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Absorption of Mercuric Chloride and Mercuric Sulphide and Their Possible Effects on Tissue Glutathione in Mice

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The toxicity of mercury to animals and man is well established and this depends greatly on the form of the mercury compounds. In general, organic mercury compounds are more poisonous than inorganic mercury (Bidstrup 1964). Even among the various types of inorganic mercury compounds, their absorption by the intestinal epithelium varies according to their solubility (Sin et al. 1983). Since glutathione is known to be involved in the metabolism and detoxification of endogenous and exogenous substances (Ketterer et al. 1983; Meister and Anderson 1983) and particularly the binding of mercury ions (Ballatori and Clarkson 1984), it is therefore of interest to study the changes in the amount of tissue glutathione in mice after they were fed high doses of inorganic mercury compounds with different solubilities.

MATERIALS AND METHODS

Animals. Young adult female Swiss albino mice weighing 20–25 g were used. For each experiment, 4 animals were used per interval and this was repeated once. All animals were fed with mouse pellets and water was given ad libitum.

Experimental design. Mercuric chloride ($HgCl_2$) and mercuric sulphide (HgS) obtained from Merck, West Germany were each prepared in a dose of 6 µg Hg^{2+} / g body weight in distilled water. Two groups of test animals were orally force-fed with this dose of either $HgCl_2$ or HgS once a day for 4 d. HgS was also prepared in a much higher dose of 108 µg Hg^{2+} / g body weight in distilled water. This dose was orally force-fed to the third group of test animals three times a day (324 µg Hg^{2+} / g/ d) for 4 d. All the prepared mercury solutions were thoroughly mixed before feeding. The volume fed ranged from 0.10 mL to 0.15 mL, depending on the weight of the animal. For comparative studies, mice were orally force-fed with distilled water for the same period. Animals were then sacrified at 3, 6, 24 and 72 h intervals after the mercury treatment. The mice were anaesthetized with ether and bled through the jugular vein. The kidney and liver were removed for mercury and glutathione determination.

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Determination of mercury. The removed organs were trimmed into small pieces and put into separate conical flasks. Mercury was extracted according to the method of Agemain and Chau (1976) and was analyzed by a Perkin-Elmer MAS 50A Mercury Analyzer System.

Determination of tissue glutathione (GSH). The amount of tissue glutathione was determined by the method of Richardson and Murphy (1975). The removed organ was immediately washed with cold physiological saline and then placed in 5% TCA in 0.001 M Na_2-EDTA. This was then homogenized with an Ultra-Turrax (West Germany) at full speed in ice-cold conditions for two intervals of 8 to 10 seconds each. The mixture was centrifuged for 15 min at 1000 g at $0^{o}C$. The supernatant was then used for the bioassay of tissue glutathione, using a Shimadzu UV-120-02 spectrophotometer (Richardson and Murphy 1975).

Statistical analysis. All the data were analyzed using one-way analysis of variance followed by a student-Newman-Keuls test. A value of $p < 0.05$ was considered to be significant.

