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Inhibition of Hepatic Epoxide Hydrolase and Glutathione S-Transferase in Rats by Bis(dimethyldithiocarbamato) Zinc (Ziram)

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Bis(dimethyldithiocarbamato) zinc (ziram) is widely used as a fungicide and rubber accelerator. Chemicals with a thiocarbamate structure act as inhibitors of several enzymes in mammalian organisms (Fishbein 1976). The goal of the present investigation was to study in rats wether ziram can influence epoxide hydrolase (EH, E.C. 3.3.2.3.) and glutathione S-transferase (GST, E.C. 2.5.1.18) activties involved in metabolic inactivation of chemicals with cytotoxic, mutagenic or carcinogenic properties.

MATERIALS AND METHODS

Ziram and methylcellulose were purchased from Fluka (Neu-Ulm / FRG). Adult female SPF Wistar rats, weighing 230 $\pm$ 10 g, were obtained from the Central Breeding Station of the University of Heidelberg / FRG, and were housed under standardized conditions (Schreiner and Freundt 1985). Following a starvation period of 2 hours the rats were given 15 ml/kg body weight of an aqueous 0.75 % (w/v) solution of methylcellulose which contained as a suspension between 0.12 and 4.16 mmol/kg ziram. Five hours after dosing the livers were removed following anaesthesia, and the microsomal fraction was separated (Schreiner and Freundt 1985). Microsomal and cytoplasmic (105 000 x g supernatant) protein was measured spectrophotometrically after reaction with Folin's reagent (Lowry et al. 1951). The activity of microsomal EH was determined using styrene oxide as substrate by measuring the styrene glycol formed (Schreiner and Freundt 1985). The

Table 1. Activities (mean $\pm$ SEM of 4 rats) of hepatic EH and GST, respectively, after oral administration of ziram given by gavage 5 h prior to sampling the livers.

Dose (mmol/kg b.w.)	Epoxide hydrolase (nmol styrene glycol/ mg protein/min)	Glutathione S-transferase (nmol styrene glutathione/ mg protein/min)	
		Cytosol	Microsomes
0 (control)	4.83 $\pm$ 0.27	52.83 $\pm$ 3.36	2.64 $\pm$ 0.18
0.12	4.80 $\pm$ 0.13	50.09 $\pm$ 2.95	2.56 $\pm$ 0.39
2.08	4.36 $\pm$ 0.20	43.44 $\pm$ 2.37[a]	2.28 $\pm$ 0.16
4.16	3.83 $\pm$ 0.14[a]	36.21 $\pm$ 1.58[a]	1.77 $\pm$ 0.13[a]

[a] significant (95 % level)

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Table 2. Activities (mean ± SEM of 4 rats, 3 determinations per rat) of EH and GST, respectively, 20 min after addition of ziram to reaction mixtures in vitro (Schreiner and Freundt 1985).

Concentration (µmol/ml)	Epoxide hydrolase (nmol styrene glycol/ mg protein/min)	Glutathione S-transferase (nmol styrene glutathione/ mg protein/min)	
		Cytosol	Microsomes
0 (control)	5.61 ± 0.31	51.30 ± 2.10	2.36 ± 0.22
1	1.54 ± 0.12[a]	37.08 ± 2.01[a]	1.58 ± 0.12[a]
2	1.51 ± 0.12[a]	36.75 ± 1.69[a]	1.20 ± 0.10[a]
8	1.49 ± 0.06[a]	34.82 ± 1.22[a]	1.09 ± 0.10[a]

[a] significant (95 % level)

cytoplasmic and microsomal GST activities were measured by determining the consumption of glutathione in the reaction of styrene oxide to styrene glutathione conjugate (Schreiner and Freundt 1985). The treatment groups were compared with control using Dunnett's (1955) test at the p = 95 % and p = 99 % significance levels.

RESULTS AND DISCUSSION

In vivo (Table 1) a dose of 4.16 mmol/kg ziram decreased EH activity significantly. Lower doses showed a slight tendency to lower EH activity. GST activity in cytosol was decreased significantly by 2.08 mmol/kg ziram, more effective were 4.16 mmol/kg. Microsomal GST activity was diminished significantly by 4.16 mmol/kg ziram, while doses below exerted a lesser and insignificant effect. In vitro (Table 2) concentrations of 1 - 8 µmol/ml ziram caused a steady and significant decrease of EH or GST activities. The strong inhibition of enzyme activities in vitro implicate that the inhibitory effect in vivo is due to ziram itself and not caused by its metabolites. The results have a bearing on the evaluation of the risk to human health after, e.g. occupational, longterm exposure to ziram since following accumulation an attainment of high concentrations in the organism similar to that used in the present investigation cannot be excluded.

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