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Interaction between Inorganic Mercury and Selenium on Tissue Sulfhydryl Groups and Glutathione-linked Enzymes in Rats

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The effect of selenium on the tissue sulfhydryl group content and lipid peroxide-destorying enzyme system in the liver, kidney and testis of rat treated with mercury was investigated. The male rats were injected s.c. with $HgCl_2$ (10 μmoles/kg BW) and orally received Na_2SeO_3 (13 μmoles/kg BW) simultaneously. After 3 days, liver, kidney and testis were removed and analyzed. Mercury decreased the total sulfhydryl group content in the kidney by 25% and the total glutathione content in the kidney and testis by 50% and 36%, respectively, with no changes in other tissues. There was 12% increase in the total sulfhydryl group but not in the total glutathione content in kidney by a simultaneous treatment of Se and Hg. Glutathione peroxidase (GSH-Px) activities were decreased by 63% in the liver and 69% in the kidney, and glutathione reductase (GSH-Rd) activity was increased in the tests by 16% by the Hg treatment with no changes in other tissues. Hg had no effect upon glutathione-S-transferase activites in all organs examined. Simultaneous Se treatment increased GSH-Rd activity in the kidney by 23% and GSH-Px activities in liver and kidney by 24% and 21%, respectively, compared to the Hg-treated group. These data indicate that the alleviation of Hg toxicity by Se treatmentit is well correlated with the protein sulfhydryl group content and GSH-Px activity.

Key Words: Interaction between Hg and Se.

Protection by selenium against heavy metal toxicity has been established (Rimerman *et al.*, 1979). But the mechanism of interaction between selenium and mercury has not been completely established. Although Se decreased Hg toxicity, it neither increased Hg excretion (Parizek *et al.*, 1971) nor decreased Hg absorption (Ganther *et al.*, 1972). It has been suggested that the protection by Se against Hg toxicity is due to (1) the alteration of tissue distribution of Hg (Parizek *et al.*, 1971), (2) diversion of Hg-binding from low molecular weight protein to large molecular weight ones in the liver, kidney and testis (Chen *et al.*, 1974) and in plasma (Burk *et al.*, 1974), and (3) an increase in binding affinity of serum protein for mercury chloride by the selenium treatment (Fang *et al.*, 1976).

Recently, it has been observed that mercury chloride induces lipid peroxidation (Yonaha *et al.*, 1980; Stacey and Klaassen, 1981). It is well known that lipid peroxidation accom-

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panies a wide variety of biochemical and pathological changes in various tissues. Lipid peroxide, a harmful product of lipid peroxidation, is catabolized by the glutathione peroxidase system which also decomposes hydrogen peroxide (Little and O'Brien, 1968) and by the glutathione-S-transferase system which is responsible for the detoxification of various electrophiles (Burk *et al.*, 1980). Reduced glutathione, a major acid-soluble sulfhydryl group in tissues, is a common substrate for glutathione peroxidase and glutathione-S-transferase, which is regenerated by NADPH via glutathione reductase. The objective of this study was to investigate the influence of selenium on the tissue sulfhydryl group level and peroxide-destroying enzyme system in tissues of rats given mercury.

MATERIALS AND METHODS

Twelve male albino rats weighing 200-250 gram were devided into 3 groups; control(4), Hg-treated(4) and Hg plus Se-treated(4). All rats were fed with a normal diet (0.05 ppm Se). Hg was injected s.c. as $HgCl_2$ (10 μmoles/kg BW) and Se was orally administered simultaneously as Na_2SeO_3 (13 μmoles/kg BW) using a stomach tube. After 3 days, the animals were killed by decapitation and liver, kidney and testis were removed. For enzyme assays, tissues were homogenized in 3 volumes of cold 0.25 M sucrose in TKM buffer (50 mM Tris-HCl, pH 7.5, containing 25 mM KCl and 5 mM $MgCl_2$) using a Teflon-glass Potter-Elvejehem homogenizer and the homogenate was centrifuged at 12,000 g for 20 min. The supernatant fraction was used for enzyme assays.

Total sulfhydryl group content in each tissue was measured by the method of Sedlak and Lindsay (1968) and total glutathione content was estimated by the method of Tietze (1969). Glutathione-S-transferase was assayed with 1 mM 1-chloro-2,4-dinitrobenzene according to Habig *et al.* (1974) and glutathione peroxidase acivity was determined with H_2O_2 according to the method of Paglia *et al.* (1976). Glutathione reductase was measured by the method of Racker (1955). Details of these methods are described in the previous paper (Lee and Oh, 1982). Protein concentration was estimated by the method of Lowry *et al.* (1951).

RESULTS

Mercury injection into rats caused a significant decrease in total sulfhydryl group content in the kidney with no change in other tissues as compared with the control group. When selenium was administered to the rats injected with Hg, the total sulfhydryl content in kidney was increased by 12% compared with the control (Table 1). Mercury injection lowered the total glutathione level in kidney and testis by 50% and 36%, respectively, but a simultaneous Se treatment did not increase the glutathione content in these organs (Table 2). Table 3 shows that mercury depressed the glutathione peroxidase activity in liver and kidney by 63% and 69%, respectively. But, in the testis, the activity was increased slightly. By selenium treatment, glutathione peroxidase activity was increased

Table 1. Effect of selenium on total sulfhydryl content in rats treated with mercury

Group	Liver	Kidney	Testis
Control	27.3±0.1	20.2±1.8	14.7±0.3
Hg	25.1±1.7	15.4±0.4**	13.6±1.6
Hg & Se	26.4±0.9	17.3±0.7*	13.5±0.3

Values are means ± S.D. (μmoles/g tissue).
* Significantly different from control value ($p<0.05$).
** Significantly different from control value ($p<0.01$).
Hg was injected s.c. as $HgCl_2$ (10 μmoles/kg BW) and Se was orally administered as Na_2SeO_3 (13 μmoles/kg).

