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Accelerated Methylmercury Elimination in γ-Glutamyl Transpeptidase-Deficient Mice

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The disposition and toxicity of methylmercury, a ubiquitous environmental pollutant, is modulated by binding to the endogenous tripeptide glutathione (GSH) and metabolism of the resulting methylmercury-glutathione complex by the ectoproteins γ-glutamyl transpeptidase (GGT) and dipeptidase. To evaluate the role of GGT in the whole-body disposition of methylmercury, we compared the elimination of [^{203}Hg]methylmercury in GGT-deficient mice with that in wild-type mice and mice heterozygous for this deficiency. The effects of *N*-acetylcysteine (NAC), a drug used to maintain the cysteine and GSH levels of GGT-deficient mice, were also examined. Female mice were treated with either 0.5 or 25 μmol of $CH_3{}^{203}HgCl$/kg body weight, in the presence and absence of 10 mg/ml NAC in the drinking water. There were no differences in methylmercury excretion between the wild-type and heterozygous mice; however, the GGT-deficient mice excreted methylmercury more rapidly at both dose levels. Wild-type and heterozygous mice excreted from 11 to 24% of the dose in the first 48 hours, whereas the GGT-deficient mice excreted 55 to 66% of the dose, with most of the methylmercury being excreted in urine. Urinary methylmercury excretion was further accelerated in mice that received NAC. In contrast to methylmercury, the whole-body elimination of inorganic mercury was not affected by GGT deficiency, although the tissue distribution of inorganic mercury was markedly different in GGT-deficient male mice, with only 13% of the ^{203}Hg body burden in the kidneys of GGT-deficient mice *versus* ~50% in kidneys of wild-type male mice. These findings provide direct evidence for a major role of GGT in regulating the tissue distribution and elimination of methylmercury and inorganic mercury and provide additional support for the use of NAC as an antidote in methylmercury poisoning. *(Am J Pathol 1998, 152:1049–1055)*

A major function of cellular glutathione (GSH) involves the transport, storage, and metabolism of metals, including toxic metals such as inorganic mercury and methylmercury.[1,2] GSH functions in the mobilization and delivery of metals between ligands, in the transport of metals across cell membranes, as a source of cysteine for metal binding, and as a reductant or cofactor in metal redox reactions.[1]

A key enzyme involved in the metabolism of GSH, its mercaptides, and *S*-conjugates, is γ-glutamyl transpeptidase (GGT). GGT catalyzes the initial step in the catabolism of glutathione-containing compounds, the removal of the glutamyl moiety from the tripeptide.[3,4] A dipeptidase subsequently cleaves cysteinylglycine to release glycine and either free cysteine or a cysteine *S*-conjugate when the parent compound is a cysteinylglycine *S*-conjugate. Because GGT and dipeptidase are membrane-bound enzymes with their active sites oriented extracellularly, these reactions occur in the extracellular space and the breakdown products are then reabsorbed back into the cell for further metabolism.[3,5] GGT is most abundant in epithelial tissues, including kidney, intestine, liver, and pancreas.[3,4]

GGT and dipeptidase modulate the distribution and toxicity of metals by facilitating the formation of cysteine-mercaptides, which appear to be excellent substrates for cellular uptake systems.[1,6] In liver and bile, copper, zinc, silver, lead, arsenic, chromium, inorganic mercury, and methylmercury are present in part as GSH complexes.[2] Within the biliary tree, these GSH-mercaptides are degraded by GGT and dipeptidase, and the breakdown products are subject to reabsorption from bile back into the liver. For example, the methylmercury that is secreted into bile is extensively reabsorbed from intrahepatic biliary spaces[7] and the gallbladder[8] in a process facilitated by the GGT-mediated conversion of the methylmercury-glutathione complex to the cysteine complex.

