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PROTECTIVE EFFECT OF SELENIUM ON GLUTATHIONE METABOLISM BY MERCURY TOXICITY IN THE CHO CELLS

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ABSTRACT: *The treatment with 5 ng/ml of mercuric chloride caused time-dependent decreases in the activities of GSH S-transferase and GSH-peroxidase, and in the concentration of GSH in CHO cells. Three hours after treatment of Hg^{2+}, the activity of GSH S-transferase was decreased to almost half value of control group and the activity of GSH-peroxidase was reduced significantly at 6 hr after treatment. The concentration of GSH was decreased 2 hr after treatment of Hg^{2+} and was decreased to nearly half value of control group 3 hr after treatment. The decreased enzyme activities of GSH S-transferase and GSH-peroxidase are much more severe than loss of protein, which could be related to altering membrane structure by treatment of mercuric chloride. When cells were treated with both sodium selenite (5 ng/ml) and mercuric chloride (5 ng/ml), the decreased activities of GSH S-transferase and GSH-peroxidase, and the reduced concentration of GSH by Hg^{2+} treatment were restored to normal level or slightly higher than those of control value. It is conceivable that selenium can protect from the toxicity of Hg^{2+} by increasing the concentration GSH and restoring the activities of the enzymes of GSH metabolism.*

Key words: *Hg^{2+}, Se, CHO cells, GSH, GSH S-transferase, GSH-peroxidase*

INTRODUCTION

Glutathione (GSH) is the major cellular thiol which binds electrophilic molecular species, free radical intermediates and heavy metal ions such as mercury (Tappel, 1973; Potter and Matrone, 1974; Alexander *et al.*, 1979). Early studies by Clarkson (1972) implied the involvement of GSH in the *in vivo* binding mercurials. Recently, the effect of Hg^{2+} on cellular GSH levels and on activities of enzymes of the glutathione metabolism pathway was shown in the laboratory animals (Eaton *et al.*, 1980; Chung *et al.*, 1982). The pattern of alteration, however, was time-

dependent decreases in the concentration of GSH by treatment of Hg^{2+}, and in the activities of γ-glutamylcysteine synthetase glutathione disulfide (GSSG) reductase, γ-glutamyl transpeptidase and GSH-peroxidase in the liver or the kidney. A similar pattern of alteration was shown in mercury dose-dependent decreases in the level of GSH and activities of enzymes of GSH metabolism. The treatment of selenium after Hg^{2+} administration prevents from blocking the depressed activities of the enzymes of GSH metabolism and the decreased concentration of GSH in the liver or kidney. The ability of selenite and its compound that are metabolized to selenite to modulate the adverse effects of mercurial compound is well established (Mitchell *et al.*, 1973; Jollow *et al.*, 1974; Plaa and Witschi, 1976; Clarkson, 1972). Recent studies (Chung *et al.*, 1982) have shownd that selenium decreased mercury toxicity related to GSH metabolism in the rats. The precise mechanism by which selenium protects against Hg^{2+} effects on the enzymes of GSH metabolims is not clear. However, there is a possibility that selenium related to increases in the activities of the enzymes GSH metabolism may have contributed significantly to the biological inactivation of Hg^{2+}. The present study was undertaken to investigate whether the treatment of Hg^{2+} in the cell culture system results in a similar trend of GSH metabolism in the rats and selenium prevents Hg^{2+} toxicity from GSH metabolism.

. MATERIALS AND METHODS

Chemicals

Glutathione, NADPH, GSSG-reductase (yeast), O-phthaladehyde, bovine serum albumin (BSA) and mercuric chloride were purchased from the Sigma chemcial Co., St. Louis, MO. Sodium selenite was purchased from the J.T. Baker chemical Co., Philipsburg, N.J. Penicillin G and streptomycin sulfite, T-75 flask, 100 mm dish, wheaton battle, Trypsin EDTA, HEPES and other cell culture apparatus were purchased from Costar, 205 Brodway/Cambridge, MASS. 02139.

