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Influence of Pb, Cd, Zn, Mn, Cu, Hg, or Be Salts on the Glutathione S-Transferases of the Rat Liver

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Glutathione S-transferases (GST, EC 2.5.1.18) catalize the metabolism of reactive substances of exogeneous or endogeneous origin (Chasseaud 1979). These enzymes are involved in physiological inactivation processes of environmentally relevant agents and their metabolites. There are several isoenzymes with different substrate specificity (Habig et al. 1974). The metabolic reactions are characterized as irreversible conjugations with glutathione (GSH), in the course of which electrophilic carbon, sulfur, nitrogen or oxygen atoms of the chemicals react with the sulfur atom of glutathione (Mannervik et al. 1983). One of the GST is the enzyme glutathione S-epoxide transferase (GSH S-epoxide transferase), which comprises about 10 percent of the total activity of all GST (Baars et al. 1978). The activity of the GSH S-epoxide transferase may be monitored with styrene oxide as the substrate (Baars et al. 1978). About 90 percent of the total GST activity is comprised of the glutathione S-aryltransferase (GSH S-aryltransferase) which may be monitored using 1-chloro-2,4-dinitrobenzene (CDNB) as the substrate (Habig et al. 1974).

Earlier investigations revealed different interactions of metal-free (Schreiner and Freundt 1985) and metal-containing (Schreiner and Freundt 1986) thiurams (fungicides, rubber accelerators) with GST of rat livers. Based on these observations the question arose whether heavy metals alone may influence the activity of GST. In vitro experiments with isolated GST showed different interactions: lead inhibited the activity of GST preparations from cestode proglottids and nematode intestinal epithelial cells (Douch and Buchanan 1978), from rat brain (Chand and Clausen 1982), from rat liver (Henry and Byington 1976), and from calf liver (Reddy et al. 1981). But other authors (Parkki et al. 1978, Dierickx 1982) could not confirm this inhibitory action of lead on isolated rat liver GST in vitro. Ions of Zn, Cd or Hg inhibited the activity of GST isolated from monkey liver (Asaoka and Takahashi 1977). Inhibition of the activity of GST isolated from rat liver has been caused by different organic Hg compounds (Dierickx 1985). The activity of the GSH S-aryltransferases in liver, kidney, lung and brain of mice were enhanced by treatment (i.v. injection) with $CdCl_2$, $HgCl_2$ or $NaVO_3$; in contrast the activity of GSH S-epoxide transferase in the liver of mice was decreased by Cd, Hg or V containing salts (Siegers et al. 1987). The objective of our study was to

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explore the effects of Pb, Cd, Zn, Mn, Cu, Hg and Be ions on the glutathione-conjugating enzyme system (GSH S-epoxide transferase, GSH S-aryltransferase) during long-term administration (3 months) as compared to in vitro exposure.

MATERIALS AND METHODS

Adult female SPF Sprague-Dawley rats, weighing 210 - 230 g, were used. The animals were housed in Macrolon cages (53 x 32 x 19 cm, 5 rats per cage) under standardized conditions: temperature 22° C, relative humidity approximately 40 %; 12-h neon lighting for simulation of light/dark cycles; standard diet from Altromin (Lage, FR Germany) and tap water ad libitum.

Rats randomized into groups of 5 and were fed with demineralized water containing 100 ppm of $Pb(CH_3COO)_2 \cdot 3H_2O$, $CdCl_2 \cdot H_2O$, $ZnSO_4 \cdot 7H_2O$, $MnCl_2 \cdot 4H_2O$, $CuCl_2$, $Hg_2(NO_3)_2 \cdot 2H_2O$, or $BeSO_4 \cdot 4H_2O$. This concentration is usual in feeding studies (Kobayashi and Kimura 1985). Demineralized water without metal salts was offered as drinking water to control animals. The water was renewed twice each week. After 15, 30 or 90 days of treatment the livers of the animals were removed for determination of GST activity. During the 90 day experiment the rats were weighed once a week on a fixed schedule and changes of the body weight were calculated for monitoring of the state of health. The intake of food (pellets) and of drinking water was determined simultaneously for each group on a weekly fixed schedule.