GGT is also involved in metal transport across the blood-brain barrier and in metal accumulation by the kidney and intestines.[1] The kidney has the highest GGT activity in the body and is a principal site of heavy metal accumulation and toxicity. The GGT on the luminal surface of the renal proximal tubular cells completely de-

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grades GSH-containing compounds that are filtered through the glomerulus or secreted by renal epithelial cells.[9] Lower levels of GGT are also present on the basolateral membrane of renal epithelium,[10,11] and this basolateral GGT mediates the catabolism of circulating GSH and GSH adducts, including GSH-mercaptides. For example, inhibition of renal GGT activity with acivicin, an irreversible inhibitor of GGT, accelerates urinary excretion of both GSH and methylmercury and decreases renal methylmercury and inorganic mercury uptake.[12–25] Strain differences in renal methylmercury excretion are related to differences in urinary GSH excretion and renal GGT activity.[21,26] Furthermore, age, sex, and species differences in renal methylmercury accumulation correlate with differences in renal GGT activity.[21,22,27,28] Taken together, these studies indicate that the GSH-mercury complexes that are delivered to the kidney are substrates for GGT and dipeptidase and that the resulting metabolic products are readily taken up by the kidney cells. Inhibition of GGT activity with acivicin also protects the kidney from cisplatin-induced toxicity; however, this may be unrelated to a change in metal uptake by the kidney.[29] In contrast, acivicin fails to protect against dichromate-induced renal injury.[30]

To directly examine the role of GGT in the disposition and clearance of methylmercury and inorganic mercury, we used mice deficient in GGT.[31] Earlier studies relying on inhibitors such as acivicin are limited by the short duration and incomplete inhibition of GGT as well as the possibility of other unsuspected effects of acivicin.[32–35] For example, γ-glutamyl leukotrienase and a similar or identical enzyme termed γ-glutamyl transpeptidase-related (GGT-rel) are also inhibited by acivicin.[36,37] The use of GGT-deficient mice avoids these complications and allows studies of long duration.

Materials and Methods

Materials and Animals

Wild-type, heterozygous, and homozygous GGT-deficient mice (8 weeks of age) were obtained from our colony at Baylor College of Medicine. Mice were generated and genotyped as previously described.[31] They were maintained on Purina Mills laboratory chow with water *ad libitum* in a temperature-controlled room with a 12-hour alternating light cycle. Some of the mice were given *N*-acetylcysteine (NAC) in the drinking water (10 mg/ml) to maintain their cysteine and GSH status.[31] NAC was purchased from Sigma Chemical Co. (St. Louis, MO). It was dissolved in water, with the pH adjusted to 6.7 to 6.8 by addition of 5 mol/L NaOH, and diluted to a final concentration of 10 mg/ml. Fresh NAC solutions were prepared every 2 to 3 days. Experiments were conducted in accordance with the guidelines of the National Institutes of Health for care of laboratory animals.

Methylmercury chloride was obtained from ICN (Plainsville, NY), and $^{203}HgCl_2$ was purchased from Amersham Corp. (Arlington Heights, IL). $CH_3{}^{203}HgCl$ was synthesized from $^{203}HgCl_2$ by the method of Toribara.[38] Purity of the synthesized [^{203}Hg]methylmercury was confirmed using cold vapor atomic absorption.[39] The product contained less than 1% inorganic mercury impurity.

Whole-Body Elimination of Methylmercury and Inorganic Mercury

Mice were placed individually in stainless steel metabolic cages (Lab Products, Rochelle Park, NJ) and were allowed to acclimate to the cages for 4 days. Urine and feces were collected onto plastic-lined absorbent bench paper placed underneath each cage. Feces was separated from the urine-stained paper, and each was stored in small plastic bags. Female mice were injected intraperitoneally (i.p.) with ^{203}Hg-labeled methylmercuric chloride (0.5 or 25 μmol/kg body weight), and male mice with $^{203}HgCl_2$ (0.2 μmol/kg, i.p.). Injection solutions were prepared in Krebs-Henseleit buffer and administered in a volume of 10 ml/kg body weight (20 μCi/kg). Body burden was measured daily in a Packard model 3002 whole-body gamma counter. ^{203}Hg standards prepared from the dosing solutions were counted daily in the same instrument to correct for decay and counter efficiency.