Cell Cultures

Cells were grown in Ham's F-12 nutrient media supplemented with 10% heat-inactivated fetal calf serum and 1% penicillin-streptomycin in a humidified atmosphere containing 5% CO_2. Cells were separated with solution containing 0.05% trypsin and 5.0×10^6 cells were seeded on each plate by counting with hematocytometer. Chinese hamster ovary cell line (CHO, Strain K_1-BH_4) was obtained from Toxicology Center at Korea Research Institute of Chemical Technology. Sodium selenite and mercuric chloride were dissolved in Ham's Media and were treated right after seeding.

Glutathione and Enzyme Assays

Glutathione concentration was measured by a modification of the method of Cohn and Lyle (1966). Cells were disrupted by 5 vol of extraction mixture (1/1/1, v/v, 0.01 N HCl-5% trichloroacetic acid-1 mM EDTA). Protein was removed by centrifugation at 5000 g for 10 min. To a 50 μl sample of the supernatant fraction, 1.0 ml of 0.5 M Na_2HPO_4 and 10 μl of O-phthaldehyde in methanol (1 mg/ml)

were added. The GSH values were detected fluorometrically using an Perkin-Elmer LS/3B. The excitation wavelength was 328 nm and the emission wavelength was 430 nm. Cells were disrupted by freezing and thawing or sonication method. Harvested cells were washed twice with phosphate-buffered saline and centrifuged 2000 g for 10 min. The supernatants were aspirated, and pelleted cells were resuspended with 0.25 M sucrose. The resuspended solution were sonicated and centrifuged 15,000 g for 20 min at 4C. The supernatant were collected and used for enzyme assay. The activity of GSH S-transferase (GST) was determined by monitering changes in formation of products at 340 nm in Beckman Du-7 spectrophotometer using 1-Chloro-2,4-dinitrobenzene (CDNB) as a substrate according to the method of Habig *et al.* (1974). The enzyme activity is given as n mole product formed per mg protein per minute. The assay of GSH peroxidase (GSP) was conducted by a modified coupling method of Paglia and Valentine (1967). The assay medium contained enzyme source, GSSG-reductase, sodium azide (NaN_3, 1.0 mM), EDTA(3.0 mM), NADPH(0.1 mM) and potassium phosphate buffer (0.1 M, pH 7.0). The reaction was initiated by the addition of H_2O_2(0.12 mM) and the reaction rate was measured at room temperature. The blank did not contain H_2O_2. One unit of enzyme activity (E.U.) was defined as 1 nmole NADPH oxidized per milligram protein per minute. Protein concentration were measured by the method of Lowry *er al.* (1951) with bovine serum albumin as the standard. All experiments were performed at least four petridishes per experiment, and the data were analyzed using Students T-test. The results are presented as means ± S.D.

RESULTS AND DISCUSSION

The effect of mercuric chloride concentration on glutathione was shown in Table 1. The significant adverse effect of mercuric chloride was shown between 4 ng and 40 ng/m*l*. The treatment of $HgCl_2$ more than 8 ng/m*l* caused significant cell death (Data not shown). Therefore, concentration of $HgCl_2$ was 5 ng/m*l* in these following experiments. As shown in Table 1, the concentration of GSH was decreased at 2 hr after treatment of Hg^{2+} and was decreased to nearly half value of control group at 3 hr after the treatment. A similar trend was shown at 6 hr

Table 1. Effect of mercuric chloride on the GSH concentration in CHO cells

Time (hr)	Control		Hg^{2+} (5 ng/m*l*)	
	GSH (nM)	Protein conc. (mg/m*l*)	GSH (nM)	Protein conc. (mg/m*l*)
1	104.66±5.99	2.30±0.03	103.45±4.94	2.12±0.02
2	111.76±8.78	2.51±0.20	88.10±0.41*	1.88±0.13*
3	100.34±2.60	2.28±0.02	48.10±2.84*	1.55±0.11*
6	110.06±9.19	2.49±0.03	46.64±4.46*	1.38±0.06*

The concentration of mercuric chloride is 5 ng/m*l*
Each value is expressed as the mean ± S.D.
The asterisk (*) indicates $P \leq 0.05$ when compared to the control values.

