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Changes in Tissue Glutathione and Mercury Concentrations in Rats Following Mercuric Chloride Injection Through the Hepatic Portal Vein

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The kidney is known as a primary target organ for mercury deposition (Wisniewska et al 1970, Sin et al 1983). However, it is also known as an important organ for the elimination of the absorbed mercury (Gregus and Klaassen 1986). Tanaka and her collaborators (1987) showed that inorganic mercury when injected through caudal vein is transported to the kidney as mercury-GSH complex. If that is so, liver which contains the highest level of tissue GSH than any other organs in normal animals (Lauterburg et al 1984) would appear to be a prime site for the complexion of mercury ions with GSH before they are released and transported to the kidney. In view of this, it is of interest to establish the interrelative changes of the amounts of GSH and mercury in between liver and kidney at the earlier time intervals after a direct injection of a low dosage of mercuric chloride ($HgCl_2$) into the hepatic portal vein.

MATERIALS AND METHODS

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

Experimental Design. The mercuric chloride (Merck, West Germany) was prepared in a dose of 0.15µg Hg^{2+}/g body weight in 0.9% saline for the rats of the test groups. It was sterilized through millipore filter chamber before use. The rats of test groups were anaesthetized with ether and their hair around the abdominal skin was shaved and swabbed with 70% alcohol twice. A 2 cm longitudinal slit was made along the abdominal wall from the level of xiphisternum towards the tail. Stomach and intestines were pushed to one side so that hepatic portal vein was exposed for mercury injection. 1 ml tuberculin syringe was used and the needle was inserted into the hepatic portal vein through the pancreatic tissue. The needle was left in situ for half a minute after the injection and then pulled out. Immediately following this, the injected site was pressed with a small sterile cotton ball for another half a minute to ensure no bleeding occurred at the injection site. The slit was then closed with Mitchell clips and swabbed with 70% alcohol twice. For control animals, they were

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also surgically treated as the test animals except that they were injected with sterile 0.9% saline. All the test and control animals were returned to the cages before they were killed at 0.5, 1 and 3 h intervals after the treatments. The animals were then anaesthetized and bled through the jugular vein. The liver and kidney were immediately removed and washed in ice-cold 0.9% saline for mercury and glutathione analysis.

Determination of tissue glutathione. The amount of tissue glutathione in both kidney and liver was determined by the method of Richardson and Murphy (1975). The cold saline washed organ was placed in 5% TCA in 0.001 M Na -EDTA. This was homogenized with ultra-turrax (Germany) at full speed in ice cold conditions for 5-8 sec intervals. The mixture was centrifuged for 15 min at 1000g and $0^{o}C$. The supernatant was then used for the bioassay of tissue glutathione using a Shimadzu UV-120-02 spectrophotometer (Richardson and Murphy 1975).

Determination of tissue mercury. The 0.9% saline washed organs were trimmed into small pieces and put into separate conical flasks. The tissue mercury was determined by the method of Agemain and Chau (1976) using a Perkin-Elmer MAS 50A Mercury Analyzer System.

Statistical analysis. The data was summarized and tabulated as mean $\pm$ standard error (S.E.) The significance of the result was analyzed by Student's t-test. A value of $p < 0.05$ was considered to be significant.

RESULTS AND DISCUSSION

Table 1 shows that the amounts of mercury in the rat liver of the test groups increased significantly at the different time intervals

Table 1. Concentration of mercury in liver at different time intervals after injection of either 0.9% saline or $HgCl_2$