The rat livers were removed under urethane anaesthesia (5 ml/kg b.w. i.p. of 25 % (w/v) urethane in demineralized water) after washing the tissue blood-free by perfusion with 20 ml physiological saline (0.15 mol/l NaCl) via the portal vein. The livers were minced and homogenized (0° C, 3 min) in sucrose-KCl-Tris buffer pH 7.35 (5 ml/g tissue; 0.35 mol/l sucrose, 0.025 mol/l KCl, 0.01 mol/l Tris). The homogenate was ultra-centrifuged (105,000 x g, 90 min). The supernatant containing the cytosol with the soluble GST was separated and used as enzyme preparation after standardizing the protein content by dilution with sucrose-KCl-Tris buffer pH 7.35 to 10 mg protein/ml cytosol for the investigations with styrene oxide as substrate (GSH S-epoxide transferase), and to 2 mg protein/ml cytosol for the investigation with 1-chloro-2,4-dinitrobenzene as substrate (GSH S-aryltransferase). All preparations were carried out at 0° C in an ice bath.

The in vitro reaction mixture (total volume: 1 ml) contained successively at 0° C (ice bath): 0.58 ml KCl-Tris buffer pH 8, 0.2 ml GSH solution (20 µmol/ml), 0.1 ml metal salt solution (5, 10, 50, 250 µmol/l), 0.1 ml cytosol preparation (protein concentration is given above) 0.02 ml substrate solution (500 µmol/ml; blank mixture without substrate). The reaction was started at 37° C. The total incubation time was 20 min. The reaction was stopped by addition of 0.5 ml methanol and cooling at 0° C (ice bath). After centrifugation (15,000 x g, 2 min) 0.5 ml supernatant were used for determination of the enzyme activity. An identical reaction mixture without addition of metal salts was used for the enzyme determination after in vivo treatment.

The activity of GSH S-epoxide transferase was determined colorimetrically (412 nm) using styrene oxide as substrate (Baars et al. 1978). The activity of GSH S-aryltransferase was determined colorimetrically (412 nm) using 1-chloro-2,4-dinitrobenzene (CDNB) as substrate (Habig et al. 1974). Both GST isoenzymes catalyse the conjugation of glutathione with the substrate. The glutathione not reacted in the incubation mixture was colorimetrically determined (Ellmann 1959) and the enzyme activity was calculated. The protein content of the enzyme preparations was colorimetrically analysed (standard: albumin, bovine, fraction V, Sigma, St. Louis, MO, USA) with the Folin reagent (Lowry et al. 1951).

Styrene oxide was purchased from EGA-Chemie, Steinbruck/FR Germany. 1-chloro-2,4-dinitrobenzene was obtained commercially from Aldrich-Chemie, Steinheim/FR Germany. Folin reagent and all other substances of reagent grade were from E. Merck, Darmstadt/FR Germany.

The means $\pm$ SEM were calculated from the corresponding individual values determined. The treatment groups were compared with controls using Dunnett's (1955) test. A p value below 0.05 was considered as significant.

RESULTS AND DISCUSSION

The activity of GST with styrene oxide as substrate was strongly decreased in vitro by low metal salt concentrations (5 µmol/l). Increasing concentrations (up to 250 µmol/l) further enhanced this inhibition; the salts of Mn and Be were less effective than the other metal salts (Table 1). In vitro experiments with 1-chloro-2,4-dinitrobenzene as substrate showed about 45 % inhibition by low metal salt concentrations (5 µmol/l). Higher concentrations (up to 250 µmol/l) further increased this inhibition by about 15 %. Only higher concentrations of Mn and Be salts were without pronounced additional inhibitory effects (Table 1). In these in vitro experiments the metals used inhibited the GSH S-aryltransferase activity to a similar degree as the activity of the GSH S-epoxide transferase. The effect of Cu on GSH S-aryltransferase activity was smaller as compared to that on GSH S-epoxide transferase, and that of Be was more pronounced as compared to the effect on GSH S-epoxide transferase (Table 1). This in vitro inhibition of both GST isoenzymes is in agreement with observations of other authors regarding inhibition of isolated GSH S-aryltransferase (substrate: CDNB) from rat liver by organic Hg compounds (Dierickx 1985) and Et_3PbCl (Henry and Byington 1976), from calf liver by $CdCl_2$, MeHgCl, $HgCl_2$ and Pb-acetate (Reddy et al. 1981), or from monkey liver by $ZnCl_2$, $CdCl_2$ and $HgCl_2$ (Asaoka and Takahashi 1977).