RESULTS AND DISCUSSION

Tables 1 and 2 show that the kidney and liver of mice after orally force - feeding with $HgCl_2$ (6 µg Hg^{2+} / g / d) for 4 d exhibited a significantly ($p < 0.01$) higher concentration of mercury as compared to that of the HgS-treated mice killed at the various time intervals. The mean value of mercury found in the kidney of the $HgCl_2$-treated mice (Table 1) killed at 3 h was 44.78 µg Hg^{2+} / g fresh weight and this value was slightly increased at 6 and 24 h and thereafter it reduced markedly ($p < 0.05$) to 23.33 µg Hg^{2+} / g fresh weight at 72 h interval. In the liver (Table 2), the amount of mercury at 3 h was 3.67 µg Hg^{2+} / g fresh weight and this was significantly ($p < 0.05$) increased to 9.72 µg Hg^{2+} / g fresh weight at 6 h. Thereafter, it decreased gradually. On the other hand, for mice treated with HgS (6 µg Hg^{2+} / g / d), the highest concentration of mercury found in the kidney was 1.11 µg Hg^{2+} / g fresh weight at 6 h interval (Table 1) while in the liver, it was 0.24 µg Hg^{2+} / g fresh weight at 24 h interval (Table 2). In the present study, mice treated with an ultra high dose of HgS (324 µg Hg^{2+} / g/ d) for 4 d could only show a relatively higher value (1.57 µg Hg^{2+} / g fresh weight in the kidney and 0.62 µg Hg^{2+} / g fresh weight in the liver) than that of the mice treated with a lower dose of HgS (6 µg Hg^{2+}/ g / d). These data therefore strongly support our previous findings that the low solubility of HgS would result in a lower absorption rate of mercury via the gastrointestinal tract as compared to the $HgCl_2$ (Sin et al. 1983).

The kidney is known as a primary target organ for mercury and it is also an important organ for the elimination of absorbed mercury (Gregus and Klaassen 1986; Tanaka et al. 1987). Therefore, higher concentration of mercury was found in the kidney as compared to the liver. However, at 72 h after the last treatment of $HgCl_2$, the kidney showed a significant ($p < 0.05$) decrease in the mercury concentration (Table 1). This decrease suggests two possible explanations: (1) It indicates that there is lesser

Table 1. Concentration of mercury in the kidney at different intervals after the last treatment

Treatment (4 d)	Mean ± S.E. (µg Hg^{2+}/g F Wt) Time intervals (h) 3	6	24	72
Control (Distilled water)	0.03 ± 0.03	0.05 ± 0.03	0.05 ± 0.01	0.04 ± 0.01
High dose HgS (6 µg Hg^{2+}/g/d)	0.75 ± 0.14	1.11 ± 0.12	0.53 ± 0.05	0.84 ± 0.37
High dose $HgCl_2$ (6 µg Hg^{2+}/g/d)	44.78^{a**} ± 3.07	49.84^{a**} ± 3.86	47.04^{a**} ± 7.50	$23.33^{a**,b*}$ ± 6.08
Ultra high dose, HgS (324 µg Hg^{2+}/g/d)	1.19 ± 0.19	1.57 ± 0.14	1.48 ± 0.16	1.24 ± 0.14

Table 2. Concentration of mercury in the liver at different intervals after the last treatment

Treatment (4 d)	Mean ± S.E. (µg Hg^{2+}/g F Wt) Time intervals (h) 3	6	24	72
Control (Distilled water)	0.07 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.05 ± 0.01
High dose HgS (6 µg Hg^{2+}/g/d)	0.16 ± 0.01	0.22^{b*} ± 0.03	0.24^{b*} ± 0.03	0.10^{b} ± 0.01
High dose $HgCl_2$ (6 µg Hg^{2+}/g/d)	$3.67^{a**,b*}$ ± 0.65	$9.72^{a**,b}$ ± 0.81	6.98^{a**} ± 2.31	$2.99^{a**,b*}$ ± 0.20
Ultra high dose, HgS (324 µg Hg^{2+}/g/d)	0.37 ± 0.01	0.62 ± 0.14	0.43 ± 0.04	0.32 ± 0.03

Table 3. Concentration of glutathione in kidney at different intervals after the last treatment

Treatment (4 d)	Mean ± S.E. (µg GSH/g F Wt) Time intervals (h) 3	6	24	72
Control (Distilled Water)	956.05 ± 32.00	834.19 ± 33.58	877.19 ± 48.84	889.75 ± 26.29
High dose HgS (6 µg Hg^{2+}/g/d)	966.20 ± 33.41	795.15 ± 37.53	899.99 ± 43.27	820.47[a] ± 68.47
High dose $HgCl_2$ (6 µg Hg^{2+}/g/d)	1267.36[a**] ± 36.84	1182.30[a**] ± 57.13	1275.61[a**] ± 55.03	1173.60[a**] ± 51.23
Ultra high dose, HgS (324 µg Hg^{2+}/g/d))	944.21 ± 79.12	942.70 ± 57.41	941.95 ± 55.55	994.27[a**] ± 40.71

