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CELLULAR GLUTATHIONE AS A DETERMINANT OF SENSITIVITY TO MERCURIC CHLORIDE TOXICITY

PREVENTION OF TOXICITY BY GIVING GLUTATHIONE MONOESTER

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Abstract—Depletion of glutathione (GSH) by treatment of mice with buthionine sulfoximine (BSO), an effective inhibitor of γ-glutamylcysteine synthetase, markedly enhanced (about 10-fold) the lethal and renal toxicity of mercuric chloride. The lethal toxicity of $HgCl_2$ was prevented by administration of GSH monoester; this was observed in mice pretreated with BSO and given a low dose of $HgCl_2$, and also in untreated mice that were given a much higher dose of $HgCl_2$. In contrast, administration of GSH did not protect. Since administered GSH is not transported effectively into cells, whereas GSH monoester is transported and split intracellularly to GSH, the findings indicate that protection against $HgCl_2$ requires intracellular GSH. The experimental approaches used here suggest that cellular GSH is a major determinant of sensitivity to $HgCl_2$ toxicity, and also that administration of GSH esters may be useful for prevention of $HgCl_2$ toxicity.

Glutathione (L-γ-Glu-L-CysH-Gly; GSH), usually the most abundant cellular thiol, functions in metabolism, transport, as a coenzyme, and in the protection of cells against the toxic effects of a variety of compounds including oxygen and certain exogenous compounds such as heavy metals [1, 2]. The development of a method that leads to selective cellular GSH deficiency [3–6], and also of a procedure that selectively reverses such a deficiency [7–12], has made it possible to approach a closer understanding of the cellular functions of GSH (see Fig. 1). Thus, administration of buthionine sulfoximine (BSO), a selective inhibitor of γ-glutamylcysteine synthetase, inhibits GSH synthesis, and leads to decreased cellular levels of GSH. The rates at which cellular levels of GSH decrease are determined by the rates of GSH utilization and export.

Administration of GSH monoesters increases cellular levels of GSH because, in contrast to GSH itself, the GSH esters are transported efficiently into cells and split intracellularly to yield GSH [7–12]. When GSH is administered intraperitoneally the extracellular (but not the intracellular) level of GSH is increased substantially. Thus, the plasma levels increase markedly, but the GSH levels of various tissues do not increase appreciably; the small increase that may occur is associated with extracellular degradation of GSH, transport of the products, and cellular synthesis of GSH.

The toxicity of mercuric ions has been examined extensively, especially its effect in producing renal failure (see, for example, Refs 13–15). Administration of various thiol compounds has been reported to decrease Hg^{2+} toxicity, and a number of studies have appeared on the accumulation of Hg^{2+} by tissues. In the present work, we have examined the effects of GSH deficiency produced by inhibition of GSH synthesis, and of administration of GSH and of GSH monoester on the toxicity of Hg^{2+}.

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EXPERIMENTAL PROCEDURES

Materials. GSH and $HgCl_2$ were obtained from the Sigma Chemical Co. (St. Louis, MO). L-Buthionine-*SR*-sulfoximine (BSO) [3, 4, 16] and GSH monoisopropyl ester 1/2 (H_2SO_4) [7, 9, 10, 17] were prepared as described. Swiss–Webster male mice (24–28 g) were obtained from Taconic Farms (Germantown, NY) and housed according to NIH guidelines.

Methods. The mice were injected subcutaneously with solutions of $HgCl_2$ in saline. They were injected subcutaneously with BSO (3 mmol/kg of body weight) twice daily, 16.5 hr and 4.5 hr prior to injection of $HgCl_2$. The mice were given drinking water containing 10 mM BSO for 24 hr starting at 20.5 hr prior to injection of $HgCl_2$. Solutions of GSH and of GSH ester were prepared just prior to use and were administered intraperitoneally 2 hr before giving $HgCl_2$. GSH ester was dissolved in water, and the pH of the solution was carefully adjusted to 6.8 with NaOH. A solution containing GSH, isopropanol, and Na_2SO_4 in a molar ratio of 1:1:0.5 was prepared and adjusted to pH 6.8 with NaOH. The mice were fasted starting at 20.5 hr prior to injection of $HgCl_2$ to standardize the tissue levels of GSH.

Blood serum urea nitrogen [18], serum creatinine levels [19], and serum aspartate aminotransferase activity [20] were determined as described. GSH was determined by the 5,5′-dithiobis(2-nitrobenzoic acid)-glutathione disulfide (DTNB-GSSG) reductase method [21]. The data were analyzed by Student's *t*-test.