After 5 days, mice were anesthetized with sodium pentobarbital (55 mg/kg, i.p.), and selected tissues (liver, kidney, brain, and whole blood) were taken for GSH and ^{203}Hg analysis. The animals were killed between 1:00 and 4:00 pm. Whole blood was withdrawn from the abdominal vena cava. The median lobe of the liver, one kidney, the brain, and ~0.2 to 0.3 ml of whole blood were each placed in tared tubes containing 5 ml of 2.5% sulfosalicylic acid/0.25 mmol/L acivicin for subsequent GSH determination, as described previously.[40] The brain and blood samples were counted in a Packard model 3002 gamma counter for 30 seconds each and then homogenized, whereas the liver and kidney samples were homogenized immediately. The rest of the liver and the other kidney were removed, weighed, and used for ^{203}Hg determination.

Results

GGT-Deficient Mice Clear Methylmercury More Rapidly Than Wild-Type Mice

We measured methylmercury excretion in female wild-type mice and mice homozygous and heterozygous for GGT deficiency after treatment with 0.5 μmol/kg body weight of $CH_3{}^{203}HgCl$ i.p. (Figure 1). Mice of each genotype were also given 10 mg/ml NAC in the drinking water starting 48 hours after methylmercury administration and continuing until the end of the experiment (Figure 1A) or from 48 hours before to 48 hours after methylmercury administration (Figure 1B).

No differences in methylmercury excretion were seen between the wild-type and heterozygous mice; however, the GGT-deficient mice excreted methylmercury much faster (Figure 1A). Wild-type and heterozygous mice excreted 22 to 24% of the dose during the first 48 hours

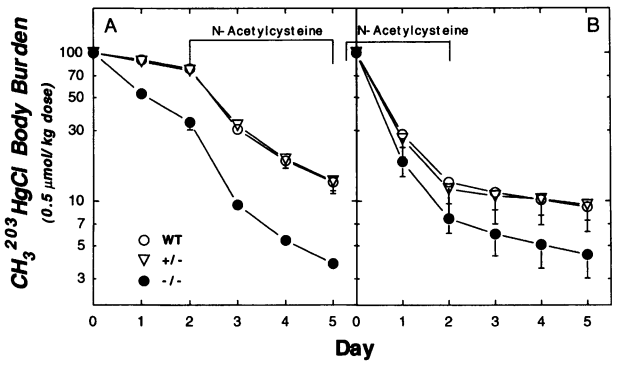

Figure 1. Body burden of methylmercury in wild-type (WT) female mice and female mice that are heterozygous (+/−) and homozygous (−/−) for GGT deficiency. Mice were injected intraperitoneally with 0.5 μmol/kg body weight of $CH_3{}^{203}HgCl$ at day zero. Mice of each genotype were also given 10 mg/ml *N*-acetylcysteine in the drinking water starting either 48 hours after methylmercury administration (**A**) and continuing until the end of the experiment or from 48 hours before to 48 hours after methylmercury administration (**B**). Values are means ± SEM of five mice for the wild-type and heterozygous mice and three for the GGT-deficient mice. Error bars are shown only if they are larger than the size of the symbols.

(Figure 1A), which corresponds to a biological half-life of ~6 days. This value is comparable to that reported in previous studies in mice.[41,42] In contrast, GGT-deficient mice excreted 66% of the dose during the same time period (a $t_{1/2}$ of just over 1 day; Figure 1A). Most of the methylmercury was excreted in urine (Figure 2A). Wild-type mice excreted 4% of the dose in urine during the first 2 days, as compared with 45% in GGT-deficient mice (Figure 2A). There were no differences in fecal ^{203}Hg excretion (Figure 3A).

