Bull. Environ. Contam. Toxicol. (1989) 42:15–21

Bulletin of Environmental Contamination and Toxicology

Effects of Selenium and Mercury on Glutathione and Glutathione–Dependent Enzymes in Experimental Quail

Paolo Di Simplicio and Claudio Leonzio

Dipartimento Biologia Ambientale, Via delle Cerchia 3, Università Degli Studi Di Siena, 53100 Siena, Italy

The protecting effect of selenium against the toxicity of methylmercury (MM) and mutual Se-Hg antagonism has long been known (see in Spallholz et al, 1981).

The defensive action of Se does not occur by greater Hg body excretion but by a rearrangement in the accumulation pattern of Hg in organs; for example when Se and Hg are administered together in the diet the absence of symptoms of toxicity can be accompanied by high levels of Hg in the brain (Stoewsand et al, 1974).
According to some Authors the Se-Hg interaction is brought about by endogenous glutathione (GSH) which in reducing selenite to selenide favors the formation of bis-methylmercury selenide (Iwata et al, 1981). The high lipoaffinity of this compound alters the metabolism and distribution of mercury in critical tissues and thus its toxicity (Masukava et al, 1982). The function of GSH as a protective agent againts MM toxicity has recently been confirmed by Balthrop and Braddon (1985) who observed depletion of GSH in the mouse liver and an increase in its levels after injection of Se prior to MM.
The same Authors laid particular emphasis on the protective effect of Se in altering the MM metabolism, and a modified activity of glutathione tranferases (GST) was found in animals treated with Se + MM compared to those treated with Se or MM alone.

GST is an important family of isoenzymes with binding properties which metabolize drugs and xenobiotics. These enzymes could indeed have an important function in Se-Hg antagonism. Their participation in conjugation with GSH and bile excretion of MM has been suggested by Refsvik (1983), although other authors seem to exclude it (Dierickx,1985).

Send reprint requests to P. Di Simplicio at the above address.

In the present paper the behavior of GST isoenzymes in quail liver after combined Se-Hg treatment was studied; in addition other enzymes which regulate the GSH metabolism as well as the hepatic levels of Se, Hg, GSH and oxidized glutathione (GSSG) have been investigated

MATERIALS AND METHODS

Eight groups of 4 adult male Japanese quail (Coturnix coturnix japonica) were given a diet containing the following concentrations of selenium and mercury (as sodium selenite and methylmercury chloride) for 3 weeks: group SE1, SE2, SE3 = 2.5, 5.0, 10 µg/g of Se; group SM1, SM2, SM3 = 2+10, 4+10, 8+10 µg/g Se+Hg corresponding to a Se/Hg molar ratio of 0.5, 1, 2 respectively; group ME = 10 µg/g Hg.
The Se and Hg compounds were dissolved in ethanol and mixed with the food in a homogenizer at room temperature until all the ethanol evaporated. Concentrations were randomly verified by the analysis of total mercury (Hg) and selenium (Se) with atomic absorption spectroscopy (AAS).
After 21 days of this diet all the animals were sacrified. Part of the liver was kept frozen at -80°C for AAS and GSH determination and part of it immediatly processed for enzyme assays.
For analysis of total selenium and mercury an aliquot of about 0.5 g liver was mineralized in teflon vessels with 2.0 ml HNO_3 at 115 °C under pressure (Stoeppler and Bakhaus; 1978). The solution was then processed for AAS. Hg was determined by the cold vapor stream system, Se by the hydride generation and electrothermal atomization technique.
Activities of glutathione transferases (GST), glutathione reductase (GSSg-Red)), Se-dependent glutathione peroxidase (GSH-Px), glyoxalase (GLY) and thioltransferase (TT) were determined by the methods reported in Di Simplicio and Mannervik (1983). GST were determined using 1-chloro-2,4-dinitrobenzene (CDNB) which is considered the common substrate for all GST isoenzymes; specific isoenzyme activity was tested by ethacrinic acid (EA) and cumene hydroperoxide (Cu-OOH). Analysis of GSH and GSSG was performed by the method of Di Simplicio (1982) slightly modified.
Proteins were quantified by the method of Bradford (1976).

All data were processed by parametric statistical analysis (analysis of variance, ANOVA) using SPSS software (IBM).

