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Effect of Mercury on Tissue Glutathione Following Intrarenal Injection of Mercuric Chloride

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The kidney is a primary target organ for mercury deposition (Wisniewska et al. 1970) and also an organ for mercury excretion (Gregus and Klaassen 1986). However, when animals were administered with either organic or inorganic mercury via different routes (Richardson and Murphy 1975; Congiu et al. 1979; Chung et al. 1982; Sin et al. 1989a) the renal tissues not only showed the highest concentration of tissue mercury but also exhibited a significant increased level of renal glutathione (GSH). The latter is known to be involved in the metabolism and detoxication of toxic substances (Ketterer et al.1983; Meister and Anderson 1983) and particularly the binding of mercury ion (Ballatori and Clarkson 1984). Richardson and Murphy (1975) proposed that renal GSH may be a determinant in the deposition of mercury in the kidney. They showed that the decreased renal GSH was accompanied by decreases in mercury deposition in the kidney. On the other hand, Sin et al. (1989b) showed that by injecting a small amount of mercuric chloride ($HgCl_2$) into rats through the hepatic portal vein , there was a significant increase of renal mercury with no significant change of renal GSH. This study was attempted to examine whether an increase of renal GSH, is a prerequisite for the accumulation of the high level of mercury in the kidney.

MATERIALS AND METHODS

Animals. Young adult male Sprague-Dawley rats weighing about 200g were used. Three animals were used for each group per interval and the experiment was repeated once. All animals were fed with rat pellet and water ad libitum.

Experimental Design. The $HgCl_2$ (Merck, West Germany) was prepared in a dose of 0.15 μg Hg^{2+}/g body weight in 0.9% saline for the test group. The solution was sterilised through millipore filter chamber and maintained at 37°C. The rats of the test groups were anaesthesized and abdominal wall was surgically operated as previously described (Sin et al. 1989b). The $HgCl_2$ preparation was divided into two equal portions of 0.05 ml, and each injected

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into the cortical tissues of each kidney. The inserted needle was left in situ for 15 sec after each injection and then pulled out. The slit of the abdominal wall was then stitched and closed with Mitchell clips and swabbed with 70% alcohol twice. The control animals were subjected to a similar treatment except that they were injected with sterile 0.9% saline. All the test and control animals were returned to their cages before they were sacrificed at 0.5, 1 and 3 hr intervals after the injections. The animals were anaesthetized and bled through the jugular vein. The blood of each rat was collected in EDTA and centrifuged at 200 g at 4°C for 15 min. The liver and kidneys were immediately removed and washed in ice-cold 0.9% saline for mercury and tissue GSH analysis.

The removed organs were 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.

The amount of tissue GSH was determined by the method of Richardson and Murphy (1975). The removed organ was immediately placed in 5% TCA in 0.001 M Na_2-EDTA and 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 then centrifuged for 15 min at 1000 g at 0°C. The supernatant was then used for the bioassay of tissue GSH, using a Shimadzu UV-120-02 spectrophotometer(Richardson and Murphy 1975) .

All data were summarized and tabulated as mean ± standard errors (S.E) and were analysed by 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. 1989b), it was shown that there was no direct correlation between the increased amounts of mercury and GSH levels in the renal tissues, when a small amount of $HgCl_2$ was injected into rats through hepatic portal vein. However, a significant increase of tissue GSH was only found in the liver at 0.5 hr and 1 hr after the $HgCl_2$ injection. Since, under such experimental conditions, the liver is the first organ to receive the entire amount of the injected $HgCl_2$, we concluded that the increased mercury in the kidney for deposition is most likely derived from the liver in the form of GSH-mercury complexes via the peripheral blood circulation. This view is further supported by the results of the present study by injecting $HgCl_2$ directly into the renal tissues.

Table 1 shows that there was no significant difference ($p>0.05$) in the amount of renal GSH in the test groups as compared with the control for all the three time intervals. In the test animals, the kidney contained 853.97 ± 41.48 μg GSH/g fresh weight at 0.5 hr interval, 868.19 ± 81.14 μg GSH/g fresh weight at 1 hr and 936.83 ± 67.94 μg GSH/g fresh weight at 3 hr interval, while the renal GSH of the saline-injected controls ranged from 836.43 ± 36.25 to

Table 1. Concentration of glutathione in kidney at different time intervals after intrarenal injection of 0.9% saline or $HgCl_2$.

