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Change in the Level of Tissue Selenium after a Single Administration of Mercuric Chloride in Mice

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Since Parizek and Ostadalova (1967) found that the toxicity of inorganic mercury (Hg) was decreased by a simultaneous injection of selenite, many studies have been carried out to examine the mechanism of this interaction and the role of selenium (Se) in detoxification of Hg. It was clearly shown that when relatively large amounts of $HgCl_2$ and Na_2SeO_3 were administered simultaneously to rodents, an inert HgSe complex, containing equimolar amounts of Hg and Se, was formed rapidly in the bloodstream and that this complex was hardly taken up by the kidney, the target organ of Hg toxicity (see Naganuma 1983). Other studies using simultaneous administration also showed that the formation of this HgSe complex was responsible for renotoxicity alleviation (Magos et al. 1987)

When the exposure to Hg with or without Se was prolonged, however, various findings were obtained. First, when Hg and Se were administered to rats for one month via drinking water, equimolar amounts of Hg and Se were found in the kidney with alleviated renotoxicity (Groth et al. 1976). A similar accumulation pattern was seen in the tissues (including kidney) of several retired mercury miners (Costa et al. 1975). Second, a study with Se-deficient rats (Burk et al. 1977) suggested that dietary selenium alleviated the toxicity of inorganic mercury without changing the pattern of subcellular distribution of Hg.

To understand the interrelations of these various results, elucidation of the kinetics of physiological level of Se is essential. Since most experiments that used simultaneous administration examined the behavior of large amount of injected Se (usually radioisotopically labelled), behavior of the "physiological" Se, i.e., Se existed in the tissues and/or derived from the diet,

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remains unknown. The long-term experiments and the human observations usually determined the Se level at least several days after Hg dosage started. Therefore it remains unknown how the physiological Se behaves shortly after Hg administration.

Since few studies (e.g.,Mogami et al. 1982) have directly evaluated the effect of Hg on the level of the physiological Se, especially shortly after Hg administration, we examined the tissue concentration of Se after $HgCl_2$ injection in mice. A relatively small dose of Hg (1 μmol/kg) was used, and tissue Se levels were determined several hr after Hg injection, a period for which no observation has been made before.

MATERIALS AND METHODS

Male and female ICR mice (10-12 wk-old) were purchased from Nihon SLC (Hamamatsu). They were housed in plastic cages with *ad libitum* access to food and water. The light cycle was 12:12 hr (0800-2000 hr; light), and the room temperature was maintained at 21-23°C.

Mice of experimental groups were given 1 μmol/kg of $HgCl_2$ (Reagent grade, Wako Chemical, Co, Osaka) s.c, dissolved in saline. Control groups were given saline s.c. In both cases, the injected volume was 50 μL per 10 g of body weight.

At 1, 3 (expts A and B), or 24 hr (expt C) after Hg injection, blood was collected from the jugular vein under light ether anesthesia, then liver and kidneys were removed. To minimize the possible confounding effect of circadian rhythm, controls and treated animals were sacrificed in randomized order. Se concentrations in the kidney, liver, plasma and RBC were determined with the Watkinson's spectrofluorometric method (Watkinson 1966) and the Hg concentration with cold-vapor atomic absorption spectrometry. To assure the accuracy of determinations, bovine liver (NIST 1577a; National Institute of Standard and Technology, USA) and powdered human hair (NIES #5; National Institute of Environmental Studies, Japan) were used as reference materials for Se and Hg, respectively. Determined values fell within the range of certified values.

RESULTS AND DISCUSSION

Table 1 shows the mean Se concentrations in the tissues. At 1 and 3 hr after Hg injection (expt A and B), the liver Se concentration of Hg-treated groups were higher than those of the control groups. The difference between pairs (control and Hg-treated) was rather small (5-15%)

but significant in most cases. In the kidney, a similar degree of significant increase was found in the Hg-treated groups. In contrast, the plasma Se level decreased in the Hg-treated groups, though the difference was significant only in the females. At 24 hrs after Hg (expt C), Hg-treated groups showed higher Se concentrations in the liver and kidney, though the differences were not statistically significant. In the plasma, a significant increase of Se was observed in the Hg-treated group, which was opposite to what was found at 1 and 3 hr. In some tissues, fluctuation of Se levels among the control groups was observed. This might have arisen from unspecified differences in the experimental condition such as housing conditions, the lot of the animals or diet.

Table 1. Tissue concentration of Se after $HgCl_2$ injection

	N	KIDNEY	LIVER	RBC	PLASMA
expt A: males					
cont	6	16.0±3.7	20.2±2.6	6.3±0.5	6.2± 1.6
1 hr	6	not done	22.8±1.8	6.6±1.1	4.7± 2.1
3 hr	6	21.8±2.0*	23.7±1.9*	7.2±0.8	4.5± 1.0
expt B: females					
cont	6	21.0±0.6	20.9±0.5	4.6±0.4	4.5± 0.1
1 hr	6	22.7±0.9*	22.5±0.9*	4.3±0.2	4.1± 0.2*
3 hr	6	22.3±1.2	22.5±0.8*	4.6±0.3	4.2± 0.3*
expt C: 24 hr (males)					
cont	5	20.0± 2.8	18.7± 1.9	4.5± 0.7	3.4±0.5
24 hr	5	22.5± 1.2	20.4± 1.4	4.0± 0.3	4.2±0.3*

NOTE: Values are expressed as nmol/g wet tissue (MEAN ± SD). Asterisks indicate significant differences from respective control group; ($p<0.05$ by Dunnet's multiple comparison). For kidney of both groups in expt C, n=6.

