Bull. Environ. Contam. Toxicol. (1992) 49:847–854

Environmental Contamination and Toxicology

Effect of Long-Term Uptake of Mercuric Sulphide on Thyroid Hormones and Glutathione in Mice

Y. M. Sin and W. F. Teh

Department of Zoology, National University of Singapore, Lower Kent Ridge Road, Singapore 0511, Republic of Singapore

Inorganic mercurials are toxic to animals and man. However, the biological effects of these inorganic mercuric compounds appear to vary according to their solubility. So far, little work has been done on the toxicity of mercuric sulphide (HgS) because of its extremely low solubility (1×10^{-6} g/100 ml) which accounts for the low absorption rate via gastrointestinal tract (Sin et al. 1983, 1989). Since mercuric sulphide is widely used in Chinese medicine in a crude form known as cinnabar (Koh et al. 1977), it is therefore of great interest to investigate the possible adverse effects of this mercuric compound on animals. In our previous work (Sin et al. 1990, Tan et al. 1990, Ryan et al. 1991) we have demonstrated that mice orally fed with mercuric sulphide (6ug Hg^{2+}/g/d ; a dose about 10 times more than that consumed by man) for 4 to 10 d did not show any significant change in tissue glutathione (GSH) and UDPglucuronyltransferase (UDPGT) activity as compared to those of the controls. On the contrary, mice treated with a highly soluble mercuric chloride ($HgCl_2$) showed a significant drop of thyroid hormones (T_3 and T_4) but a significant increase of kidney GSH and UDPGT (Sin et al. 1990, Tan et al. 1990). The aim of this work was to further examine whether a prolonged treatment of HgS would have any effects on the levels of thyroid hormones and the tissue GSH in mice.

MATERIALS AND METHODS

Forty young female Swiss albino mice weighing about 20-25g were used. All animals were supplied with mouse pellets and water ad libitum.
HgS obtained from Merck, West Germany was prepared in a dose of 6 ug Hg^{2+}/g body weight in distilled water

Send reprint requests to Dr. Y.M. Sin at the above address

(D.W.). The animals were allowed 1 wk to acclimatise to the experimental conditions and separated into two groups, the test and the control. The animals of the test group received a dose of HgS once a day for 4 wk via gavage. The prepared mercury solution was thoroughly mixed immediately before feeding and each animal was fed an approximate volume of 0.1 ml. The control group was similarly fed but with distilled water. The body weights of both groups were recorded weekly for 5 wk ; once before the onset of treatment and 4 times after the onset of treatment. Five animals from each group were then sacrificed at 1, 2, 3 and 4 wk intervals after the last treatment. The mice were anaesthetized with ether and bled through the jugular vein. The blood was collected in EDTA for determination of plasma T_3 and T_4. The brain, kidney and liver were removed for mercury and glutathione determination.

Tissue mercury was extracted from the above organs according to the method of Agemain and Chau (1976). In brief, the removed organs were pre-weighed and oven-dried in conical flasks and digested with concentrated H_2SO_4 (analytical grade, Merck). A Perkin-Elmer (model MAS 50B) Mercury Analyzer was used for mercury analysis.

The amount of tissue GSH was determined by the method of Richardson and Murphy (1975). The removed organs were immediately washed with cold physiological saline and then placed in 5% TCA in 0.001 M Na_2-EDTA. They were then homogenized with an Ultra-Turrax homogenizer (West Germany) at full speed in ice-cold conditions for 2 intervals of 8 to 10 sec each. The mixture was centrifuged for 15 min at 1000 g at 0^{0}C. The supernatant was then used for the bioassay of tissue GSH and a Shimadzu UV-120-02 spectrophotometer was used to record the absorbance.

The concentrations of plasma T_3 and T_4 were analyzed using enzyme immunoassay (EIA) kits purchased from Biomerica, Inc, California. The blood collected in EDTA was centrifuged at 200 g for 15 min at 0°C. The plasma was then carefully collected and used for the determination of T_3 and T_4. A Shimadzu UV-120-02 spectrophotometer was used to record the absorbance.

All results were expressed as a mean ± standard error (S.E.). The significance of the results was determined using Student's T-test. A value of $p<0.05$ was considered to be significant.