Group (treatment)	Mean ± S.E. (μg GSH/g F Wt)		
	Time intervals (hr)		
	0.5	1	3
Control (0.9% saline)	836.43 ± 36.25	871.28 ± 98.82	891.38 ± 26.06
Test ($HgCl_2$)	853.97 ± 41.48	868.19 ± 81.14	936.83 ± 67.94

F Wt: Fresh weight of the organ; n = 6

Table 2. Concentration of mercury in kidney at different time intervals after intrarenal injection of 0.9% saline or $HgCl_2$.

Group (treatment)	Mean ± S.E. (μg Hg^{2+}/g F Wt)		
	Time intervals (hr)		
	0.5	1	3
Control (0.9% saline)	0.18 ± 0.01	0.25 ± 0.06	0.32 ± 0.04
Test ($HgCl_2$)	5.65 ± 0.79 **	5.55 ± 1.23 **	6.04 ± 1.20**

** $p < 0.01$ significantly different from controls
F Wt: Fresh weight of the organ; n = 6

891.38 ± 26.06 μg GSH/g fresh weight. On the other hand, the mercury concentrations in the renal tissue of the test animals (Table 2) were significantly higher ($p < 0.05$) than those of the different controls. Since the kidney under the present experimental design, is the first organ which received the injected $HgCl_2$, the findings therefore suggest that endogenous renal GSH production caused by the $HgCl_2$ does not respond in the same magnitude as the liver which was shown to increase tissue GSH significantly at 0.5 h after the hepatic portal vein injection of $HgCl_2$(Sin et al. 1989b). Since no correlation was found between the amount of endogenous renal GSH and renal mercury concentration in the present study, it is therefore concluded that endogenous renal GSH is not a prerequisite for the accumulation of the increased deposition of the renal mercury as suggested by Richardson and Murphy(1975) and Congiu et al.(1979). Therefore, it

is highly possible that metallothionein is responsible for binding most of the injected mercury ions in the kidney(Wisniewska et al. 1970). However, one cannot rule out the possibility that renal GSH also binds to the injected mercury ions in insignificant amounts. If most of the endogenous GSH binds with the mercury ions then a significant decrease of GSH should have occurred. This was not observed. Therefore an increase of GSH in the kidney of mercury-treated animals as reported by others (Richardson and Murphy 1975; Congiu et al. 1979; Chung et al. 1982) was most likely derived from the blood circulation. Since the kidney is known to be the major organ that extracts plasma GSH (Hahn et al. 1978) and, as proposed by Meister(1981), there is an interorgan cycle of GSH from the liver to the kidney.

Table 3. Concentration of glutathione liver at different time intervals after intrarenal injection of 0.9% saline or $HgCl_2$.

Group (treatment)	Mean ± S.E. (μg GSH/g F Wt)		
	Time intervals (hr)		
	0.5	1	3
Control (0.9% saline)	1295.69 ± 85.88	887.58 ± 49.99	1086.52 ± 171.59
Test ($HgCl_2$)	1429.15 ± 83.32	967.66 ± 49.87	1166.88 ± 184.16

F Wt: Fresh weight of the organ; n = 6

In the present study a small increase in liver GSH was found in the test animals as compared with those of the control animals(Table 3). This was most likely due to the action of escaped $HgCl_2$ from the injected site of the renal tissues via blood circulation. This assumption is in fact well supported by our previous finding (Sin et al. 1989b) that liver GSH became significantly increased at 0.5 hr after hepatic portal vein injection of a small dose of $HgCl_2$.

The data of Table 4 and 5 also indicate that a small amount of $HgCl_2$ had escaped from the damaged renal tissues at the injected sites. At 0.5 hr interval after the $HgCl_2$ injection, the levels of mercury in the liver and blood plasma (Table 3 and 4) of the test animals showed significant increases ($p<0.05$) as compared with the controls. It has been reported that blood mercury immediately complexes with various components in the blood (Lau and Sarkar 1979). In the tissues or organs particularly the liver, the mercury forms complexes with the tissue GSH and is subsequently released into the blood circulation and bile for excretion (Ballatori and Clarkson 1984; Tanaka et al. 1987). Hence, it is most likely that these GSH-mercury complexes in the blood circulation might contribute to the slight increase of mercury in

Table 4. Concentration of mercury in liver at different time intervals after intrarenal injection of 0.9% saline or $HgCl_2$.

