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Effect of Mercury on Glutathione and Thyroid Hormones

Y. M. Sin,[1] W. F. Teh,[1] M. K. Wong,[2] and P. K. Reddy[1]

[1]Department of Zoology, [2]Department of Chemistry, National University of Singapore, 10 Kent Ridge Crescent, Singapore 0511

In our previous work (Sin, et al 1983), oral administration of mice with mercuric chloride ($HgCl_2$) or mercuric sulphide (HgS) showed that the low solubility of HgS would result in a lower absorption rate of mercury via the gastrointestinal tract as compared to the $HgCl_2$. Therefore, the biological effects of these two mercuric compounds towards animals appear to vary according to the levels of tissue accumulation of the absorbed ionic mercury (Sin, et al 1989). Friberg and Vostal (1974) reported that the absorbed ionic mercury forms complexes with SH groups in the tissues of the body. This leads to the suggestion that cysteine alone might be a critical factor in the control of mercury deposition in body tissues (Thomas and O'Tuama, 1979). However, cysteine is also used for synthesis of glutathione (GSH) which, in turn, may serve as a reservoir of cysteine (Higashi, et al 1977). Sulfoxidation of the cysteine will lead to the formation of inorganic sulphate (Stipanuk, 1986). On the other hand, thyroid hormones (T3 and T4) are also known to conjugate with glucuronide or sulphate (Roche and Michel, 1960, Tan and Wong, 1989) for biliary or urinary excretion. If that is the case, it would be interesting to study the changes of glutathione and thyroid hormones in animals treated with mercuric compounds of different solubilities.

MATERIALS AND METHODS

Animals. Young adult female Swiss albino mice weighing 20–25 g were used. For each experiment, 6 animals were used. All animals received mouse pellets and water ad libitum.

Experimental design. $HgCl_2$ and HgS obtained from Merck, West Germany were each prepared in a dose of 6 µg Hg^{2+}/g body weight in distilled water. 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. Two groups of test animals received doses of either $HgCl_2$ or HgS once a day for 10 d via gavage. For controls, two groups of mice were used. One group received distilled water via gavage for the same period, while the other group was not subjected to any treatment. Animals were

send reprint requests to Prof Sin Yoke Min at the above address

then sacrificed 24 hr after the last mercury treatment. The mice were anaesthetized with ether and bled through the jugular vein. The brain, kidneys and liver were immediately removed for mercury and glutathione determination.

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

Determination of tissue GSH. The amount of tissue GSH 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 sec 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 GSH, using a Shimadzu UV-120-02 spectrophotometer (Richardson and Murphy 1975).

Determination of Plasma T_3 and T_4. The blood collected in EDTA was centrifuged at 200 g. for 15 min at $0^{o}C$. The concentrations of T_3 and T_4 were measured by specific radioimmunoassay method as described by Dickhoff, et al (1978) with slight modifications. The antisera were purchased from Radioassay Systems Laboratories, Inc. California and ^{125}I-labelled T_3 and T_4 from Amersham.

Statistical analysis. All results were expressed as a mean $\pm$ standard error. The significance of the results was determined using one-way analysis of variance followed by a Student - Newman-Keuls multiple range test. A value of $p<0.05$ was considered to be significant.

RESULTS AND DISCUSSION

The toxicity of mercury to animals and man is well established and depends greatly on the form of the mercury compounds. In the present study, $HgCl_2$ and HgS were used; their solubilities are 6.9g/100 ml and 1 x 10^{-6} g/100 ml, respectively. Because of these different solubilities, accumulation of mercury into liver, kidney and brain tissues of the $HgCl_2$-treated mice was significantly higher ($p<0.05$) than those of the HgS-treated mice, although mice of both groups were treated with the same amount of Hg^{2+} (Table 1). One would then expect the normal physiological activities of the organs studied to be affected differently by the two different mercuric compounds.

Table 2 shows that the amounts of tissue GSH in the liver of $HgCl_2$ and HgS-treated mice were both similarly affected, showing lower mean values but not significantly different from that of the controls. On the other hand, the amount of tissue GSH in kidney of $HgCl_2$-treated mice was significantly higher ($p<0.05$) than the controls. However, this increase was not seen in the same organ of

Table 1. Concentration of mercury in various organs of mice subjected to different treatments.

Group (treatment)	Amount of Hg^{2+} (µg/g F. Wt.) ± S.E.		
	Kidney	Liver	Brain
Control (No feeding)	0.22±0.03	0.06±0.02	0.13±0.02
Control (Distilled water)	0.20±0.03	0.05±0.01	0.12±0.02
Test ($HgCl_2$)	58.04±7.82*	8.46±0.70*	0.56±0.06*
Test (HgS)	1.34±0.20	0.30±0.04	0.22±0.04

* = $p<0.05$ significantly different between treatments.
F.Wt. = Fresh weight of the organ.
n = 6

Table 2. Concentration of glutathione in various organs of mice subjected to different treatments.

Group (treatment)	Amount of GSH (µg/g F.Wt.) ± S.E.		
	Kidney	Liver	Brain
Control (No feeding)	1041.29 ± 53.76	1719.34 ± 90.15	784.57 ± 59.84
Control (Distilled water)	1016.82 ± 54.22	1605.61 ± 88.58	726.99 ± 52.78
Test ($HgCl_2$)	1387.75 ± 98.17*	1525.23 ± 97.88	998.11 ± 57.68
Test (HgS)	956.61 ± 34.22	1526.77 ± 81.04	781.98 ± 65.09

* = $p<0.05$ significantly different between treatments.
F.Wt. = Fresh weight of the organ.
n = 6

the HgS-treated mice. In interpreting this discrepancy, it is obvious that the increase of tissue GSH is most likely due to the greater amount of mercury accumulated in the studied organs of the $HgCl_2$-treated mice. Since tissue GSH is mainly synthesized in the liver and transported to other organs, particularly the kidney via systemic circulation (Meister, 1981), it is therefore logical to assume that the increase of tissue GSH in both the kidney and brain of the $HgCl_2$-treated mice is most likely derived from the liver via blood circulation.