Table 2. Activity of GSH S-transferase in CHO cells treated with mercuric chloride

Time (hr)	Control		Hg^{2+}	
	Enzyme activity (E.U.)	Protein conc. (mg/ml)	Enzyme activity (E.U.)	Protein conc. (mg/ml)
0	1.02±0.12	2.55±0.05	1.04±0.09	2.53±0.04
3	1.09±0.06	2.54±0.02	0.53±0.08*	1.78±0.03*
6	1.49±0.04*	2.59±0.04	0.74±0.04*	1.89±0.02*
9	2.02±0.09*	2.58±0.03	0.98±0.02*	1.82±0.15*

E.U. is defined as 1 μmole GSH conjugated/min.
Each value represents the mean ± S.D.
The asterisk (*) indicates P≤0.05 when compared to the control values.

Table 3. Effect of mercuric chloride on the activity of GSH-peroxidase in CHO cells

Time (hr)	Control		Hg^{2+} (5 ng/ml)	
	Enzyme activity (E.U.)	Protein conc. (mg/ml)	Enzyme activity (E.U.)	Protein conc. (mg/ml)
0	34.10±4.25	2.49±0.06	33.54±5.04	2.51±0.06
3	31.13±7.40	2.52±0.04	37.20±5.62	1.82¢0.05
6	51.44±3.19	2.61±0.05	27.32±5.47*	1.54±0.07*

E.U. is defined as 1 nmole NADPH oxidized/min.

Table 4. Effect of sodium selenite and mercuric chloride on the activities of GSH S-transferase and GSH-peroxidase in CHO cells 6 hrs after treatment

Treatment	GSH-S transferase (E.U./mg protein)*	GSH-peroxidse (E.U./mg protein)**	Protein conc. (mg/ml)
Control	1.09±0.04	50.60±2.61	2.52±0.16
$HgCl_2$	0.53±0.07*	26.42±5.72*	1.54±0.21*
Se	1.38±0.28	57.27±5.32*	2.58±0.04
$HgCl_2$+Se	1.24±0.31	60.52±6.25*	2.38±0.02

The concentration of mercuric chloride and sodium selenite is 5 ng/ml.
*E.U. is defined as 1 μmole GSH conjugated/min.
**E.U. is defined as 1 nmole NAPH oxidized/min.
Each value represents the mean ± S.D.
The asterisk (*) indicates P≤0.05 when compared to the control values.

after treatment. The activities of GST were measured at 3, 6 and 9 hrs after treatment of mercuric chloride (shown in Table 2). The activity of GST was reduced to almost half value of control group at 3 hr after treatment and a similar reduction was shown 6 and 9 hrs after the treatment. The activities of GSP were measured 3, 6 and 9 hrs after treatment of mercuric chloride (Table 3). There was no effect of GSP activity at 3 hr after treatment of mercuric chloride but a slight increased activity of the enzyme was shown. The activity of GSP significantly

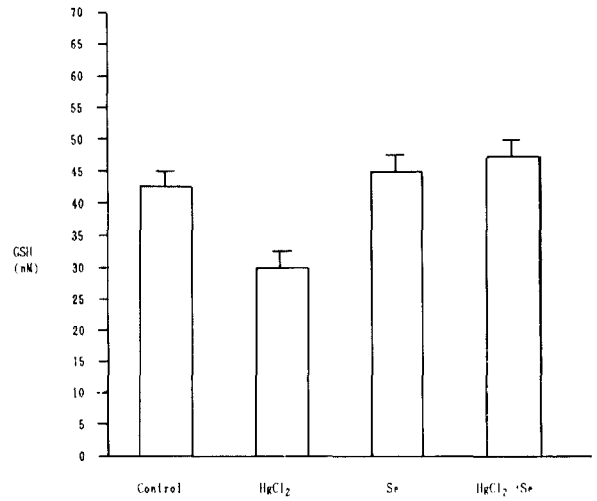

Fig. 1. Effect of sodium selenite and mercuric chloride on the concentration of GSH in CHO cells 6 hours after treatment.
The concentration of mercuric chloride and sodium selenite was 5 ng/ml.
The bar indicates the mean ± S.D.
The asterisk (*) indicates $P \leq 0.05$ when compared to the control values.

reduced at 6 hr after the treatment of mercuric chloride. A similar trend was shown in the activity of enzyme at 9 hr after the treatment.