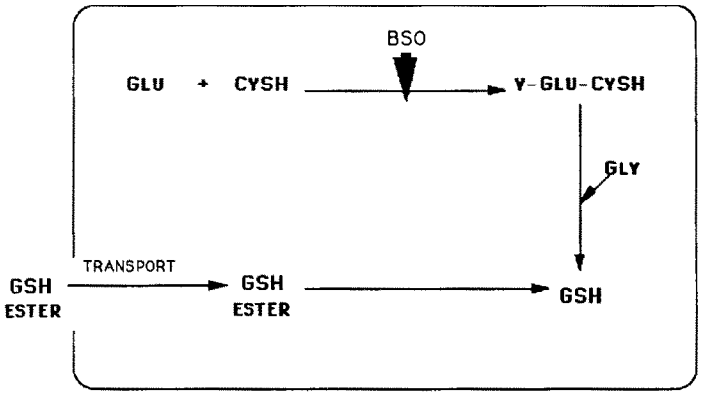

Fig. 1. Formation of intracellular glutathione by synthesis and by treatment with glutathione ester. Glutathione (GSH) is synthesized intracellularly by the consecutive actions of γ-glutamylcysteine and glutathione synthetases. Buthionine sulfoximine (BSO) is an irreversible inhibitor of γ-glutamylcysteine synthetase. Glutathione ester is transported into cells and hydrolyzed intracellularly to give GSH.

Table 1. Effect of treatment with buthionine sulfoximine on the toxicity of mercuric chloride*

Dose of $HgCl_2$ (μmol/kg)	Survival of mice: Controls %	Controls No./Total No.	Treated with BSO %	Treated with BSO No./Total No.
0	100	(6/6)	100	(6/6)
1.0			100	(6/6)
1.5			63	(5/8)
2.0			54	(7/13)
2.25			0	(0/5)
2.5	100	(4/4)	0	(0/5)
10	100	(8/8)		
15	100	(6/6)		
17.5	67	(4/6)		
20	0	(0/12)		
25	0	(0/6)		

* The mice were treated with saline (controls) or with BSO (see Methods) prior to administration of $HgCl_2$. The number and percentage of mice that survived after 10 days and the total number of mice in each group are given. The GSH levels of the blood plasma, liver and kidney were determined in parallel studies at the same time that the other mice were injected with $HgCl_2$. Control GSH levels: 34.5 ± 3.2 μM (plasma), 5.23 ± 0.41 μmol/g (liver), and 3.17 ± 0.26 μmol/g (kidney); after BSO treatment, 4.30 ± 0.80 μM (plasma), 0.57 ± 0.07 μmol/g (liver) and 0.37 ± 0.04 μmol/g (kidney).

Table 2. Effect of glutathione monoester on the buthionine sulfoximine-enhanced toxicity of mercuric chloride*

Dose of GSH ester (mmol/kg)	Survival of mice: %	No./Total No.
Control	100	(6/6)
0	0	(0/6)
0.5	17	(1/6)
1.0	33	(2/6)
2.0	67	(4/6)
3.5	83	(5/6)
5.0	100	(6/6)
7.5	100	(6/6)

* Mice were injected with GSH monoester 2 hr prior to injection of $HgCl_2$ (2.3 μmol/kg). The control mice were also given BSO. For experimental details, see Table 1 and Methods. The number and percentage of mice that survived after 10 days are given.

RESULTS

In these studies mice were treated with BSO to decrease tissue GSH synthesis and levels of GSH, and were treated with GSH monoester to increase tissue levels of GSH (Fig. 1). The experimental protocols used here closely parallel those used previously to achieve, respectively, a GSH depletion of tissues [5, 7, 8, 10] and an increase of tissue GSH levels [7, 8, 10, 22]. Under these conditions, plasma, liver, and kidney GSH levels decrease to 10–13% of the controls ([5, 7, 8, 10], Table 1) after BSO treatment. After treatment with GSH monoester, GSH levels of kidney and liver are typically increased 50–400% as compared to appropriate controls (see, for example, Refs 7, 8 and 22). In the present work, the lethal dose of $HgCl_2$ for the controls was about 20 μmol/kg, whereas the lethal dose for the BSO-treated mice was about 2.25 μmol/kg. Treatment with BSO was not accompanied by significant weight loss or change in behavior. Administration of GSH monoester to BSO-treated mice protected against lethal $HgCl_2$ toxicity (Table 2). The effect of GSH monoester was dose-dependent over the range 0.5 to 5 mmol/kg; complete protection was found at doses of 5 mmol/kg and higher. As indicated by the data given in Table 3, GSH monoester also protected untreated mice against $HgCl_2$ toxicity. Thus, in Expt. A-4 no deaths occurred within 10 days after administration of a dose of $HgCl_2$ (20 μmol/kg) that was fatal to all of the controls within 5 days (Expt. A-2). Analogous studies in which GSH was given did not lead to significant protection (Expt. A-3). In Expt. B (Table 3), mice treated with BSO were given a lethal dose of $HgCl_2$ (2.3 μmol/kg) and also either GSH (Expt. B-3) or GSH ester (Expt. B-4). The data