Tissue GSH is known to be involved in the metabolism and detoxification of endogenous and exogenous substances (Ketterer, et al 1983, Meister and Anderson 1983) particularly in the binding of mercury ions (Ballatori and Clarkson, 1984). Therefore, a change in the amount of GSH in organs could at least reflect the possible influence of the deposited mercury on the organ's function. Gregus et al (1988) showed that a decrease of hepatic GSH in rats may impair formation of inorganic sulphate. They showed that severe GSH depletion decreased sulfation of the phenolic compounds. However, there was an increase in glucuronidation which may compensate for the impaired sulfation. If that is so, it would be interesting to see to what extent the circulating thyroid hormones can be affected by the different amount of mercury accumulated in the various studied organs.

Table 3. **Concentration of thyroid hormones in plasma of mice subjected to different treatments.**

Group (treatment)	Mean ± S.E. (ng/ml plasma)	
	T4	T3
Control (No feeding)	78.44 ± 5.17	1.45 ± 0.09
Control (Distilled water)	82.99 ± 9.61	1.37 ± 0.14
Test ($HgCl_2$)	45.72 ± 6.13*	0.43 ± 0.08*
Test (HgS)	76.88 ± 10.58	0.59 ± 0.17*

* = $p<0.05$ when compared to the controls.
n = 6

There are a number of pathways by which the thyroid hormones (T3 and T4) are degraded and excreted (DeGroot, et al 1984). Enzymes are mainly involved in the deiodination of T4 to T3. Glucuronidate or sulphate conjugation of the phenolic hydroxyl of the thyroid hormones also takes place (Eelkman Rooda, et al 1989). Table 3

shows that the amounts of circulating thyroid hormones T3 and T4 in the $HgCl_2$-treated mice were significantly reduced ($p<0.05$) as compared to those of the controls. The decrease of both hormones in plasma suggests that the deposited mercury had exerted its effect not only on the liver but also possibly the thyroid and other organs. As pointed out by Kawada et al (1980) methyl-mercury caused a coupling defect in the synthesis of iodothyronines. In fact, methylmercury was shown to be concentrated in the thyroidal cells and not in its colloid fraction (Suzuki, et al 1966). Although we had not determined the mercury concentration in the thyroid of both mercury-treated groups, the fact emerging from the present study of the mercury concentration in other organs suggests that the thyroidal cells of the $HgCl_2$-treated mice were probably accumulating more mercury as compared with the HgS-treated mice. This might explain why the amount of circulating T4 was only significantly reduced in the $HgCl_2$-treated but not the HgS-treated mice. This, in turn, might reduce the uptake of the T4 by the liver cells from the circulating blood and thus significantly decrease the circulating T3 which is derived from the conversion of T_4 in the peripheral organs. However, similar decrease of the circulating T3 was also found in the HgS-treated mice.

There are various possibilities to explain these interesting findings: (1) Since the liver is an important detoxifying organ, and is the first organ to receive the toxic mercury by gavage, it is obvious that the absorbed mercury from both mercuric compounds not only directly binds to liver GSH but also influences enzymes which are involved in the conversion of T4 to T3. As pointed out by Webb (1966), mercury would seem to inhibit most of the enzymes in the organ. Therefore, it is highly possible that the deposited mercury in the liver of $HgCl_2$ and HgS treated animals would inhibit the 5'-Thyroxine deiodinase, Type II which is involved in the conversion of T4 to T3; (2) The non-significant decrease of liver GSH might enhance the synthesis of glucuronide as reported by Gregus et al (1988). This, in turn, might increase the rate of conjugation between T4 and the glucuronide, particularly since the latter is formed predominantly in the liver. It is also very likely that there is an increase of glucuronide in the kidney and the brain of the $HgCl_2$-treated mice where tissue mercury was significantly ($p<0.05$) higher than that of the HgS-treated mice. Hence, the conjugation between T4 and the glucuronide might lead to a faster rate of the biliary and urinary excretion of the T4 hormone; (3) T3 is known to conjugate with sulphate and accounts for the T3 disposal (Roche & Michel, 1960, Eelkman Rooda, et al 1989). It is not likely that this process was increased in the mercury-treated mice since decreased amount of liver GSH might impair the formation of inorganic sulfate in the liver (Gregus, et al 1988). However, it is not known whether this will be increased in the kidney which showed significant increase of tissue GSH in $HgCl_2$-treated mice and (4) It is also known that stress and starvation could decrease the liver uptake of circulating T4. In this study, we used an extra control group for comparative study in order to find out whether stress created by gavage would affect the amount of GSH and the thyroid hormones. The findings seem to indicate that they were not affected under the present experimental

conditions. It may be mentioned here that we had also measured the feed conversion rate of these animals throughout the period of the experiment (unpublished data). It shows no sign of anorexia in the mercury-treated animals. Therefore, the decrease of circulating T3 and T4 must be attributed to the effect of the absorbed mercury. However, this effect is largely dependent upon the amount of mercury accumulated in the organs analysed.

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