RESULTS AND DISCUSSION

Se in the diet at increasing concentrations resulted in a parallel

Table 1. Total selenium and mercury concentrations (μg/g ww) in the liver of treated quails ($\bar{x}$=mean; SD=standard deviation; n=4).

Group:		CON.	ME	SE1	SE2	SE3	SM1	SM2	SM3
Se	$\bar{x}$	0.19	0.56	1.72	2.08	2.46	0.78	1.25	2.44
	SD	0.01	0.12	0.56	1.13	0.89	0.30	0.90	0.82
Hg	$\bar{x}$	0.16	12.26	0.18	0.13	0.31	14.45	14.12	23.21
	SD	0.01	2.54	0.07	0.03	0.05	6.10	6.43	8.88

increase in hepatic total mercury levels, likewise increasing amounts of Hg were taken up by the liver of animals given the same dose of MM and increasing doses of Se (Tab.1). These data confirm previous data on the Se-Hg interactions reported by El Begearmi (1977).

No change in hepatic concentrations of GSH and GSSG was found in any treated group (control values: GSH=3.35±0.65 μmoles/g ww, n=4; GSSG= 39.4 ± 8.12 nmoles/g).

All enzymatic actvities are reported in Fig. 1.

No change in CDNB activity was found in the SE or ME groups. A significant decrease with respect to controls was found in SM1, a significant increase in SM3, and no change in SM2.

In the SE and ME groups, GST response assayed with the Cu-OOH substrate showed an increase and a decrease with respect to the control group, respectively. In SM treated quails, isoenzyme expression conformed to that obtained in the SE or ME treatment depending on the Se/Hg ratio.

GST sensitive to EA showed an activity pattern substantially different from that obtained with CDNB and Cu-OOH. In the SE and ME treatments an increase in EA activity was recorded; the same increase was found in the SM groups when Hg (significant) or Se (not significant) were in excess. No change in activity was noted when the molar ratio Se/Hg was equal to 1 (SM2).

GSH-Px and TT activities were elevated in all groups and significantly correlated ($p<0.001$, $r=0.875$, $n=32$).

GR activity was significantly decreased after ME treatment; in all the other groups no variation was observed with respect to the control group.

Gly was unmodified in all the treated groups (mean control value ± standard deviation = 345±19 mU/mg n=4)