NAC Accelerates Methylmercury Clearance in Wild-Type and GGT-Deficient Mice

When NAC was added to the drinking water starting 48 hours after methylmercury administration, it produced a dramatic acceleration of whole-body methylmercury elimination in both wild-type and GGT-deficient female mice (Figure 1A). These findings are in agreement with our previous findings in wild-type mice of both sexes.[43]

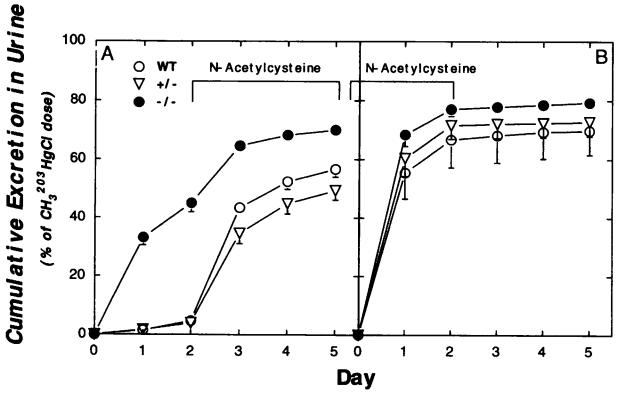

Figure 2. Cumulative urinary [^{203}Hg]methylmercury excretion in female mice that received 0.5 μmol/kg body weight of $CH_3{}^{203}HgCl$. Other explanatory information is provided in the legend for Figure 1. Values are means ± SEM of five mice for the wild-type and heterozygous mice, and three for the GGT-deficient mice.

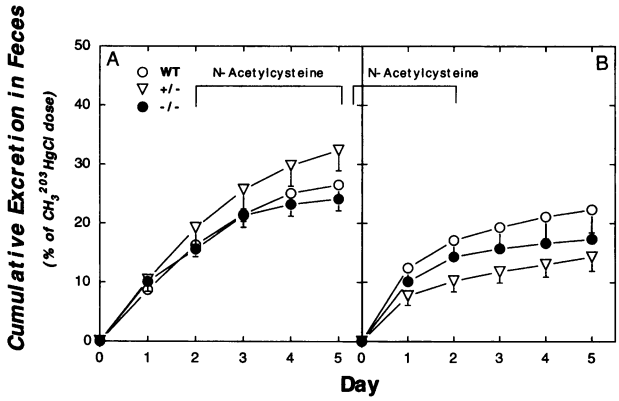

Figure 3. Cumulative fecal [^{203}Hg]methylmercury excretion in female mice that received 0.5 μmol/kg body weight of $CH_3{}^{203}HgCl$. Other explanatory information is provided in the legend for Figure 1. Values are means ± SEM of five mice for the wild-type and heterozygous mice, and three for the GGT-deficient mice.

Most of the methylmercury was excreted in urine (Figure 2A). NAC had no effect on fecal excretion (Figure 3A).

NAC was even more effective when given continuously from the time of methylmercury administration (Figure 1B). NAC accelerated methylmercury elimination even in GGT-deficient mice (Figure 1B). Once again, most of the methylmercury excreted after NAC administration appeared in urine (Figure 2B). When NAC was removed from the drinking water at 48 hours after dosing with methylmercury, there was an abrupt decrease in methylmercury excretion (Figure 1B). The biological half-life for methylmercury after NAC withdrawal was similar to that seen in mice that did not receive NAC.

GGT Deficiency and NAC Administration Enhance Elimination of High Doses of Methylmercury

Methylmercury elimination was also faster in GGT-deficient female mice given a 50-fold larger dose of methylmercury, 25 μmol/kg body weight (Figure 4). This dose is at the threshold of toxicity in mice,[44] but no clinical signs of toxicity were noted in the present study. The results at this higher dose (Figure 4) are similar to those at the lower dose (Figure 1), except that the biological half-life was somewhat longer. Wild-type and heterozygous mice excreted 11 to 16% of the methylmercury dose during the first 2 days, corresponding to a $t_{1/2}$ of ~9 days (open symbols in Figure 4A). In contrast, GGT-deficient mice excreted 55% of the dose in the first 2 days (Figure 4A). NAC had similar effects at the high methylmercury dose as at the lower dose (compare Figures 1 and 4).