RESULTS AND DISCUSSION

In our previous work (Sin et al. 1990), we have

demonstrated that mice treated with the highly soluble $HgCl_2$ for 10 d and sacrificed 24 h after the last treatment showed a significant decrease of plasma T_3 and T_4 but a significant increase of kidney GSH as compared to those of the controls. On the contrary, mice treated with the poorly soluble HgS showed no significant change in the amounts of kidney GSH and plasma T_4 but a decrease of plasma T_3 was detected. This raises an interesting question why the level of T_4 in the HgS-treated animals was not similarly affected as in $HgCl_2$-treated animals. Was the decrease in T_3 of the HgS-treated animals (Sin et al. 1990) implying that it was just a transient phenomenon because the experimental animals were sacrificed 24 h after the last treatment of HgS and thus affected by the continuous uptake of mercury ions from the gastrointestinal tract where HgS particles were possibly still trapped in the intestinal folds? In order to avoid this possibility, animals of the present experiment were sacrificed at different wk after the last treatment of the HgS. This would certainly ensure that any changes that occur in the animals can be considered to be attributed to the prolonged treatment of HgS.

Table 1 shows that the levels of mercury in brain, kidney and liver of the test (HgS-treated) animals were very low. With the exception of kidney mercury in mice sacrificed at 1 wk, the amounts of mercury in the various organs at the various time intervals after the last HgS treatment were insignificantly ($p>0.05$) different from those of the controls.

However, reduction in the levels of T_4 but not T_3 was found in the test mice sacrificed at the different time intervals (Table 2). The amount of T_4 was significantly ($p<0.05$) lower at 1 and 4 wk as compared to their controls. The decrease is obviously due to the longer treatment of HgS because a similar decrease was not encountered in mice treated with HgS for 10 d (Sin et al.1990). Hormone T_4 is the predominant secretory product of the thyroid gland (Angler and Burger 1984). Therefore, it is highly possible that the absorbed mercuric ions might concentrate in the thyroidal cells and cause a coupling defect in the synthesis of iodothyronines as suggested by Kawada et al.(1980) in the methylmercury-treated animals. However, one cannot rule out the possibility that the continuous presence of mercury in brain tissue might also affect the hypothalamus-pituitary axis which regulates the normal activity of the thyroid gland. Since a decrease in T_3 was seen in mice at 24 h after the last HgS treatment (Sin et al. 1990), it is not

Table 1. Concentration of mercury in various organs of mice at different wk after the last HgS treatment.

Organ / Time (wk)	Mean Hg^{2+} content ± S.E.(ug/g F.Wt) Group (Treatment) Control (D.W.)	Test (HgS)
Kidney		
1	0.074 ± 0.008	0.143 ± 0.010**
2	0.045 ± 0.002	0.071 ± 0.011
3	0.066 ± 0.007	0.074 ± 0.008
4	0.035 ± 0.002	0.053 ± 0.012
Liver		
1	0.035 ± 0.003	0.034 ± 0.003
2	0.029 ± 0.001	0.029 ± 0.002
3	0.047 ± 0.006	0.066 ± 0.009
4	0.014 ± 0.002	0.022 ± 0.001
Brain		
1	0.032 ± 0.002	0.050 ± 0.007
2	0.043 ± 0.008	0.021 ± 0.007
3	0.049 ± 0.007	0.033 ± 0.006
4	0.027 ± 0.001	0.030 ± 0.001

** $p < 0.01$ significantly different from controls
F.Wt = fresh weight ; n = 5

Table 2. Concentration of thyroid hormones in plasma of mice at different wk after the last HgS treatment.

Thyroid hormones / Time (wk)	Mean thyroid hormones ± S.E.(ng/ml plasma) Group (treatment) Control (D.W.)	Test (HgS)
T_3		
1	1.19 ± 0.01	1.08 ± 0.08
2	1.17 ± 0.08	1.03 ± 0.05
3	1.06 ± 0.03	1.05 ± 0.08
4	1.13 ± 0.08	0.95 ± 0.04
T_4		
1	41.40 ± 4.50	28.40 ± 1.20*
2	41.40 ± 9.50	29.40 ± 5.00
3	40.67 ± 3.61	29.30 ± 3.20
4	39.30 ± 5.00	23.10 ± 2.20*