Table 4. Concentration of glutathione in liver at different intervals after the last treatment

Treatment (4 d)	Mean ± S.E. (µg GSH/g F Wt) Time intervals (h) 3	6	24	72
Control (Distilled water)	1716.35 ± 52.31	1760.91 ± 44.86	1783.98 ± 54.58	2007.63[b*] ± 86.05
High dose HgS (6 µg Hg^{2+}/g/d)	1747.56[a] ± 54.67	1557.66[b] ± 90.35	1882.04[b**] ± 112.95	1993.86[b**] ± 73.03
High dose $HgCl_2$ (6 µg Hg^{2+}/g/d)	1512.10[a*] ± 29.47	1550.54 ± 94.00	1615.17 ± 116.12	1755.67 ± 95.86
Ultra high dose, HgS (324 µg Hg^{2+}/g/d)	1680.47 ± 82.92	1686.51 ± 64.16	1635.95 ± 57.80	1847.63 ± 40.78

Footnotes for Tables 1-4.
a = significant differences between treatments.
b = significant differences between time intervals.
** = $p < 0.01$
* = $p < 0.05$
F Wt = Fresh Weight of organs
n = 8

gastrointestinal absorption of mercury 24 h after $HgCl_2$ treatment. This assumption is entirely based on the difference between the absorption rate and the excretion rate of the mercury. (2) Severe damage has taken place in the kidney 24 h after the $HgCl_2$ treatment. This would result in a rapid loss of mercury in urine through the damaged tubules.

On the contrary, the kidney of HgS-treated mice showed a fairly consistent low level of mercury throughout the various intervals (Table 1). According to the low solubility and absorption rate of the HgS as discussed previously, one might expect that there will be a marked decrease of kidney mercury at 72 h. But this is not so even when the mice were treated with an ultra high dose of HgS (324 µg Hg^{2+} / g / d) for 4 d. This phenomenon could be explained by either (1) as shown by Cember (1962) that elimination rate of mercury was to some extent dose dependent so that high dose tended to be eliminated faster than a lower dose. However, the level of mercury in liver as shown in Table 2 at 72 h interval decreased markedly in $HgCl_2$ and HgS treated animals. Therefore, whether the elimination rate of mercury was dose dependent as proposed by Cember (1962) or it occurs differently in the different organs of mice treated with mercury compounds of different solubilities remains to be verified; or (2) it might be due to the continuous presence of the HgS in the gastrointestinal tract. This hypothesis is highly possible because fine particles of HgS might be trapped within the intestinal epithelial foldings. This was supported by our unpublished data that intestinal tissue with its content at 72 h after the HgS treatment still showed a considerably high value of mercury, although one cannot ignore the fact that part of this was derived from biliary excretion (Yonaga and Morita 1981; Gregus and Kalassen 1986). The daily feeding with high doses of HgS contaminated diet (Yeoh et al. 1986) to the mice might explain why the kidney and the liver of these mice accumulated significantly increased amount of tissue mercury as compared to their control. Yeoh and his co-workers (1986) reported that each of his experimental mice consumed HgS in a dose of about 324 µg Hg^{2+} / g / d for 7 d. This value is about 600 times higher than the dose (0.54 µg Hg^{2+}/ g/ d) found in some Chinese medicines which contained the highest amount of HgS (Koh et al. 1977). By using the dose of HgS similar to that of Yeoh and his collaborators in the present study, one can see that the mercury accumulation in the kidney and liver (Table 1 and 2) is still very low when compared to that of the $HgCl_2$. With such a low solubility and absorption rate, and with intervals allowed in between the administering of HgS, insignificant accumulation of mercury was found in both the kidney and liver of these HgS-treated animals even when the treatment was prolonged to eight weeks as shown in our previous

study (Sin et al. 1983).