GGT Deficiency and NAC Administration Have No Effect on the Whole-Body Elimination of Inorganic Mercury in Male Mice

Excretion of inorganic mercury was faster than that of methylmercury (a $t_{1/2}$ of <2 days), and wild-type, heterozygous, and GGT-deficient mice showed similar rates

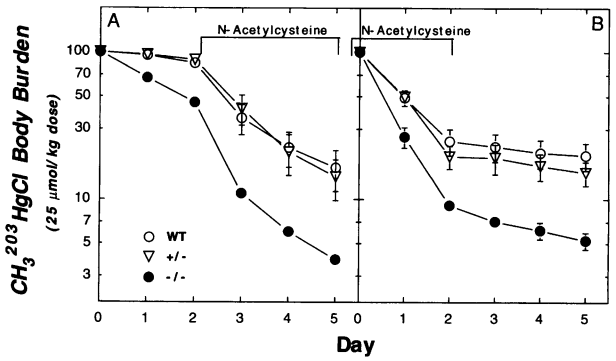

Figure 4. Body burden of methylmercury in wild-type (WT) female mice and mice that are heterozygous (+/−) and homozygous (−/−) for GGT deficiency that were injected intraperitoneally with 25 μmol/kg body weight of $CH_3{}^{203}HgCl$ at day zero. Mice of each genotype were also given 10 mg/ml *N*-acetylcysteine in the drinking water starting either 48 hours after methylmercury administration and continuing until the end of the experiment (**A**) or from 48 hours before to 48 hours after methylmercury administration (**B**). Values are means ± SEM of five mice for the wild-type and heterozygous mice and three for the GGT-deficient mice.

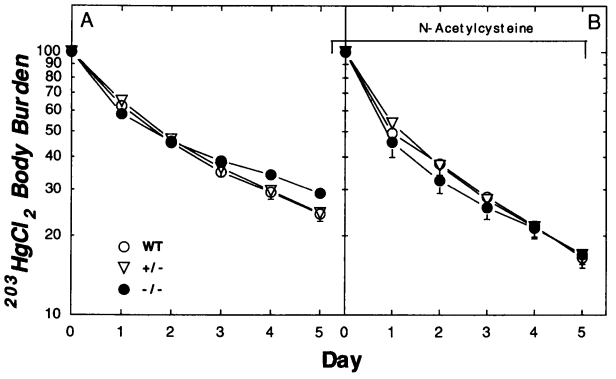

Figure 5. Body burden of inorganic mercury in wild-type (WT) male mice (n = 3) and mice that are heterozygous (+/−; n = 5) and homozygous (−/−; n = 4) for GGT deficiency. Mice were injected intraperitoneally with 0.2 μmol/kg body weight of $^{203}HgCl_2$. One group of mice received *N*-acetylcysteine in the drinking water (10 mg/ml; **B**). Values are means ± SEM.

of clearance, both in the absence (Figure 5A) and presence (Figure 5B) of NAC. In this experiment, NAC was given continuously over the 5-day period (Figure 5B). A comparison of the data in Figure 5, A and B, also demonstrates that NAC did not stimulate excretion of inorganic mercury, in agreement with our previous findings in wild-type mice of both sexes.[43]

GGT Deficiency Alters Retention of Inorganic Mercury in Individual Organs

Despite the lack of effect of GGT deficiency on whole-body elimination of inorganic mercury, the ^{203}Hg tissue distribution was markedly different, with only 13% of the body burden residing in the kidneys of GGT-deficient mice *versus* ~50% in kidneys of the wild-type and heterozygous mice (Table 1). The lower kidney levels in GGT-deficient mice was compensated nearly quantitatively by higher levels of ^{203}Hg in the liver (Table 1), suggesting a redistribution of inorganic mercury from kidney to liver with no net change in whole-body content. Despite this tissue redistribution, there were no differences in urinary or fecal excretion of inorganic mercury between wild-type, heterozygous, and GGT-deficient mice (data not shown). These findings confirm previous studies showing that acivicin-pretreated animals accumulate less inorganic mercury in their kidneys.[18,20,25]

Measurement of glutathione levels in liver, kidney, brain, and whole blood indicated that these levels were not different in wild-type or heterozygous mice that received NAC for 7 consecutive days *versus* mice that did not receive NAC (Table 1). Thus, although some of the NAC may be converted to cysteine,[45] this additional cysteine does not increase tissue GSH levels in wild-type or heterozygous mice fed *ad libitum*, supporting previous observations in wild-type mice,[46] (Table 1). In contrast, glutathione levels were lower in GGT-deficient animals (Table 1), as expected.[31]