Rats were given 100 ppm heavy metal salts for up to 90 days via their drinking water. On day 15 the GSH S-epoxide transferase activity was found to be decreased, after further 15 days the inhibition was less pronounced. On day 90 of the continuous heavy metal salt treatment the activity of GSH S-epoxide transferase had returned to almost normal level in most cases. Under identical supplementations of the drinking water with heavy metal salts the GSH S-aryltransferase activity was inhibited on day 30 more than on day 15 and less on day 90 than on day 30; exception: With Pb-acetate this effect occurred on day 15 (Table 2).

Table 1. Activity of GSH S-epoxide transferase (nmol styrene oxide/mg protein/min) or GSH S-aryltransferase (nmol CDNB/mg protein/min) in vitro under the influence of heavy metal salts (5 - 250 µmol/l). Means ± SEM of 3 observations with treatment or 9 control observations each.

Metal salt	Control	nmol Styrene oxide/mg protein/min after 5 µmol Metal salt/l	10 µmol Metal salt/l	50 µmol Metal salt/l	250 µmol Metal salt/l
$Pb(CH_3COO)_2$	1.74 ± 0.12	1.01 ± 0.06 (a)(-42)	0.97 ± 0.05 (a)(-44)	0.89 ± 0.05 (a)(-49)	0.73 ± 0.05 (a)(-58)
$CdCl_2$	1.74 ± 0.11	0.84 ± 0.03 (a)(-52)	0.80 ± 0.02 (a)(-54)	0.77 ± 0.03 (a)(-56)	0.64 ± 0.03 (a)(-63)
$ZnSO_4$	1.59 ± 0.08	0.95 ± 0.01 (a)(-40)	0.92 ± 0.01 (a)(-42)	0.85 ± 0.02 (a)(-47)	0.67 ± 0.01 (a)(-58)
$MnCl_2$	1.63 ± 0.05	0.98 ± 0.04 (a)(-40)	0.97 ± 0.04 (a)(-40)	0.91 ± 0.05 (a)(-44)	0.85 ± 0.06 (a)(-48)
$CuCl$	1.92 ± 0.06	0.77 ± 0.02 (a)(-60)	0.69 ± 0.02 (a)(-64)	0.61 ± 0.02 (a)(-68)	0.48 ± 0.02 (a)(-75)
$HgNO_3$	1.92 ± 0.05	0.92 ± 0.02 (a)(-52)	0.89 ± 0.02 (a)(-54)	0.84 ± 0.03 (a)(-56)	0.66 ± 0.02 (a)(-66)
$BeSO_4$	1.31 ± 0.06	0.94 ± 0.03 (a)(-28)	0.94 ± 0.03 (a)(-28)	0.90 ± 0.02 (a)(-31)	0.84 ± 0.02 (a)(-36)
		nmol CDNB/mg protein/min			
$Pb(CH_3COO)_2$	1.74 ± 0.03	0.94 ± 0.03 (a)(-46)	0.91 ± 0.04 (a)(-48)	0.79 ± 0.06 (a)(-55)	0.72 ± 0.02 (a)(-59)
$CdCl_2$	1.74 ± 0.03	0.92 ± 0.02 (a)(-47)	0.87 ± 0.03 (a)(-50)	0.80 ± 0.07 (a)(-54)	0.75 ± 0.08 (a)(-57)
$ZnSO_4$	1.76 ± 0.04	0.92 ± 0.03 (a)(-48)	0.89 ± 0.03 (a)(-49)	0.79 ± 0.07 (a)(-55)	0.70 ± 0.08 (a)(-60)
$MnCl_2$	1.69 ± 0.06	0.97 ± 0.01 (a)(-43)	0.97 ± 0.01 (a)(-43)	0.94 ± 0.01 (a)(-44)	0.91 ± 0.01 (a)(-46)
$CuCl$	1.67 ± 0.04	0.84 ± 0.01 (a)(-50)	0.79 ± 0.02 (a)(-53)	0.76 ± 0.02 (a)(-54)	0.63 ± 0.02 (a)(-62)
$HgNO_3$	1.68 ± 0.04	0.95 ± 0.02 (a)(-43)	0.91 ± 0.01 (a)(-46)	0.90 ± 0.01 (a)(-46)	0.66 ± 0.01 (a)(-61)
$BeSO_4$	1.69 ± 0.05	0.97 ± 0.01 (a)(-43)	0.93 ± 0.01 (a)(-45)	0.93 ± 0.01 (a)(-45)	0.92 ± 0.01 (a)(-46)