In the present study, no toxicity tests were carried out in these mice after oral feeding with $HgCl_2$ or HgS. However, the level of tissue mercury accumulation after oral feeding with HgS appears to be in the normal range found in normal human tissues. Sumino and his co-workers (1975) showed that the mercury content in kidney obtained from normal subjects killed by traumatic brain injury ranged between 0.18 - 2.60 µg Hg^{2+} / g fresh weight with a mean of 1.10 ± 0.67 µg Hg^{2+} / g fresh weight, while the liver ranged between 0.16 - 1.30 µg Hg^{2+} / g fresh weight with a mean of 0.47 ± 0.26 µg Hg^{2+} / g fresh weight.

In this study, we examine the effect of these mercuric compounds on tissue GSH, a tripeptide which is important in the metabolism of endogenous and exogenous toxins.In mice treated with $HgCl_2$(6 µg Hg^{2+} / g / d) for 4 d, a significant ($p < 0.01$) increase in cellular concentration of GSH in the kidney at the various intervals was observed (Table 3). On the other hand, there was no significant decrease of tissue GSH in the liver as compared to the control (Table 4). Our results are therefore not in good agreement with the findings of Chung et al. (1982) who showed that in rats, a marked depletion in tissue glutathione was found in both kidney and liver 24 h after subcutaneous injection with $HgCl_2$ (6 µg Hg^{2+} / g). In contrast to the liver, lower dose of $HgCl_2$(2 µg Hg^{2+} / g) increased cellular GSH concentration in the kidney. This discrepancy might be due to the different routes of administering the $HgCl_2$ into the animals.

The significantly increased levels of GSH found in the kidney of these $HgCl_2$-treated animals throughout the various intervals (Table 3) can be explained in two ways: (1) Rapid synthesis of GSH occurs in the kidneys tissue. As pointed out by Chung and his co-workers (1982) that there was an increase of GSH in kidney tissue when rats were subcutaneously injected with a lower dose of $HgCl_2$. In the present study, the $HgCl_2$ was administered via the gastrointestinal tract. According to the findings of Clarkson (1971) the net absorption of $HgCl_2$ across the gastrointestinal tract in mice is on the average less than 2% of the daily intake. If that is so, the dose of $HgCl_2$ (6 µg Hg^{2+} / g) used for oral administration in the present study might produce a similar effect as the lower dose used by Chung et al (1981). However, the marked increase of GSH in the kidney of $HgCl_2$-treated mice was not encountered with that of HgS-treated animals, even though the dose of treatment was increased tremendously. Therefore, the increase of GSH in the kidney of $HgCl_2$-treated mice whether it is directly produced by the kidney cells or indirectly absorbed from the blood circulation remains to be verified. (2) Rapid synthesis and excretion of GSH has taken place in the liver. In the present study, liver is the organ which first receives the mercury from the digestive tract through the portal vein. Therefore, it is likely that the hepatic GSH is rapidly synthesized in order to cope with the absorbed mercury and at the same time released into the blood stream and then tranported to the kidney. As proposed by Meister (1981) there is an interorgan cycle of GSH from liver to the kidney. The rapid

increase of GSH in kidney might not be responsible for the high accumulation of mercury since metallothionein is responsible for binding most of the mercury in the rat kidney when $HgCl_2$ was administered (Wisnieska et al. 1970). Therefore one cannot rule out the possibility that the marked increase of GSH in the kidney tissue is also due to the functional disturbances in the kidney cells induced by the high accumulation of the mercury in situ. This might upset the cells to demand for more GSH probably absorbed from the blood circulation for cellular detoxification and elimination of toxic substances other than mercury such as peroxides and free radicals arised from the mercury poisoning (Gstraunthaler et al. 1983).

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