Methylmercury was more evenly distributed among the tissues analyzed (Table 2). Note that at necropsy (5 days) the GGT-deficient mice had excreted more than 96% of the original dose, whereas the other mice had excreted approximately 85% of the dose (Figure 4). Thus, although the amount of [^{203}Hg]methylmercury remaining in the tissues of GGT-deficient mice was generally much lower, the percentage of the body burden was comparable (Table 2). Similar to inorganic mercury, the percentage of the [^{203}Hg]methylmercury body burden in liver and brain was elevated in GGT-deficient mice (Table 2). Wild-type and heterozygous mice had 8 to 10% of the [^{203}Hg]methylmercury body burden in the liver, as compared with 23 to 31% for GGT-deficient mice (Table 2). Kidneys had

Table 1. Inorganic Mercury (^{203}Hg) and Glutathione (GSH) Content at 5 Days after Administration of 0.2 μmol of $^{203}HgCl_2$/kg to GGT-Deficient (−/−), Heterozygous (+/−), and Wild-Type (WT) Male Mice, in the Presence and Absence of 10 mg/ml *N*-Acetylcysteine (NAC) in Their Drinking Water

		Whole blood		Kidneys		Liver		Brain	
	n	GSH	^{203}Hg	GSH	^{203}Hg	GSH	^{203}Hg	GSH	^{203}Hg
WT	3	1.3 ± 0.1	2.0 ± 0.1	4.6 ± 0.5	53.1 ± 1.8	7.9 ± 0.5	6.4 ± 1.1	2.5 ± 0.1	0.8 ± 0.1
+/−	5	1.3 ± 0.1	2.2 ± 0.7	5.4 ± 0.2	50.3 ± 1.5	8.0 ± 0.4	6.8 ± 0.8	2.7 ± 0.1	0.6 ± 0.1
−/−	4	1.4 ± 0.1	1.4 ± 0.4	4.2 ± 0.4	12.9 ± 0.7*	2.8 ± 0.3*	41.6 ± 3.9*	1.7 ± 0.1*	1.0 ± 0.2
WT + NAC	3	1.2 ± 0.1	1.2 ± 0.2	5.0 ± 0.1	31.9 ± 0.6	7.1 ± 0.3	14.0 ± 1.2	2.7 ± 0.1	0.9 ± 0.2
+/− + NAC	5	1.4 ± 0.1	1.3 ± 0.3	5.2 ± 0.2	38.7 ± 1.8	7.0 ± 0.2	9.8 ± 0.5	2.6 ± 0.1	0.8 ± 0.1
−/− + NAC	4	1.8 ± 0.3	1.4 ± 0.3	6.5 ± 0.4	14.0 ± 1.5*	3.7 ± 0.6*	19.0 ± 1.3	3.0 ± 0.2	1.9 ± 0.3

Values are means ± SEM. GSH concentration is expressed as μmol/g tissue and ^{203}Hg as a percentage of the body burden at 5 days. Blood ^{203}Hg content was calculated assuming a blood volume of 55 ml/kg body weight.
*Significantly different from the respective wild-type mice ($P < 0.025$), using Student's t-test.

Table 2. [^{203}Hg]Methylmercury and Glutathione (GSH) Content at 5 Days after Administration of 25 μmol of CH_3 ^{203}HgCl/kg Body Weight to GGT-Deficient (−/−), Heterozygous (+/−), and Wild-Type (WT) Female Mice, in the Presence and Absence of 10 mg/ml *N*-Acetylcysteine (NAC) in Their Drinking Water (from 2 to 5 Days after Methylmercury Administration)