(a) = p below 0.05 (-) = decrease as percent of control

Table 2. Activity of GSH S-epoxide transferase (pmol styrene oxide/mg protein/min) or GSH S-aryltransferase (pmol CDNB/mg protein/min) in rat liver after oral intake of drinking water containing 100 ppm of heavy metal salts for 15, 30 or 90 days. Means ± SEM of 5 observations (rats) per group.

Metal salt	pmol Styrene oxide/mg protein/min after 15 days		30 days		90 days	
Control	198 ± 2		165 ± 8		166 ± 5	
$Pb(CH_3COO)_2$	86 ± 4 (a)	(-57)	207 ± 5 (a)	(+25)	180 ± 10	(+8)
$CdCl_2$	87 ± 2 (a)	(-56)	188 ± 4 (a)	(+14)	161 ± 10	(-3)
$ZnSO_4$	145 ± 3 (a)	(-27)	151 ± 8	(-8)	157 ± 3	(-5)
$MnCl_2$	158 ± 8 (a)	(-20)	138 ± 4 (a)	(-16)	162 ± 5	(-2)
$CuCl_2$	141 ± 4 (a)	(-29)	170 ± 10	(+3)	176 ± 5	(+6)
$HgNO_3$	167 ± 7 (a)	(-16)	149 ± 29	(-10)	150 ± 1 (a)	(-10)
$BeSO_4$	155 ± 2 (a)	(-22)	168 ± 2	(+2)	169 ± 10	(+2)
	pmol CDNB/mg protein/min					
Control	253 ± 2		236 ± 5		255 ± 6	
$Pb(CH_3COO)_2$	232 ± 2 (a)	(-8)	236 ± 4		243 ± 3	(-5)
$CdCl_2$	240 ± 7	(-5)	215 ± 5 (a)	(-9)	232 ± 6 (a)	(-9)
$ZnSO_4$	257 ± 4	(+2)	208 ± 4 (a)	(-12)	233 ± 11	(-9)
$MnCl_2$	251 ± 2	(-1)	219 ± 6	(-7)	245 ± 5	(-4)
$CuCl_2$	252 ± 5	(0)	219 ± 3 (a)	(-7)	240 ± 5	(-6)
$HgNO_3$	245 ± 4	(-3)	220 ± 2	(-7)	228 ± 6 (a)	(-11)
$BeSO_4$	246 ± 3	(-3)	216 ± 3 (a)	(-8)	236 ± 4 (a)	(-7)

(a) = p below 0.05
(-) = decrease or (+) = increase as percent of control

Evidently there was an adaptation to the permanent heavy metal salt burden by both isoenzymes of GST.

The concentrations of the metals determined in the liver (unpublished data) after intake of the metal salts via drinking water (100 ppm) were much lower compared to the metal concentrations of the in vitro experiments calculated for liver tissue on the basis of the liver protein used in the reaction mixture. However, the concentrations of Cd are in the same range: after 30 day intake (100 ppm $CdCl_2$ in drinking water) - 6492 ± 664 ng Cd/g liver (control: 10 ± 1 ng/g), or after 90 day intake (100 ppm $CdCl_2$) - 20074 ± 1824 ng Cd/g liver (control: 10 ± 1 ng/g) (means ± SEM, n = 5 each); concentrations calculated from in vitro administration of 5 µmol $CdCl_2$/l - 3934 ng Cd/g liver, or 10 µmol

$CdCl_2$/l - 7868 ng Cd/g liver (determination of GSH S-epoxide transferase activity), or from administration of 5 µmol $CdCl_2$/l - 19670 ng Cd/g liver (determination of GSH S-aryltransferase activity).