Table 3. Effect of glutathione monoester and of glutathione on the toxicity of mercuric chloride*

Expt. No.	Treatment	Survival of mice: Days after giving $HgCl_2$ 0	1	2	3	4	5	6	7	8	9	10
A-1	Control	6	6	6	6	6	6	6	6	6	6	6
A-2	$HgCl_2$	6	6	4	2	2	0	0	0	0	0	0
A-3	$HgCl_2$ + GSH	6	6	3	2	0	0	0	0	0	0	0
A-4	$HgCl_2$ + GSH ester	6	6	6	6	6	6	6	6	6	6	6
B-1	BSO	6	6	6	6	6	6	6	6	6	6	6
B-2	$HgCl_2$ + BSO	6	6	0	0	0	0	0	0	0	0	0
B-3	$HgCl_2$ + BSO + GSH	6	6	3	0	0	0	0	0	0	0	0
B-4	$HgCl_2$ + BSO + GSH ester	6	6	6	6	6	6	6	6	6	6	6

* Mice were treated with GSH monoester (7.5 mmol/kg) or with GSH (7.5 mmol/kg) 2 hr prior to administration of $HgCl_2$ (20 μmol/kg, Expt. A; or 2.3 μmol/kg, Expt. B). In Expt. B, the mice were treated with BSO as described in Experimental Procedures. Each group consisted of six mice.

Table 4. Effect of buthionine sulfoximine, glutathione monoester and glutathione on $HgCl_2$-induced toxicity

Expt. No.	Treatment	Dose	Serum urea nitrogen* (mg/100 mL)	Serum aspartate amino transferase* (units/mL)
1	None		21.4 ± 3.3 (8)	0.26 ± 0.06 (4)
2	$HgCl_2$	(2.3 μmol/kg)	19.1 ± 4.5 (7)	0.22 ± 0.01 (3)
3	$HgCl_2$	(20 μmol/kg)	104 ± 12.7 (5)	0.58 ± 0.38† (4)
4	$HgCl_2$ + GSH	(20 μmol/kg + 7.5 mmol/kg)	96.1 ± 12.0 (5)	
5	$HgCl_2$ + GSH monoester	(20 μmol/kg + 7.5 mmol/kg)	59.2 ± 19.2 (5)	
6	BSO		20.7 ± 7.5 (4)	0.30 ± 0.09 (4)
7	BSO + $HgCl_2$	(2.3 μmol/kg)	133 ± 7.5 (4)	1.01 ± 0.48 (4)
8	BSO + $HgCl_2$ + GSH	(2.3 μmol/kg + 7.5 mmol/kg)	117 ± 7.4 (4)	1.27 ± 0.23 (4)
9	BSO + $HgCl_2$ + GSH monoester	(2.3 μmol/kg + 7.5 mmol/kg)	46.3 ± 17.4 (4)	0.37 ± 0.26 (3)

* Determined 24 hr after $HgCl_2$ injection. The values given are means ± SD. Numbers in parentheses give the number of mice. The differences between the data found for BUN in Expts. 3 and 1 ($P < 0.0004$), Expts. 5 and 3 ($P < 0.003$), Expts. 7 and 6 ($P < 0.0004$) and Expts. 9 and 7 ($P < 0.0004$) are statistically significant. The P values for the differences between the data found for serum amino transferase are: Expts. 3 and 1 ($P < 0.006$), Expts. 7 and 6 ($P < 0.026$), Expts. 8 and 6 ($P < 0.0003$), and Expts. 9 and 7 ($P < 0.09$).

† The dose of $HgCl_2$ was 25 μmol/kg.

indicate complete protection by GSH monoester and no significant protection by GSH.

In the studies described in Table 4, the renal function of mice with BSO, $HgCl_2$, GSH, and GSH monoester was evaluated by determinations of blood serum urea nitrogen (BUN). When mice were treated with a low dose of $HgCl_2$ (2.3 μmol/kg), no increase in BUN was found (Expt. 2). In contrast, a higher dose of $HgCl_2$ (20 μmol/kg) led to about a 5-fold increase in the BUN value (Expt. 3), and similar values were obtained when GSH was also given (Expt. 4). On the other hand, significantly lower BUN levels were found after giving GSH monoester (Expt. 5). In Expts. 6–9, the mice were treated with BSO. The results were similar to those found in Expts. 1–5; treatment with BSO alone had no effect on the BUN values (Expt. 6), whereas increased values of BUN were found after giving BSO and $HgCl_2$ (Expt. 7), or BSO, $HgCl_2$, and GSH (Expt. 8). Administration of BSO, $HgCl_2$ and GSH monoester gave less elevation of BUN (Expt. 9) than found in Expts. 7 and 8. Studies in which serum creatinine levels were determined gave results that were similar to those found with determinations of BUN (Table 5). Thus, in Expt. 3 (Table 5) there was less increase in serum creatinine after giving GSH monoester (Expt. 3) than found after giving GSH (Expt. 4). Although GSH monoester was protective as judged by survival and determinations of BUN and serum creatinine, the data suggest that sublethal kidney damage may have occurred even after treatment with GSH monoester. Similarly, determinations of serum aspartate aminotransferase activity (Table 4) indicate partial protection (presumably of liver) by GSH monoester.