Group	Mean $\pm$ S.E. ($\mu g Hg^{2+}$/g F Wt)		
	Time intervals(h)		
	0.5	1	3
Control (0.9% saline)	0.01 $\pm$ 0.01	0.02 $\pm$ 0.01	0.02 $\pm$ 0.02
Test ($HgCl_2$)	0.32 $\pm$ 0.04^{a**}	0.46 $\pm$ 0.15^{a*}	0.36 $\pm$ 0.03^{a**}

a* $p < 0.05$; a** $p < 0.01$ when compared to the control values
F Wt: Fresh weight of the organ
n = 8

after the $HgCl_2$ injection as compared to those of the controls injected with 0.9% saline. The liver contained 0.32 ± 0.04 $\mu gHg^{2+}/g$ fresh weight at 0.5 h interval, 0.46 ± 0.15 $\mu gHg^{2+}/g$ fresh weight at 1 h interval and 0.36 ± 0.03 $\mu gHg^{2+}/g$ fresh weight at 3 h interval. However, the results indicate that the amounts of liver mercury of the test groups at different time intervals were not significantly different from one another. This decrease suggests that liver is not an organ which accumulates the injected mercury although it is the first visceral organ to receive the entire injected $HgCl_2$. In the kidney, the amounts of mercury of the test groups (Table 2) at the various time intervals were significantly higher than those of the controls. It has to be pointed out that the amounts of the kidney mercury of the test groups were about 5 to 10 folds greater than those found in their liver. The results (Table 2) also show that the levels of kidney mercury of the test groups were significantly ($p < 0.05$) increased at 3 h interval (3.71 ± 0.56 $\mu gHg^{2+}/g$ fresh weight) as compared to the other two earlier intervals of the same treatment (2.66 ± 0.57$\mu gHg^{2+}/g$ fresh weight at 0.5 h interval and 2.52 ± 0.67$\mu gHg^{2+}/g$ fresh weight at 1 h interval). This finding is in accord with the observations reported by others (Wisniewska et al 1970) that kidney is the major site of mercury deposition.

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

Group	Mean ± S.E. ($\mu gHg^{2+}/g$ F Wt)		
	Time interval(h)		
	0.5	1	3
Control (0.9% saline)	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
Test ($HgCl_2$)	2.66 ± 0.57^{a**}	2.52 ± 0.67^{a**}	3.71 ± 0.56^{a**b*}

a** $p < 0.01$ when compared to the control values
b* $p < 0.05$ when compared to the same treatment at different time intervals
F Wt Fresh weight of the organ
n = 8

Since liver is the first organ to receive the entire amount of the injected $HgCl_2$, one may ask what form the inorganic mercury is being transported from the liver to the kidney for deposition. Is the $HgCl_2$ mostly complexed with the liver GSH before release since mercury ions show high affinity for GSH (Ballatori and Clarkson 1984) or is there a substantial amount of the $HgCl_2$ still remained as various hydroxide forms (Endo et al 1984) after leaving the liver blood circulation? Since the dosage of the mercury used in the present study was rather low, it is most likely that the

majority of the injected $HgCl_2$ would bind to the liver tissue GSH before leaving the organ. Table 3 shows that amounts of the liver

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

Group	Mean ± S.E. (μgGSH/g F Wt)		
	Time intervals(h)		
	0.5	1	3
Control (0.9% saline)	1380.26±109.09	1666.03±105.62	1517.74±25.68
Test ($HgCl_2$)	1714.01±24.09^{a**}	2066.20±51.16^{a**b**}	1582.45±23.36

a** $p < 0.01$ when compared to the control values
b** $p < 0.01$ when compared to same treatment at different time intervals
F Wt: Fresh Weight of the organ
n = 8