The effect of sodium selenite on the inhibition of the activities of GST and GSP and the decrease in the concentration of GSH by mercuric chloride were shown in Table 4 and Fig. 1, respectively. The activities of GST and GSP and concentration of GSH by Se treatment were slightly elevated than those of control but the effect of selenium on those parameter was not significant. Selenium restored or increased the activities of above enzymes and the concentration of GSH by the treatment of mercuric chloride. A possible mechanism for the marked depression of GST and GSP by treatment of Hg^{2+} may alter membrane structure since GST and GSP are soluble enzymes and Hg^{2+} is impermeable to cell membrane. Recently it has been shown that mercury and other metal ions can interact with sulfhydryl groups of cell membrane protein and interaction is responsible for increased cation permeability in renal proximal tubules (Kone *et al.*, 1990). The observation suggests that Hg^{2+} treatment could damage membrane structure and the change of membrane structure might be related to a loss of the enzyme proteins from the cells. The enzyme activities of GST and GSP are much more decreased than loss of protein. Further it has been shown the *in vitro* studies that Hg^{2+} can interfere with protein synthesis inhibiting DNA-dependent RNA polymerase as well as direct interaction with nuclear material (Jennetle *et al.*, 1975, Barton and Lippard, 1980). Mercury may change the membrane structure and interfere with protein synthesis. The precise mechanism by which selenium protects against Hg^{2+} effect on the enzymes of GSH metabolism is not clear. However, there is a distinct possibility that selenium-related increases in the activities of

enzymes of GSH metabolism may have contributed significantly to the biological inactivation of Hg^{2+}. Chung and Maines (1981) have demonstrated that the treatment of rats with 10 μmol/kg(s.c.) of meruric chloride caused time-dependent decreases in the activities of enzymes of the glutathione metabolism pathway in the kidney. When rat was given 30 μmol/kg of mercuric chloride, the same pattern of enzyme response was noted. However, the decrease in the specific activity of enzymes was accompanied by great losses in the cellular protein concentration in both the liver and kidney. When rats were given 10 μmol of sodium selenite 30 min after Hg^{2+} treatment, the Hg^{2+}-related depression in the activities of the enzymes of GSH metabolism in the liver and kidney were blocked. Sener *et al.* (1979) have shown that giving selenite to rats treated with Hg^{2+} decreased the excretion of γ-glutamyl transpeptidase in the urine. A similar decrease in the activity of γ-glutamyl transpeptidase in the kidney by mercury treatment was observed and the activity of the enzyme was reversed to almost normal level by administration of selenium. It was demonstrated that, after feeding experiments, Hg^{2+} and selenium were found in equimolar amounts macrophages, nervous tissues, the brain and liver of mammals (Groth *et al.*, 1976; Koeman, 1973; Kosta *et al.*, 1975). According to these findings, there is a possibility that the direct selenium-Hg^{2+} interaction or their common affinity for -SH group of tissue protein or membrane may have been involved in the presently observed protective action of selenium against Hg^{2+}. The concentration of GSH after treatment of Hg^{2+} in the cell was decreased by time-dependent manner. There were no difference of GSH concentration 1 hr after mercury treatment, but 2 hr after mercury treatment GSH concentration decreased slightly, markedly 3 hr after mercury treatment, and almost 60% of control value 6 hr after. The activities of GSH S-transferase and GSH-peroxidase in mercury treated cells were decreased more than those of normal values, and the protein concentration reduced less than the activities of above enzymes. There are some possibilities that mercury altered membrane structure, these by the amount of GSH and the protein of those enzymes could be leaked out. However, it appears that such interaction could not constitute the sole mechanism for the observed blockade by selenium of the inhibitory effects of Hg^{2+} on the enzymes of GSH metabolism. Moreover, the failure of selenium *in vitro* to prevent the inhibition of GSSG-reductase activity by Hg^{2+} is indicative of the involvement of mechanisms other than a direct interaction between selenium and Hg^{2+} in the cell protection offered by selenium against inhibition of GSSG-reductase activity of Hg^{2+}. The treatment of rats with selenium caused increases in the activites of GSH S-transferases (Bark and Chung, 1989) and the amount of glutathione in rats (Chung and Maines, 1981). Selenium after protection against toxic effects of mercury in many marine vertebrates and GSH compeletely nullifies the toxic effect of Hg^{2+}, both *in vivo* and *in vitro* in marine vertebrates and in marine lamellibranchs (Patel, *et al.*, 1988, Lee *et al.*, 1989). Furthermore, the elevation of intracellular GSH level and GST activity in arsenic-resistant CHO cells may be responsible for the resistance to arsenite or other metal such as mercury. It was not possible to detect the activity of GSSG-reductase or other enzymes related to GSH metabolism in CHO cell except above two enzymes. The observed inhibitory effects of Hg^{2+} on the enzymes of GSH metabolism and the