Table 2 shows the mean Hg concentrations in the tissues, which varied according to the Hg treatment. Livers of the Hg-treated groups contained several to tenfold higher concentrations of Hg than in the control groups. In the kidney, a more than 50-fold higher concentration was found in the Hg-treated groups.

To examine whether the observed changes of tissue Se could be explained by the inflow of the HgSe complex formed in the plasma (Naganuma 1983), the change of Se was compared with that of Hg (Table 3). If the above was the case, changes of tissue concentrations of these two elements should be equimolar to each other since the complex contained equimolar amount of Se and Hg (Naganuma 1983). In fact, in the liver, the ratio was more than two in all the cases, suggesting that the inflow of the HgSe complex, if any, could account only less than half

of the Se increase. In the kidney, the observed increase of Se could not be explained by the inflow of the complex, since the HgSe complex formed in plasma was hardly taken up by this organ (Naganuma 1983). It is noteworthy that, though the ratio in the kidney was much smaller than in the liver, the percent increase of Se (over the control level) was similar in the two organs.

Table 2. Tissue concentration of Hg after $HgCl_2$ injection

	KIDNEY	LIVER	RBC	PLASMA
expt A: male				
cont	0.2±0.0	0.1±0.1	0.0±0.0	0.0±0.0
1 hr	not done	0.7±0.2	0.8±0.4	0.7±0.1
3 hr	31.7±8.3	1.1±0.3	1.5±0.5	0.6±0.2
expt B: females				
cont	0.2±0.1	0.1±0.0	0.0±0.0	0.0±0.0
1 hr	0.3±2.6	0.5±0.0	0.5±0.2	0.8±0.2
3 hr	15.2±3.5	0.8±0.2	1.7±1.2	0.6±0.1
expt C: 24h (males)				
cont	0.4±0.2	0.1±0.1	0.2±0.1	0.2±0.3
24 hr	28.1±5.3	1.0±0.2	0.6±0.2	1.1±0.6

NOTE: Values are expressed as nmol/g wet tissue (MEAN ± SD). All the differences between the control and Hg-treated groups were statistically significant ($p<0.05$ by Dunnet multiple comparison). For all the groups, n=6.

These results showed that the physiological Se could respond to low doses of Hg at as early as 1 or 3 hr. The observed changes in Se concentrations in the liver and kidney coincided with the observations in mice after repeated doses of $HgCl_2$ (10 μmol/kg/day x 7 days) (Wada et al. 1976). In this case (Wada et al. 1976), the authors speculated that the increased Se was derived from the diet. An increase of kidney Se was also observed in rats exposed to $HgCl_2$ via drinking water for 4 weeks, but liver Se did not appear to increase (Mengel and Karlog 1980). It remains to be elucidated how these changes are related to the earlier changes observed in the present study. A study using rats showed that little change was found in the gel filtration pattern of Se in the liver cytosol at 24 hr after a large dose (25 μmol/kg) of $HgCl_2$ (Mogami et al. 1982). The tissue Se level was not evaluated in their study, and it was possible that this Se level changed without changing the pattern of subcellular Se distribution, as observed in Se-deficient Hg-treated animals (Burk et al. 1977).

To support the increases in liver and kidney, redistribution of Se among various tissues might occur. For the shorter periods (expts A and B), the decrease of plasma Se concomitant with the increase of liver and kidney

suggested that this was the case and that plasma Se might served as a source of the increased tissue Se. It should be noted that, in rats, more than half amount of plasma Se exists as selenoprotein P (Read et al. 1990) for which an Se-transporting function was proposed (Gomez and Tappel 1989). Another possible source of Se is muscle, which has the largest pool of Se in the mammalian body (Levander 1986). For the longer period (expt C), Se derived from the diet consumed after Hg injection could also be a source of the increased Se. In this case, the kinetic behavior of dietary Se, including absorption and/or initial distribution, might also be modified by the injected Hg. For expts A and B, given the short interval between Hg injection and sacrifice, such contribution of diet-derived Se appeared to be of minor importance though it could not be excluded entirely.

Table 3. Ratio of Se increase to Hg increase in the liver and kidney after $HgCl_2$ injection

	KIDNEY	LIVER
expt A: male		
1hr	not done	4.0
3hr	0.2	3.5
expt B: females		
1hr	0.2	3.9
3hr	0.1	2.3
expt C: 24 h (males)		
24hr	0.1	2.0

NOTE: The values are calculated as following:
Ratio $([Se]/[Hg]) = ([Se]_{exp} - [Se]_{cont}) / ([Hg]_{exp} - [Hg]_{cont})$
where $[Se]_{exp}$ denotes the mean concentration of Se in the experimental group.

It should be noted that, besides the increased inflow of Se to liver and kidney as discussed above, a decreased outflow of Se from these organs might also result in an apparent increase of Se. For example, the liver synthesizes the selenoprotein P mentioned above and rapidly releases it into blood (Motsenbocker and Tappel 1982). Therefore, if Hg inhibited this releasing process, Se may increase in the liver and decrease in the bloodstream. This possibility deserves to be further evaluation.

The present results showed that physiological Se responded to Hg injection as early as one or three hours after injection. The change could not entirely be explained by the formation of an HgSe complex, as is true for simultaneous administration of these two elements. The mechanism,as well as toxicological significance, of this observation remains to be elucidated.

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