* $p < 0.05$ significantly different from controls
n = 5

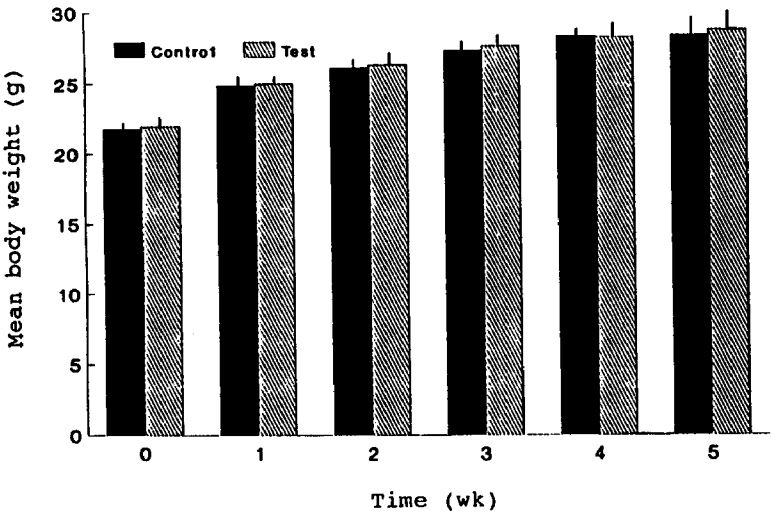

Figure 1. The growth rate of test (HgS-treated) and control mice. n = 20

clear why a decrease in T_4 levels would not lead to a reduction in T_3 value at different wk after the last HgS treatment. There are two possibilities to explain this discrepancy: (1) it is well established that peripheral conversion of T_4 is the major source of circulating T_3 in normal humans and rats (Engler and Burger 1984). Since animals of the present study were sacrificed at least 1 wk and not 24 h after the last HgS treatment, the amount of tissue mercury might not be sufficiently high to exert its influence on the levels of enzymes which are involved in the normal peripheral metabolism of hormone T_4. (2) the daily requirement of T_3 in the normal body is very low (Bernal and Refetoff 1977) and thus a decrease in T_4 value at the present study might not have reached the critical low level that would affect the conversion. As shown in Table 2 the level of T_4 remained high in the test animals even though it is significantly lower than that of the controls. This view is further supported by the fact that only a small amount of T_4 in normal animals was being used for conversion into T_3, the remainder is metabolized to form various intermediates (Bernal and Refetoff 1977). The bioactive T_3 is known to play an important role in animal growth and development. Since there is no change in the level of T_3 after HgS treatment, this might explain why the body weights of the test group showed no difference as compared to those of the control group at the various time intervals (Fig.1).

It has been reported that both glutathione synthesis and the rate of conversion of T_4 to T_3 in the liver are dependent upon the availability of hepatic nonprotein sulfhydryl groups (DeGroot et al. 1984) and the latter has a high affinity for mercury ions. Since absorbed mercury ions are known to bind tissue GSH (Ballatori and Clarkson 1984) and also able to inhibit many tissue enzymes (Webb 1966), one might expect the levels of tissue GSH to be affected to some extent.

Table 3. Concentration of GSH in various organs of mice at different wk after the last HgS treatment.

Organ / Time (wk)	Mean GSH (ug/g F.Wt.) ± S.E. Group (treatment) Control (D.W.)	Test (HgS)
Kidney		
1	668.27 ± 37.31	716.11 ± 25.34
2	762.31 ± 32.53	827.72 ± 50.73
3	617.46 ± 21.97	629.10 ± 42.34
4	682.69 ± 23.16	724.31 ± 30.81
Liver		
1	2324.49 ± 59.20	2081.87 ± 65.83
2	2487.33 ± 38.36	2439.46 ± 83.44
3	2392.84 ± 48.25	2275.55 ±103.02
4	2401.55 ± 31.85	2265.62 ± 60.10
Brain		
1	499.56 ± 32.18	500.42 ± 14.18
2	591.06 ± 29.85	718.31 ± 38.28*
3	513.01 ± 40.30	707.88 ± 38.12**
4	534.53 ± 21.32	642.22 ± 31.88