concentration of GSH are involved in the general cellular toxicity of Hg^{2+}. It is conceivable that the increased the activities of these enzymes concentration of glutathione by Se may inactivate Hg^{2+} and mediate detoxification of Hg^{2+}.

REFERENCES

Alexander, J., Hostmark, A.T. Forre, O. and von Kraemer Bryn, M. (1979): The influence of selenium on methyl mercury toxicity in rat hepatoma cells, human embryonic fibroblasts and human lymphocytes in culture, *Acta Pharmacol. Toxicol.*, **45**, 379-386.

Bark, Y.Y. and Chung, A.S. (1989): Induction of some glutathione S-transferases in rat liver by selenium, *Korean Biochem. J.*, **22**, 61-67.

Barton, J.K., S.J. Lippard (1980): Heavy metal interactions with nucleic acids, *in Nuclei* Acid Metal Ion Interaction VI. (T.G. Spiro (ed, Wiley and Sone. New York), pp. 31-113.

Black, S. and Hudson, B. (1961): Flavin and thiol groups of yeast glutathione reductase, *Biochem. Biophys. Res. Commun.*, **5**, 135-138.

Burk, R.F., Foster, K.A., Greenfield, P.M. and Kiker, K.W. (1974): Binding of simultaneously administered inorganic selenium and mercury in a rat plasma protein, *Proc. Soc. Exp. Biol. Med.*, **145**, 782-785.

Clarkson, T.W. (1972): The pharmacology of mercury compounds, *Ann. Rev. Pharmacol.*, **12**, 375-406.

Chung, A.-S. and Maines, M.D. (1981): Effect of selenium on glutathione metabolism: Induction of γ-glutamylcysteine synthetase and glutathione reductase in the rat liver, *Biochem. Pharmacol.*, **30**, 3217-3223.

Chung, A.-S., Maines, M.D. and Reynolds, W.N. (1982): Inhibition of the enzymes of glutathione metabolism by mercuric chloride in the rat kidney: Reversal by selenium, *Biochem. Pharmacol.*, **31**, 3093-3100.

Cohn, V.H. and Lyle, J. (1966): A fluorometric assay for glutathione, *Anal. Biochem.*, **14**, 434-440.

Crowley, C., Gillham, B. and Thorn, N.M. (1975): A direct enzymatic method for the determination of reduced glutathione in Blood and other tissues, *Biochem. Med.*, **13**, 287-292.

Dalvi R.R. and Robbins, T.J. (1978): Comparative studies on the effects of cadmium, cobalt, lead and selenium on hepatic microsomal monooxygenase enzymes and glutathione levels in mice, *J. Environ. Path. Toxicol.*, **1**, 601-607.

Eaton D.L., Stacey, N.H., Wong, K.L. and Klaassen, C.D. (1980): Dose peptides effects of various ions on rat liver metallothionein, glutathione, heme oxygenase and cytochrome P-450, *Toxicol. App. Pharmacol.*, **55**, 393-402.

Ganther, H.E., Goudi, C., Sunde, M.K., Kopecky, M.J., Wagner, P., Sang-Hwan, Oh and Hoekstra, W.G. (1972): Selenium: Relation to decrease toxicity of methyl mercury added to diets containing Tuna, *Science*, **175**, 1120-1124.