Our data show a change in the MM metabolism in the presence of Se

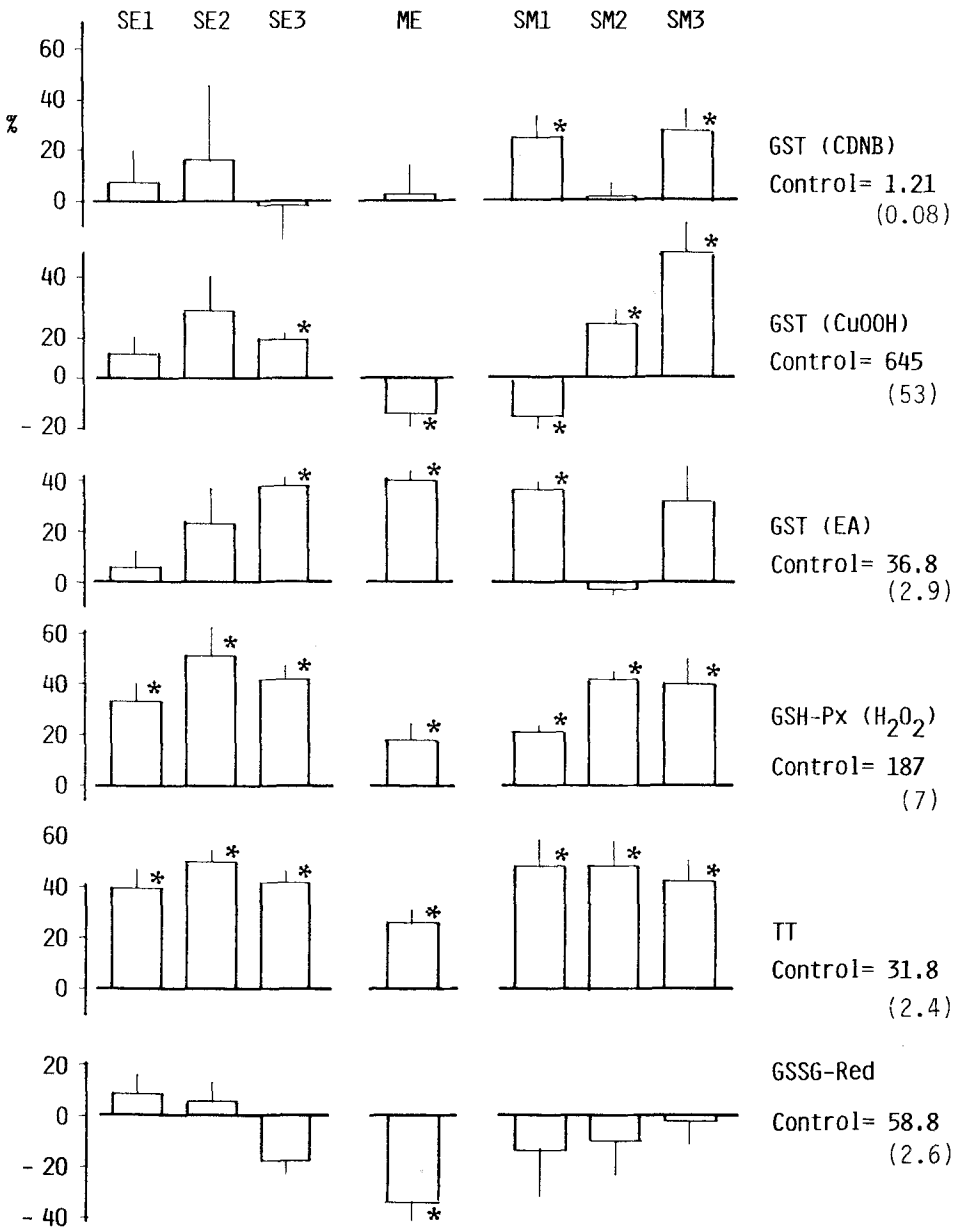

Figure 1. Percentage variation in enzymatic activity with respect to controls in quail liver after selenium and mercury treatments. Average control activities (standard deviation in parenthesis) are expressed as U/mg prot for GST(CDNB) and mU/mg prot for the others; asterisk indicates significant difference ($p<0.005$) with respect to control.

accompanied by modified GST activity as recently reported (Balthrop and Braddon, 1985). Further information was gathered by the observation of some GST isoenzymes. For example the increase in GST activity assayed with EA and the decrease in CuOOH activity for ME and SM1 groups indicate that GST isoenzymes are differently influenced by MM or Se treatments; in this case results based only on the unchanged value of CDNB activity observed for the ME group may lead to ambiguous interpretation of the toxic effect of MM.

The behaviour of CDNB, CuOOH, and EA activities may be considered a sensitive biochemical method to study the mutual Se-Hg antagonism at cytoplasmic level; this aspect has not yet been extensively studied. The literature is mainly concerned with the effect of GST isoenzymes after Se or MM treatment rather than in combined administration.

The increased CuOOH and EA activities observed in quail liver after Se treatment confirm previous results obtained in mice (Reiter and Wendel,1985).

The unchanged values of GSH and GSSG in all treated groups are unexpected, considering that GSH has been demonstrated to be sensitive to MM (Balthrop and Braddon, 1985) and Se treatment (Chung and Maines, 1981). Perhaps GSH and GSSG hepatic levels represent the final result of various interactions (i.e. GSH synthesis and efflux) which regulate their intracellular concentration.

According to our findings the GSH-Px activity is stimulated in animals fed supplementary Se (Reiter and Wendel, 1985). It is known that MM lowers the GSH-Px activity (Prohaska and Ganther, 1977; Hirota et al, 1980). On the other hand Chang and Suber (1982) found no variation in GSH-Px activity 3 weeks after MM treatment in rats and a significant decrease after 6 weeks; Kling and Soares (1978) did not detect any change in GSH-Px in liver and blood of quails treated with methylmercury. These studies and our results seem to indicate that GSH-Px inhibition due to MM poisoning could be influenced by several factors such as the dose administered, the time of exposure and even the natural presence of selenium in the diet.

TT shows a pattern of activity similar to that observed for GSH-Px. This enzyme regulates the amount of GSH bound to proteins by mixed disulfides (Axelsson and Mannervik, 1980) and has been little investigated in Se-Hg interactions; Reiter and Wendel (1985) did not find any variations in TT activity after Se administration.