		Whole blood		Kidneys		Liver		Brain	
	n	GSH	^{203}Hg	GSH	^{203}Hg	GSH	^{203}Hg	GSH	^{203}Hg
WT	5	0.9 ± 0.1	2.4 ± 0.7	4.3 ± 0.1	6.1 ± 1.0	7.8 ± 0.5	9.5 ± 1.5	2.0 ± 0.1	1.8 ± 0.1
+/−	5	1.0 ± 0.1	1.8 ± 0.8	4.3 ± 0.1	5.2 ± 1.4	6.2 ± 0.3	9.0 ± 1.9	2.2 ± 0.1	3.4 ± 1.0
−/−	3	1.0 ± 0.1	1.6 ± 0.5	2.4 ± 0.1*	4.2 ± 0.6	1.6 ± 0.1*	22.9 ± 2.3*	1.6 ± 0.1	3.2 ± 0.6
WT + NAC	5	1.0 ± 0.1	1.8 ± 0.9	3.9 ± 0.2	4.4 ± 1.4	6.9 ± 0.4	8.5 ± 2.6	2.2 ± 0.1	2.2 ± 0.6
+/− + NAC	5	0.9 ± 0.1	2.6 ± 0.3	4.0 ± 0.1	6.1 ± 1.0	7.6 ± 0.4	9.8 ± 1.8	2.0 ± 0.1	1.5 ± 0.2
−/− + NAC	3	0.9 ± 0.1	1.6 ± 1.1	5.8 ± 0.6*	3.5 ± 0.6	6.6 ± 0.6	31.3 ± 5.0*	2.6 ± 0.1	5.2 ± 0.1*

Values are means ± SEM. GSH concentration is expressed as μmol/g tissue and ^{203}Hg as a percentage of the body burden at 5 days. Blood ^{203}Hg content was calculated assuming a blood volume of 55 ml/kg body weight.
*Significantly different from the respective wild-type mice ($P < 0.025$), using Student's *t*-test.

a slightly lower percentage of the body burden, but the difference was not statistically significant.

Discussion

The present study demonstrates that GGT-deficient mice excrete methylmercury significantly faster than wild-type mice and provides direct support for the hypothesis that GGT modulates the disposition of metals that complex with GSH. This accelerated excretion was seen both in the presence and absence of NAC in the drinking water, indicating that this difference is not explained by the lower GSH and cysteine levels in GGT-deficient mice. Oral NAC administration maintains tissue cysteine and GSH concentrations at near normal levels in the GGT-deficient mice[31] (Tables 1 and 2).

The accelerated methylmercury excretion observed in GGT-deficient mice confirms previous reports that used acivicin in short-term studies to inhibit GGT activity in mercury-treated animals[12–25] but provides a more compelling argument for a role of this enzyme in regulating mercury distribution and excretion. Although acivicin is an excellent irreversible inhibitor of GGT activity, this compound only partially inhibits GGT activity *in vivo*, even when administered in relatively high doses. The extent of enzyme inhibition also differs among tissues. In addition, acivicin inhibits other enzymes, including enzymes related to GGT.[36,37] Moreover, at high doses, acivicin has been shown to have additional effects that complicate interpretation of results.[32–35] Thus, these studies in GGT-deficient mice provide more definitive evidence for the role of this enzyme in regulating methylmercury and inorganic mercury distribution and excretion, although it is possible that factors other than differences in GGT activity may have also contributed to the changes in mercury disposition.

Most of the methylmercury excreted by the GGT-deficient mice appeared in urine, demonstrating a central role for kidney GGT in regulating methylmercury excretion. Although there are several mechanisms by which methylmercury may enter the tubular lumen, including glomerular filtration, secretion as a glutathione complex, or secretion as a complex with another ligand, the high urinary methylmercury and GSH excretion observed in GGT-deficient mice is consistent with secretion as a GSH complex. Tubular secretion most likely predominates over glomerular filtration owing to both the high rate of GSH secretion by kidney cells[9] and the low plasma GSH and methylmercury concentrations.[1] Recent studies demonstrate that the methylmercury-GSH complex is a substrate for GSH transport systems, at least in the liver.[47] Regardless of the mechanism by which methylmercury enters the renal tubular lumen, it is likely that it binds to GSH, which is present in high concentrations at this site in GGT-deficient mice.[31]