Group (treatment)	Mean ± S.E. ($\mu g\ Hg^{2+}/g$ F Wt)		
	Time intervals (hr)		
	0.5	1	3
Control (0.9% saline)	0.02 ± 0.01	0.02 ± 0.00	0.03 ± 0.00
Test ($HgCl_2$)	0.21 ± 0.03 **	0.15 ± 0.02 **	0.20 ± 0.03 **

** $p < 0.01$ significantly different from controls.
F Wt: Fresh weight of the organ; n = 6

Table 5. Concentration of plasma mercury at different time intervals after intrarenal injection of 0.9% saline or $HgCl_2$.

Group (treatment)	Mean ± S.E. ($\mu g\ Hg^{2+}/g$ F Wt)		
	Time intervals (hr)		
	0.5	1	3
Control (0.9% saline)	0.13 ± 0.03	0.08 ± 0.02	0.08 ± 0.02
Test ($HgCl_2$)	0.65 ± 0.12**	0.49 ± 0.07**	0.46 ± 0.05**

** $p < 0.05$ significantly different from controls.
F Wt: Fresh weight of the organ; n = 6

the kidney at 3 hr interval after the $HgCl_2$ injection (Table 2) Therefore, it is reasonable to assume that if the test animals of the present study were killed at a much later period, the renal mercury of these $HgCl_2$-treated rats could certainly be increased with time, because more and more escaped mercury from the injected sites would be in the form of GSH-mercury complexes and ended up in the kidneys via blood circulation.

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REFERENCES

Agemain H, Chau ASY (1976) An improved digestion method for the extraction of mercury from environmental samples. Analyst 101:91-95.

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

Chung AS, Maines MD, Reynolds WA(1982) Inhibition of the enzymes of glutathione metabolism by mercuric chloride in the rat kidney: reversal by selenium. Biochem Pharmacol 31:3093-3100.

Congiu L, Corongiu FP, Dore M, Montaldo L, Vargiolu S, Casula D, Spiga G (1979) The effect of lead nitrate on the tissue distribution of mercury in rats treated with methylmercury chloride. Toxicol Appl Pharmacol 51:363-366.

Gregus Z, Klaassen CD(1986) Disposition of metals in rats: A comparative study of faecal, urinary and biliary excretion and tissue distribution of eighteen metals. Toxicol Appl Pharmacol, 85:24-38.

Hahn R, Wendel A, Flohe L (1978) The fate of extracellular glutathione in the rat. Biochem Biophys Acta 539:324-337.

Ketterer B, Coles B, Meyer DJ(1983) The role of glutathione in detoxication. Environ Health Perspect 49:59-69.

Lau S and Sarkar B(1979) Inorganic mercury(II) - binding components in normal human blood serum. J Toxicol Environ Health 5(5):907-916.

Meister A (1981) On the cycles of glutathione metabolism and transport. Curr Top Cell Reg 18:21-58.

Meister A, Anderson ME (1983) Glutathione. Ann Rev Biochem 52:711.

Richardson RJ, Murphy SD (1975). Effect of glutathione depletion on tissue deposition of methylmercury in rats. Toxicol Appl Pharmacol 31:505-519.

Sin YM, Teh WF, Wong MK (1989a) Absorption of mercuric chloride and mercuric sulphide and their possible effects on tissue glutathione in mice. Bull Environ Contam Toxicol 42:307-314.

Sin WC, Wong Mk, Sin YM (1989b) 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.

Tanaka T, Naganuma A, Imura N (1987) Mechanism of mercury uptake by kidneys. Eisei kagaku 33:14.

Wisniewska JM, Trojanowska B, Piotrowksi J, Jakubowski M.(1970). Binding of mercury in the rat kidney by metallothionein. Toxicol Appl Pharmacol 16:754-763.

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