GSH of the test group increased significantly ($p < 0.01$) when compared with the controls at 0.5 and 1 h intervals after the injection of the $HgCl_2$ (Table 3). This finding indicates that liver cells can rapidly respond to the challenge of the injected $HgCl_2$ by producing a greater amount of GSH. However, the results also showed that there was a significant ($p < 0.01$) decrease in the amount of the GSH at 3 h interval. Therefore it is highly possible that the increased liver GSH will eventually be released into the blood circulation and ends up in the kidney (Meister 1981). Since there was no more continuous challenge of $HgCl_2$ from the hepatic portal vein after the single injection of the small dosage of $HgCl_2$, one can understand why the GSH production in the liver at 3 h interval was reduced to the low level as in that of the control animals. On the contrary, the kidney of the test groups which accumulated signifcantly higher amount of mercury than the liver showed no significant increase of their kidney GSH at the various time intervals (Table 4). Our results are therefore not in accord with the finding of Chung et al (1982) who showed that a marked depletion in tissue GSH was found in both rat kidney and liver 24 h after subcutaneous injection with a higher dosage of $HgCl_2$ (30μmHg^{2+}/kg). However, when they injected 10μmHg^{2+}/kg subcutaneously into the rats they found there were increased GSH concentration in the kidney. It has to be pointed out that similar marked increase in the amounts of GSH in kidney were also found in mice when they were orally force-fed with $HgCl_2$ (6μgHg^{2+}/g) for 4 d and killed at 3, 6, 24 and 72 h intervals after the last treatment (Sin et al, in press). This discrepancy is possibly due to the different routes of mercury injection, the

mercury dosage used and the time intervals of removing organs from animals after the initial injection of $HgCl_2$.

Table 4. Concentration of glutathione in kidney at different time intervals after injection of 0.9% saline or $HgCl_2$

Group	Mean ± S.E. (µg GSH/F Wt)		
	Time intervals (h)		
	0.5	1	2
Control (0.9% saline)	949.32 ± 13.20	899.94 ± 96.90	903.45 ± 41.99
Test ($HgCl_2$)	1048.14 ± 40.60	1016.97 ± 79.49	1052.34 ± 160.96

F Wt: Fresh weight of the organ
n = 8

The results of Tables 2 and 4 showed that there were no direct correlation between the increased amount of mercury and GSH levels in the kidney tissues of the test groups at the 3 h interval. The results therefore do not agree well with the findings of Conjiu et al (1979) who showed that increased kidney GSH resulted in increased mercury concentration in the kidney. This discrepancy can possibly be explained in the following way. As discussed previously, a low dosage of $HgCl_2$ was used in the present studies and this was injected into the animal through the hepatic portal vein. This means that all the injected $HgCl_2$ has to pass through the liver before disseminating throughout the body. Our experimental design therefore allows most of the injected $HgCl_2$ to react with the liver GSH before release. As a result of this, a majority of the mercury that arrived at the kidney for deposition is likely to be in the form of GSH-mercury complexes as proposed by Tanaka et al (1987). Under such circumstance, it seems that there is no need for the kidney to markedly enhance its GSH production to cope with the GSH-mercury complexes, particularly the kidney itself is also known to respond to the mercury challenge by producing metallothionein which is reported to be responsible for binding most of the mercury in the rat kidney when $HgCl_2$ was administered (Wisniewska et al 1970). However, the above situation of kidney respone to $HgCl_2$ might be changed if a large dosage of $HgCl_2$ is used for the hepatic portal vein injection. It is highly possible that the excess of $HgCl_2$ might be able to sneak through the binding capability of liver GSH, and leave the liver for other organs. If this happens. the kidney cells will respond to $HgCl_2$ challenge by increasing their GSH production. This might explain why there were significant increases of kidney GSH after the administration of a high dosage of $HgCl_2$ into rodents through caudal vein (Conjiu et at 1979), subcutaneously (Chung et al 1982), intraperitoneally

(Richardson and Murphy 1975) and orally (Sin et al, in press.) Hence, it is very likely when the $HgCl_2$ was administered into the animal body other than the route of hepatic portal vein injection, the $HgCl_2$ will immediately complex with various components in the blood (Lau and Sarkar 1979) or perhaps some may remain as the hydroxide forms and become disseminated throughout the body organs. Under such circumstance, the increased amounts of GSH in the renal tissues may be a determining factor in the deposition of mercury as proposed by Richardson and Murphy (1975). Nevertheless, one cannot ignore the increased amount of kidney GSH is also due to the interorgan cycle of GSH from the liver to the kidney as proposed by Meister (1981).

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