Table 2. Effect of selenium on tissue glutathione concentrations in rats intoxicated with Hg

Group	Liver	Kidney	Testis
Control	5.66±0.69	5.35±0.40	5.46±0.90
Hg	4.68±0.98	2,64±0.16**	3.49±0.48*
Hg & Se	5.61±0.70	3.04±0.16**	3.66±0.16*

Values are μmoles GSH/g wet tissue
Significantly different from control value in the same column (*P<0.05 ; **P<0.01).

Table 3. Effect of selenium on glutathione peroxidase activities in rats treated with Hg

Group	Liver	Kidney	Testis
Control	374.0±2.0	232.7±9.0	39.2±2.1
Hg	140.0±5.6[b]	72.4±5.7[b]	46.4±2.2[a]
Hg & Se	184.0±4.0[b*]	91.8±5.1[b*]	49.4±1.9

All values are specific activities (nmoles NADPH oxidized/min/mg protein) ± S.D.
a: Significantly different from control value (P<0.05).
b: Significantly different from control value (P<0.01).
* Significantly different from Hg treated group (P<0.05).

Table 4. Effect of selenium on glutathione reductase activities in rats intoxicated with Hg

Group	Liver	Kidney	Testis
Control	81.4±0.8	120.6± 5.8	15.7±0.6
Hg	82.0±9.6	114.5±12.0	18.6±1.4[a]
Hg & Se	76.0±4.4	148.9±12.0[a*]	17.8±1.0

All values represent specific activities (nmoles NADPH oxidized/min/mg protein) ± S.D.
a: Significantly different from control value (P<0.05).
* Significantly different from Hg treated group (P<0.05).

Table 5. Effect of selenium on glutathione-S-transferase activities in rats intoxicated with Hg

Group	Liver	Kidney	Testis
Control	637.0±17.0	231.0±13.0	922.0±83.0
Hg	585.0± 7.9	252.0±27.0	1087.0±48.0
Hg & Se	613.0± 6.2	218.0±11.0	1103.0±60.0

All values are specific activities. (nmoles product formed/min/mg protein with 1-chloro-2,4-dinitrobenzene as substrate) ± S.D.

in liver and kidney by 24% and 21%, respectively, compared with the Hg-treated group. However, no change was observed in the testis after Se treatment. Glutathione reductase activity was slightly increased in testis by Hg treatment with no change in other tissues examined. There was a 23% increase in glutathione reductase activity in the kidney by Se treatment (Table 4). No significant effect of mercury and selenium on the activity of glutathione-S-transferase was observed (Table 5).

DISCUSSION

When mercury was injected into rats, most of the mercury was present in kidney. And selenium pretreatment increased Hg contents in blood and testis, but decreased it in kidney with no change in the liver (Chen *et al.*, 1974). The toxicity of mercury is variable and the kidney is one of the target organs for its toxicity. The present data indicate that the total sulfhydryl group content in kidney was affected by Hg treatment and much of the affected sulfhydryl group was glutathione. However, there was no significant difference in the glutathione content between Hg-treated group and the group of a simultaneous administration of Hg and Se. From these results, it is assumed that protein-bound sulfhydryl group is probably protected from Hg by the Se pretreatment. Our present results agree well with the idea of Chen *et al.* (1074) who suggested that Se is attached to a sulfhydryl group of the protein and Hg is attached to Se. Of the enzymes tested in this experiment, glutathione peroxidase

(assayed with H_2O_2) was most affected by Hg treatment. Decreased activity of glutathione peroxidase in liver and kidney by Hg treatment in the present experiment is consistent with previous results (Black *et al.*, 1970; Wada *et al.*, 1976). In contrast, Wagner *et al.* reported that methylmercury did not depress but increased liver glutathione peroxidase (Ganther *et al.*, 1976). The decreased activity of glutathione peroxidase by Hg treatment probably resulted from the conditional selenium deficiency induced by Hg injection. The result of Froseth *et al.* (1974) who have shown that Hg participates in signs of Se deficiency in pigs, explains the induction of selenium deficiency by Hg treatment. Glutathione peroxidase activity was significantly increased in the liver and kidney of rats receiving Se plus Hg compared with the Hg-treated group. This result suggests that increased glutathione peroxidase activity by Se pretreatment may in part contribute to the protection of tissues against Hg toxicity by an efficient scavenging of H_2O_2 and organic peroxide generated in cells. There was no significant change in glutathione reductase activity by Hg treatment, but the activity of the enzyme in the kidney was slightly increased by a simultaneous treatment of Hg and Se. Mercury did not change the activity of glutathione-S-transferase (assayed with 1-chloro-2, 4-dinitrobenzene) in all organs, which agreed well with the results of Black *et al.* (1979). Relatively high activity of glutathione-S-transferase compared with glutathione peroxidase in the testis suggests that removal of organic peroxide by glutathione-S-transferase is more significant in this organ. The accumulation of organic peroxide in tissues of rats intoxicated with Hg may not be due to the defect in the destruction of organic peroxide through glutathione peroxidase system because glutathione-S-transferase activity is not decreased in tissues by Hg treatment. It is probable that the lowered glutathione peroxidase activity by Hg treatment could lead to an accumulation of hydrogen peroxide, a potential source of hydroxyl radical which can initiate lipid peroxidation (MaCay *et al.*, 1976), since catalase is predominantly localized in peroxisomes.

These data indicate that the alleviation of Hg toxicity by selenium treatment is well correlated with the increment of protein sulfhydryl group content and glutathione peroxidase activity.

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