DISCUSSION

The marked sensitization of mice to $HgCl_2$ toxicity by treatment with BSO (Table 1) suggests that cellular GSH is a major determinant of $HgCl_2$ toxicity. It is notable that administration of GSH did not protect significantly against $HgCl_2$, whereas administration of GSH monoester protected very effectively. Intraperitoneal administration of GSH increases extracellular levels of GSH greatly without appreciable

Table 5. Effect of buthionine sulfoximine, glutathione monoester, and glutathione on $HgCl_2$-induced toxicity: blood serum creatinine values

Expt.	Treatment*	Serum creatinine (mg/100 mL) 24 hr	48 hr	72 hr
1	None	0.52 ± 0.07 (4)	0.43, 0.43	0.41, 0.51
2	BSO + $HgCl_2$	1.98 ± 0.27 (4)	†	
3	BSO + $HgCl_2$ + GSH monoester	1.16 ± 0.29 (4)	1.35 ± 0.53 (4)	0.98 ± 0.44 (4)
4	BSO + $HgCl_2$ + GSH	1.85 ± 0.21 (4)	2.77 ± 0.24 (4)	†

* The compounds were given in the doses listed under Expts. 7–9, Table 4. Values are means ± SD, except where individual values are given. The differences between the data found in Expts. 2 and 1 (24 hr) ($P < 0.00005$) and Expts. 3 and 2 ($P < 0.007$) are significant.
† Mice died.

effect on cellular levels, whereas administration of GSH monoester increases cellular GSH levels [7, 8, 22]. Thus, the protective effect of GSH against $HgCl_2$ is mediated predominantly intracellularly rather than extracellularly. The present findings are different quantitatively from those obtained in studies on $CdCl_2$ toxicity in mice [10], in which treatment with BSO by a protocol similar to that used here led to only a 2- to 3-fold increase in toxicity to $CdCl_2$; however, the results on $CdCl_2$ toxicity are similar to those on $HgCl_2$ toxicity in that administration of GSH (as compared to GSH monoester) did not protect. It thus appears that the major toxic effects of both Cd^{2+} and of Hg^{2+} are exerted within cells rather than extracellularly. Although many studies have been carried out on the toxic effects of Hg^{2+}, the nature of the intracellular target (or targets) is not yet fully understood at the molecular level. Complexation of Hg^{2+} with cellular GSH [23] might be expected to lead to a deficiency of cellular GSH with consequent increased susceptibility to oxidative damage, various metabolic defects, and membrane disruption; several observations are consistent with this view [24–28]. In addition, Hg^{2+} may bind to thiol groups or to other moieties of essential cellular macromolecules; an increase of cellular GSH levels would tend to decrease such interactions. Previous studies showed that administration of $HgCl_2$ decreases renal levels of GSH and the activities of several enzymes including GSH peroxidase [24], and that administration of diethylmaleate plus very low doses (0.45 mmol/kg) of BSO increases the toxicity of $HgCl_2$ [29]. Administration of $HgCl_2$ has been found to be associated with accumulation of Hg^{2+} in the kidney and elsewhere [28]. It will be of interest to determine the effects of administration of GSH monoester on tissue accumulation of Hg^{2+} after Hg^{2+} administration.

Treatment of $HgCl_2$ toxicity by administration of thiols was suggested many years ago [30]; the thiols that have been used in studies on Hg^{2+} toxicity include dimercaprol [30], D-penicillamine [31], *N*-acetylpenicillamine [31], sulfur-containing steroids [32], α-mercapto-β-(2-furyl)acrylic acid [33], dithiothreitol [34–36], and *meso*-2,3-dimercaptosuccinic acid [37]. Although GSH would be a logical candidate for prevention and therapy of $HgCl_2$ toxicity, it is very poorly transported into cells. The properties of GSH monoesters seem to be ideal for prevention of Hg^{2+} toxicity. GSH esters may also have a role in the treatment of acute Hg^{2+} toxicity. Although GSH esters are hydrolyzed intracellularly, it is possible that GSH ester itself can interact with Hg^{2+} and thus also have a direct protective effect.

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