Our data may have important implications for human susceptibility to methylmercury; however, the expression and regulation of GGT in humans is not well understood. Unlike in the mouse in which GGT is encoded by a single gene, in humans several genes encode GGT (summarized in Ref. 4). Also, it is unknown what role each of these genes plays in tissue-specific expression in humans. Nevertheless, it is clear from our mouse data that high levels of GGT expression are related to increased methylmercury retention (see Figure 1), but the pattern is complex, with clearance being related to urinary excretion and the tissue distribution of retained methylmercury being different in wild-type and GGT-deficient mice (Table 2, liver). In mice and rats, kidney levels of GGT in males are approximately twice as high as females,[31] making male mice more vulnerable to the accumulation of methylmercury in the kidney than females. We do not have similar GGT data for humans, nor do we know the frequency of heterozygosity at any of the GGT loci in humans that control expression in different tissues. All of these factors make it difficult to extrapolate directly to humans from the mouse data. However, based on our findings and earlier experiments with acivicin inhibition of GGT, it would be surprising if clearance of methylmercury (and its toxicity) were not also dependent on genetically and hormonally regulated GGT levels. As we know that excretion of other organometallic compounds and coordination complexes is also dependent on GGT levels,[48] a better understanding of the regulation and expression of GGT in humans is necessary if we are to understand the human genetics of organometal toxicity.

Urinary methylmercury excretion was further accelerated by NAC feeding in both wild-type and GGT-

deficient female mice. This observation confirms our previous findings in wild-type mice of both sexes and supports the use of NAC as an antidote in methylmercury poisoning.[43] In addition, these data indicate that a more effective strategy to accelerate methylmercury excretion may involve the combined use of NAC and acivicin to inhibit GGT activity. NAC is currently used clinically,[49,50] and acivicin has been evaluated in phase I and phase II clinical trials as an anticancer agent.[32,34] It is worth noting that the combined effects of GGT deficiency and NAC treatment were seen at both methylmercury dose levels, indicating that this may be an effective strategy in individuals poisoned with high doses of methylmercury (Figure 4).

In contrast to methylmercury, whole-body elimination of inorganic mercury was not stimulated by GGT deficiency. Inorganic mercury excretion was similar between wild-type, heterozygous, and homozygous male mice, both in the presence and absence of NAC in the drinking water. The lack of effect of NAC on inorganic mercury excretion is consistent with previous findings.[51]

Although the body burden of inorganic mercury was not affected by GGT deficiency, the tissue distribution was markedly different in GGT-deficient animals, with only 13% of the ^{203}Hg body burden residing in the kidneys of GGT-deficient mice *versus* ~50% in kidneys of the other mice (Table 1). Conversely, liver inorganic mercury content of the wild-type animals was ~6% of the body burden *versus* 42% in livers of GGT-deficient mice. This redistribution away from kidneys, which contain from one-third to one-half of the body burden, may limit the amount of inorganic mercury that can be excreted in urine.

Despite the higher liver inorganic mercury levels in GGT-deficient mice, fecal mercury excretion was unaffected (data not shown), indicating that the higher hepatic levels do not necessarily lead to higher biliary and fecal excretion of inorganic mercury. The reason for this is not clear but may be attributed in part to the multiple pathways by which inorganic mercury both enters and is reabsorbed from the gastrointestinal tract[52,53] and the complex interorgan metabolism of GSH and its metal complexes.[1,5,6] One possibility is that biliary excretion of both inorganic mercury and methylmercury may be impaired in GGT-deficient mice due to the generally lower hepatic GSH levels (Tables 1 and 2). Our previous studies demonstrate that biliary excretion of methylmercury and inorganic mercury is dependent on the rate of biliary GSH secretion,[1,2] which is in turn proportional to cellular GSH levels.[54] Thus, the higher hepatic levels of inorganic mercury and methylmercury in GGT-deficient mice may be counterbalanced by a less efficient biliary excretory process, leading to no net change in biliary (and possibly fecal) excretion. Additional studies are needed to test this hypothesis and to elucidate the mechanisms by which mercurial compounds partition among various tissue compartments.

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