PubMed: 10699157]
29. Ishikawa T, Muller M, Klunemann C, Schaub T, Keppler D. ATP-dependent primary active transport of cysteinyl leukotrienes across liver canalicular membrane. Role of the ATP-dependent

- transport system for glutathione S-conjugates. *J Biol Chem.* 1990; 265(31):19279–19286. [PubMed: 2172249]
30. Leier I, Jedlitschky G, Buchholz U, Center M, Cole SP, Deeley RG, Keppler D. ATP-dependent glutathione disulphide transport mediated by the MRP gene-encoded conjugate export pump. *Biochem J.* 1996; 314(Pt 2):433–437. [PubMed: 8670053]
31. Gerk PM, Li W, Megaraj V, Vore M. Human multidrug resistance protein 2 transports the therapeutic bile salt tauroursodeoxycholate. *J Pharmacol Exp Ther.* 2007; 320(2):893–899. DOI: 10.1124/jpet.106.106922 [PubMed: 17108237]
32. Myint K, Li Y, Paxton J, McKeage M. Multidrug Resistance-Associated Protein 2 (MRP2) Mediated Transport of Oxaliplatin-Derived Platinum in Membrane Vesicles. *PloS one.* 2015; 10(7):e0130727.doi: 10.1371/journal.pone.0130727 [PubMed: 26131551]
33. Thiebaut F, Tsuruo T, Hamada H, Gottesman MM, Pastan I, Willingham MC. Cellular localization of the multidrug-resistance gene product P-glycoprotein in normal human tissues. *Proc Natl Acad Sci U S A.* 1987; 84(21):7735–7738. [PubMed: 2444983]
34. Johnson BM, Zhang P, Schuetz JD, Brouwer KL. Characterization of transport protein expression in multidrug resistance-associated protein (Mrp) 2-deficient rats. *Drug Metab Dispos.* 2006; 34(4): 556–562. doi:dmd.105.005793 [pii]10.1124/dmd.105.005793. [PubMed: 16204465]
35. Zalups RK, Joshee L, Bridges CC. Novel Hg²⁺-induced nephropathy in rats and mice lacking mrp2: evidence of axial heterogeneity in the handling of Hg²⁺ along the proximal tubule. *Toxicol Sci.* 2014; 142(1):250–260. DOI: 10.1093/toxsci/kfu171 [PubMed: 25145654]

Figure 1.

Uptake of PAH and Cys-*S*-Hg-*S*-Cys in the Presence of GSH

The ATP-dependent transport of 50 nM [³H]-*p*-aminohippurate into inside-out membrane vesicles containing human MRP2 was measured for 30 sec at 37°C in the presence and absence of glutathione (GSH) (A). In addition, the ATP-dependent transport of 10 μM Cys-*S*-Hg-*S*-Cys into inside-out membrane vesicles containing human MRP2 was measured for 30 sec at 37°C in the presence or absence of GSH (B).

Figure 2.

Time-course and Saturation Kinetics of MRP2-mediated Transport of Cys-*S*-Hg-*S*-Cys. The transport of 5 μ M radioactive Cys-*S*-Hg-*S*-Cys into inside-out membrane vesicles containing human MRP2 was measured for 15 sec, 30 sec, 1 min, 2.5 min, and 5 min (A). Analyses of Michaelis-Menten kinetics were also carried out in inside-out membrane vesicle containing human MRP2. Vesicles were incubated with 5 μ M radioactive Cys-*S*-Hg-*S*-Cys in the presence of buffer or 50 μ M, 100 μ M, or 500 μ M unlabeled Cys-*S*-Hg-*S*-Cys (B). The maximum velocity ($V_{\max}$) for the transport of Cys-*S*-Hg-*S*-Cys via MRP2 was estimated to be 74.3 ± 10.1 nmol mg protein⁻¹ 30 sec⁻¹ and the Michaelis constant (K_m) was 63.4 ± 27.3 μ M.

Figure 3.

Burden of Hg in the Total Renal Mass

The amount of Hg in the total renal mass of Wistar and TR⁻ kidneys was measured 15 min, 1h, or 24h after exposure to 0.5 $\mu\text{mol kg}^{-1}$ HgCl₂. *, significantly different ($p < 0.05$) from the mean of the corresponding group of Wistar rats. +, significantly different ($p < 0.05$) from the mean of the group of rats of the same strain of rats exposed for 15 min. #. significantly different ($p < 0.05$) from the mean of the group of rats of the same strain exposed for 1 h.

Figure 4.

Amount of Hg in the Renal Cortex and Outer Stripe of the Outer Medulla

The amount of Hg in the renal cortex (A) and outer stripe of the outer medulla (OSOM; B) of Wistar and TR⁻ kidneys was measured 15 min, 1h, or 24h after exposure to 0.5 $\mu\text{mol kg}^{-1}$ HgCl₂. *, significantly different ($p < 0.05$) from the mean of the corresponding group of Wistar rats. +, significantly different ($p < 0.05$) from the mean of the group of rats of the same strain of rats exposed for 15 min. #. significantly different ($p < 0.05$) from the mean of the group of rats of the same strain exposed for 1 h.

Figure 5.

Hepatic Burden of Hg

The amount of Hg in the liver of Wistar and TR⁻ kidneys was measured 15 min, 1h, or 24h after exposure to 0.5 $\mu\text{mol kg}^{-1}$ HgCl_2 . *, significantly different ($p < 0.05$) from the mean of the corresponding group of Wistar rats. +, significantly different ($p < 0.05$) from the mean of the group of rats of the same strain of rats exposed rats exposed for 15 min. #. significantly different ($p < 0.05$) from the mean of the group of rats of the same strain exposed for 1 h.

Figure 6.

Amount of Hg in Blood

The amount of Hg in the total blood of Wistar and TR⁻ kidneys was measured 15 min, 1h, or 24h after exposure to 0.5 $\mu\text{mol kg}^{-1}$ HgCl₂. *, significantly different ($p < 0.05$) from the mean of the corresponding group of Wistar rats. +, significantly different ($p < 0.05$) from the mean of the group of rats of the same strain of rats exposed rats exposed for 15 min. #. significantly different ($p < 0.05$) from the mean of the group of rats of the same strain exposed for 1 h.