The GSSG-Red activity decreases more than all the other observed enzymes; the supplementation of Se especially at the highest dose (SM3) restored the basal activity with a pattern different from the other cases. Mikkanen and Ganther (1974) found a decrease in

GSSG-Red after MM treatment *in vitro* but not *in vivo*.

In conclusion, after subcronic treatment with Se and MM which did not alter GSH levels in the liver, we obtained a sensitive variation of GST isoenzymes (CuOOH with respect to EA). Under these conditions GSH-Px and GSSG-Red showed a different pattern of activity from that obtained by other authors in acute MM intoxication. These data seem to confirm that Se protection against MM toxicity is effected by a change in MM metabolism and that a very complex response of glutathione dependent enzymes is involved.

REFERENCES

AXELSSON K, MANNERVIK B (1980) General specificity of cytoplasmic thioltransferase (thiol: disulphide oxidoreductase) from rat liver for thiol and disulphide substrates. Biochim Biophys Acta 613:324-336

BALTHROP JE, BRADDON SA (1985) Effects of selenium and methylmercury upon glutathione and glutathione-S-transferase in mice. Arch Environ Contam Toxicol 14:197-205

BRADFORD MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein using the principle of protein dye binding. Anal Biochem 72:248-254

CHANG LW, SUBER R (1982) Protective effect of selenium on methylmercury toxicity: a possible mechanism. Bull Environm Contam Toxicol 29:285-289

CHUNG AS, MAINES MD (1981) Effect of selenium on glutathione metabolism: induction of gamma-glutamylcysteine synthetase and glutathione reductase in the rat liver. Biochem Pharmacol 30:3217-3223

DIERICKX PJ (1985) In vitro interaction of organic mercury compounds with soluble glutathione S-transferases from rat liver. Pharmacol Res Comm 17:489-500

DI SIMPLICIO P (1982) Glutathione and glutathione S-transferase in rat liver and plasma after carbon tetrachloride and thiocetamide intoxication. Pharmacol Res Commun 14:909-920

DI SIMPLICIO P, MANNERVIK B (1983) Enzymes involved in glutathione metabolism in rat liver and blood after carbon tetrachloride intoxication. Toxicol Letters 18:285-290

EL-BEGEARMI MM, SUNDE ML, GANTHER HE (1977) A mutual protective effect of mercury and selenium in japanese quail. Poultry Sci 56:313-322

HIROTA Y, YADAGUSIN S, SHIRODO N, SAND K I (1980) Inhibitory effect of methylmercury on the activity of glutathione peroxidase. Toxicol Appl Pharmacol 53:174-176

IWATA H, MASUKAWA T, KITO H, HAYASHI M (1981) Involvement of

tissue sulfhydryls in the formation of a complex of methylmercury with selenium. Biochem Pharmacol 30:3159-3163
KLING LJ, SOARES JH (1978) Mercury metabolism in japanese II. The effect of dietary mercury and selenium on blood and liver glutathione peroxidase activity and selenium concentration. Poultry Sci 57:1286-1292
MASUKAWA T, KITO H, HAYASHI M, IWATA H (1982) Formation and possible role of bis (methylmercuric) selenide in rats treated with methylmercury and selenite. Biochem Pharmacol 31:75-78
MIKKANEN HM, GANTHER HE (1974) Effect of mercury on erythrocyte glutathione reductase activity. In vivo and in vitro studies. Bull Environ Contam Toxicol 12:10-16
PROHASKA JR, GANTHER HE (1977) Interaction between selenium and methylmercury in rat brain. Chem-Biol Interact 16:155-167
REFSVIK T (1983) The mechanism of biliary excretion of methylmercury: studies with methylthiols. Acta Pharmacol Toxicol 53:153-158
REITER R, WENDEL A (1985) Selenium and drugs metabolism-III: relation of glutathione peroxidase and other hepatic enzyme modulations to dietary supplements. Biochem Pharmacol 34:2287-2290
SPALLHOLZ JE, MARTIN JL, GANTHER HE (1981) Selenium in biology and medicine. AVI Publishing Co., Westport
STOEPPLER M, BACKAUS F (1978) Pretreatment studies with biological and environmental materials. I. Systems for pressurized multisample decomposition. Fresenius Z Anal Chem 291:209-223
STOEWSAND GS, BACHE CA, LISK DJ (1974) Dietary selenium protection of methylmercury intoxication of japanese quail. Bull Environ Contam Toxicol 11:152-156

Received May 17, 1988; accepted June 13, 1988.