* $p < 0.05$; ** $p < 0.01$ significantly different from controls ; n = 5

Table 3. showed that GSH levels in kidney and liver of the HgS-treated mice were insignificantly different from their control animals. In contrast, the level of brain GSH was significantly increased at 2 wk ($p<0.05$) and 3 wk ($p<0.01$) after the last HgS treatment. This suggests that the low level of tissue mercury does not upset the supply of non-protein sulfhydryl groups. This might explain why the levels of glutathione and T_3 in the HgS-treated mice did not show any decrease as in $HgCl_2$-treated mice (Sin et al. 1990). The increase of brain GSH raises an interesting question. Was the increase of GSH produced locally or derived from elsewhere? In our previous work (Sin et al. 1989, Kee et al. 1992) we have shown that the significant increase of kidney GSH in $HgCl_2$-treated rats was most

likely derived from the liver via blood circulation as suggested by Meister (1981). Although there were no direct correlation between the increased amount of brain GSH and liver GSH, one cannot rule out the possibility that the release of liver GSH to other organs (Meister 1981) might also contribute to the increase of brain GSH. The significant increase of GSH in brain tissue at 2 and 3 wk after the last HgS treatment might account for the low levels of brain mercury seen at these two time intervals as compared to those of the controls.

Mice when exposed to HgS for a period of 4 wk showed a low level of mercury in their various organs. Tissue mercury did not cause any decrease of GSH and T3. However, mercury significantly reduced T_4. This suggests that a prolonged uptake of low level of tissue mercury might interfere with the normal activities of either the thyroidal cells or the hypothalamus-pituitary axis.

Acknowledgments. This work was supported by a research grant from the National University of Singapore.

REFERENCES

Agemian H, Chau ASY (1976) An improved digestion method for the extraction of mercury from environmental samples. Analyst 100: 91-95.

Ballatori N, Clarkson TW (1984) Dependence of biliary secretion of inorganic mercury on the kidney transport of glutathione. Biochem Pharmacol 33: 1093-1098.

Bernal J, Refetoff S (1977) The action of thyroid hormone. Clin Endocrinol 6: 227-249.

DeGroot LJ, Larsen PR, Refetoft S, Stanbury JB (1984) The thyroid and its diseases. 5th Edition, John Wiley and Sons, Inc., Canada.

Engler D, Burger AG (1984) The deiodination of the iodothyronines and their derivatives in man. Endocrinol rev 5: 151-184.

Kawada J, Nishida M, Yashimura Y, Mitani K (1980) Effects of organic and inorganic mercurials on thyroidal functions. J Pharm Acol Dynamics 3: 149-159.

Kee KN, Sin YM (1992) Effect of mercury on tissue glutathione following intrarenal injection of mercuric chloride. Bull Environ Contam Toxicol 48 509-514.

Koh LL, Wong MK, Ng SC, Soh SM (1977) Mercury in Chinese medicine. Technical report 5. Institute of Natural Science, Nanyang University, Singapore.

Meister A (1981) On the cycles of glutathione

metabolism and transport. Curr Top Cell Reg 18: 21-58.
Richardson RJ, Murphy SD (1975) Effect of glutathione depletion on tissue deposition of methylmercury in rats. Toxicol Appl Pharmacol 31: 505-519.
Ryan DM, Sin YM, Wong MK (1991) Uptake, distribution and immunotoxicological effects of mercury in mice. Environ Monit Assess 19: 507-517.
Sin WC, Wong MK, Sin YM (1989) Changes in tissue glutathione and mercury concentrations in rats following mercuric chloride injection through the hepatic portal vein. Bull Environ Contam Toxicol 42: 942-948.
Sin YM, Lim YF, Wong MK (1983) Uptake and distribution of mercury in mice from ingesting soluble and insoluble mercury compounds. Bull Environ Contam Toxicol 31: 605-612.
Sin YM, Teh WF, Wong MK (1989) Absorption of mercuric chloride and mercuric sulphide and their possible effects on tissue glutathione in mice. Bull Environ Contam Toxicol 42: 307-314.
Sin YM, Teh WF, Wong MK, Reddy PK (1990) Effect of mercury on glutathione and thyroid hormones. Bull Environ Contam Toxicol 44: 616-622.
Tan MC, Sin YM, Wong KP (1990) Mercury-induced UDPglucuronyltransferase (UDPGT) activity in mouse kidney. Toxicology: 81-87.
Webb JL (1966) Enzyme and metabolic inhibitors. Vol. II. Academic Press, New York and London.

Received February 10, 1992; accepted May 30, 1992.