Groth, D.H., Settler, L. and Machay, G. (1976): Interaction of mercury, cadmium selenium, tellurium, arsenic and berylium, in Effects of Dose-Response Relationships of Toxic Metals, (G.F. Nordberg, (ed.), Elsevier, Amsterddam), pp. 527-543.

Hanes, C.S., Hird, F.J.R. and F.A.I. Sherwood, (1950): Synthesis of peptides in enzyme reactions involving glutathione, *Nature*, **166**, 288-292.

Hill, C.H. (1974): Reversal of selenium toxicity in chicks by mercury, copper and cadmium, *J. Nutr.*, **104**, 593-598.

Jennette, K.W., Lippard, S.J. and Ucko, D.A. (1975): Magnetic resonance investigation of mercury (II) bindign to nucleosides and thiolated nucleosides in dimethylsulfoxide, *Biochem. Biophys. Acta*, **402**, 402-412.

Jollow, D.J., Mitchell, J.R., Zampaglione, N. and Gillettee, J.R. (1974): Bromobenzene Induced Liver Necrosis Protective Role of Glutathione and Evidence for 3,4-Bromobenzene oxide as the Hepatotoxic Metabolite, *Pharmacology*, **11**, 151-169.

Kone, B.B., Brenner, R.M. and Gullans, S.R. (1990): Sulfhydryl-reactive heavy metals increase cell membrane K^+ and Ca^{2+} transport in renal proximal tubule, *J. Membr. Biol.*, **133**, 1-12.

Koeman, J.H. (1973): Mercury-selenium correlations in marine mammals, *Nature*, **245**, 385-386.

Kosta, A.R., Byren, B.J., Zelenko, V. (1974): Mercury-selenium association in person exposed to inorganic mercury, in Proc. of Recent Advances in the Assessment of Health Effects of Environmental Pollution, CED-EPA-WHO-Symposium, Paris, 24-28.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951): Protein measurement with folin phenol reagent. *J. Biol. Chem.*, **193**, 265-275.

Lee, T.C., Wei M.L., Cahng, W.J., Ho, I.C., Jan, K.Y. and Huang H. (1989): Elevation of glutathione levels and glutathione S-transferase activity in arsenic-resistant Chinese hamster ovary cells, *In Vitro Cell Dev. Biol.*, **25**, 442-448.

Maines, M.D. and Kappas, A. (1975): Cobalt stimulation of heme degradation in the liver. Dissociation of microsomal oxidation of heme from cytochrome P-450, *J. Biol. Chem.*, **250**, 4171-4177.

Massey, V. and William, C.H., Jr. (1965): On the reactions of yeast glutathione reductase, *J. Biol. Chem.*, **240**, 4470-4480.

Mitchell, J.R., Jollwo, D.J., Potter, Z., Gillette, J.R. and Brodie, B.B. (1973): Acetaminophen-induced hepatic necrosis. IV. Protective role of glutathione, *J. Pharmacol. Exp. Ther.*, **187**, 211-217.

Paglia, D.E. and Valentin, W.N. (1967): Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase, *J. Lab. Clin. Med.*, **70**, 158-169.

Patel, B., Chandy, J.P. and Patel, S. (1988): Do selenium and glutathione inhibit the toxic effects of Mercury in marine lamellibranchs ? *Sci. Total Environ.*, **76**, 147-165.

Plaa G.L. and Witschi, H. (1976): Chemicals, drugs and lipid peroxidation, *Ann. Rew. Pharmaco. Toxicol.*, **16**, 125-141.

Potter, S.D. and Matrone, G. (1974): Effect of selenium on the toxicity of dietary methyl mercury and mercuric in the Rat. *J. Nutr.*, **104**, 638-645.

Sener, S. Braum, J.P., Rico, A.G., Benard, P. and Burgat-Sacaze, V. (1979): Note surle'elination urinaire de la gamma-glutamyl transfe'rase lors d'injections re'

pe'es de chlorure mercurique chezle rat, *Toxicol. Eur. Res.*, **1**(4), 263-265.
Tappel, A.L. (1973): Lipid peroxidation damage to cell components, *Fed. Proc.*, **32**, 1870-1874.