The inhibitory effects of heavy metal ions on the activities of the GST isoenzymes investigated is possibly due to a direct reaction of the metals with enzyme proteins (SH-group?), while the activating effects of some of the heavy metal salts on the activities of both isoenzymes might be caused by stimulation of the de novo synthesis of the enzymes.

Since the long-term experiments with high concentrations of metal salts in the drinking water showed either low or no effects, it is to be concluded that chronic exposure to the applied heavy metals - doses below the concentration administered in this study - does not markedly alter the function of the GST-system. But it cannot be excluded that a minor reduction of the GST-bound metabolizing activity may be caused by the metals investigated.

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REFERENCES

Asaoka K, Takahashi K (1977) Monkey glutathione S-aryltransferases. J Biochem 82: 1313 - 1323

Baars AJ, Jansen M, Breimer DD (1978) The influence of phenobarbital, 3-methylcholanthrene and 2,3,7,8-tetrachlorodibenzo-p-dioxin on glutathione S-transferase activity of rat liver cytosol. Biochem Pharmacol 27: 2487 - 2494

Chand P, Clausen J (1982) Triethyl lead toxicity in relation to brain glutathione and glutathione S-transferase. Tox Lett 12: 181 - 184

Chasseaud LF (1979) The role of glutathione and glutathione S-transferases in the metabolism of chemical carcinogens and other electrophilic agents. Adv Cancer Res 29: 175 - 274

Dierickx PJ (1982) In vitro inhibition of the soluble glutathione S-transferases from rat liver by heavy metals. Enzyme 27: 25 - 32

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

Douch PG, Buchanan LL (1978) Glutathione conjugation of some xenobiotics by Ascaris suum and Moniezia expansa. Xenobiotica 8: 171 - 176

Dunnett CW (1955) A multiple comparison procedure for comparing several treatments with a control. Am Statist Ass J 50: 1096 - 1121

Ellman GL (1959) Tissue sulfhydryl groups. Arch Biochem Biophys 82: 70 - 77

Habig WH, Pabst MJ, Jakoby WB (1974) Glutathione S-transferases - The first enzymatic step in mercapturic acid formation. J Biol Chem 249: 7130 - 7139

Henry RA, Byington KH (1976) Inhibition of glutathione S-aryltransferase from rat liver by organogermanium, lead and tin compounds. Biochem Pharmac 25: 2291 - 2295

Kobayashi S, Kimura M (1985) Effects of orally administered cadmium on alkaline phosphatase isoenzymes in rat tissues. J Pharmacobio-Dyn 8: 853 - 863

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193: 265 - 275

Mannervik B, Guthenberg C, Jensson H, Warholm M, Alin P (1983) Isoenzymes of glutathione S-transferases in rat and human tissues. In: Larsson A et al. (eds) Functions of glutathione: biochemical, physiological, toxicological, and clinical aspects. Raven Press, New York, pp 75 - 88

Parkki MG, Ahotupa M, Aitio M (1978) Inhibition of epoxide hydrase and glutathione S-transferase by heavy metals in vitro. In: Aitio M (ed) Conjugation reactions in drug biotransformation. Elsevier North-Holland, Amsterdam, p 516

Reddy CC, Scholz RW, Massaro EJ (1981) Cadmium, methylmercury, mercury, and lead inhibition of calf liver glutathione S-transferase exhibiting selenium-independent glutathione peroxidase activity. Tox Appl Pharmac 61: 460 - 468

Siegers C-P, Schenke M, Younes M (1987) Influence of cadmium chloride, mercuric chloride, and sodium vanadate on the glutathione-conjugating enzyme system in liver, kidney, and brain of mice. J Toxicol Environ Hlth 22: 141 - 148

Schreiner E, Freundt KJ (1985) Activities of hepatic epoxide hydrolase and glutathione S-transferase in rats under the influence of tetramethyl thiuramdisulfide, tetramethyl thiurammonosulfide or dimethyl dithiocarbamate. Tox Lett 25: 147 - 152

Schreiner E, Freundt KJ (1986) Inhibition of hepatic epoxide hydrolase and glutathione S-transferase in rats by bis(dimethyldithiocarbamato) zinc (ziram). Bull Environ Contam Toxicol 37: 53 - 54

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