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Antidotal effects of dimethyl sulphoxide against paracetamol-, bromobenzene-, and thioacetamide-induced hepatotoxicity

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In mice the hepatotoxic effects of paracetamol (0·5–1·0 g kg^{-1}, orally) as evidenced by increased serum enzyme activities of the aminotransferases and sorbitol dehydrogenase were dose-dependently inhibited by simultaneous treatment with dimethyl sulphoxide (DMSO 0·25–1·0 g kg^{-1}, i.p.). DMSO was also active against bromobenzene- and thioacetamide-induced hepatotoxicity, but failed to protect mice against carbon tetrachloride-induced liver damage. Hepatic glutathione depletion in mice amounting to 94% after paracetamol (0·5 g kg^{-1}, orally) and to 60% after bromobenzene (0·25 ml kg^{-1}, orally) was dose-dependently reduced by the simultaneous administration of DMSO (0·25–1·0 g kg^{-1}, i.p.). This indicates less conjugation of the toxic metabolites of paracetamol and bromobenzene to liver glutathione (G-SH) in the presence of DMSO.

Dimethyl sulphoxide (DMSO) is one of the solvents frequently used in pharmacological or toxicological experiments to improve the solubility of otherwise poorly soluble compounds. Ideally, however, the drug under observation should be administered in an inert vehicle. DMSO is reported to have cryoprotective and radioprotective properties (Ashwood-Smith, 1971) and inhibitory effects on several enzyme systems (Rammler, 1971), but there is no information on the interactions of DMSO with hepatotoxic agents.

During investigations on paracetamol-induced hepatotoxicity using DMSO as a solvent, I found that it protected mice against the hepatotoxic effects of paracetamol. In this preliminary report the results on the anti-hepatotoxic properties of DMSO against paracetamol and other hepatotoxic agents are presented.

MATERIALS AND METHODS

Male NMRI-mice (dealer: P. Bäumler, Wolfratshausen) 25–35 g were kept at an ambient temperature of 23° and Altromin pellets and drinking water were freely available. Paracetamol (0·5 or 1·0 g kg^{-1}, respectively) was suspended in a 1% tylose solution and instilled by stomach tube or injected intraperitoneally (10 ml kg^{-1}). Bromobenzene (0·25 ml kg^{-1}) and carbon tetrachloride (0·1 ml kg^{-1}) were dissolved in olive oil and were also given by stomach tube (10 ml kg^{-1}). Thioacetamide (50 mg kg^{-1}) was dissolved in saline and given by stomach tube. Dimethyl sulphoxide was diluted with saline (volume: 10 ml kg^{-1}) for intraperitoneal as well as for oral administration.

Blood samples from the mice were obtained by decapitation 24 h after the treatments. Serum enzyme activities of the aminotransferases (GOT, GPT) and sorbitol dehydrogenase (SDH) were measured using the commercial reagents of Boehringer, Mannheim. For the glutathione measurements, the livers were excised rapidly 1 h after dosing and homogenized in 6 ml of ice-cold 1 M perchloric acid. To eliminate the diurnal influence of glutathione concentrations, dosing was always carried out at 11 a.m. Reduced glutathione (GSH) was measured according to Bernt & Bergmeyer (1970).

RESULTS

Serum enzymes

Serum activities of GOT, GPT and SDH measured 24 h after oral or intraperitoneal administration of paracetamol (0·5 or 1·0 g kg^{-1}, respectively) are given in Table 1. High increases of the enzymes indicated severe liver damage following paracetamol treatment at a dose of 0·5 g kg^{-1}, orally. Simultaneous treatment with dimethyl sulphoxide (i.p.) caused a dose-dependent reduction of the paracetamol-induced enzyme elevations (Table 1); after 1·0 g kg^{-1} DMSO (i.p.) the serum enzymes were almost within the nomal ranges. DMSO alone (2·0 g kg^{-1}, i.p.) did not alter the GOT, GPT- and SDH-activities of mice compared with the values of control animals.

DMSO protected mice against paracetamol induced hepatotoxicity even when applied 1 h after paracetamol administration (Table 1). In a further experiment in which paracetamol was injected intraperitoneally, and DMSO given orally, the hepa-

Table 1. *Serum enzyme activities 24 h after poisoning with paracetamol and simultaneous treatment with dimethyl sulphoxide (DMSO) in mice.* In one experiment DMSO was given 1 h after paracetamol, in another experiment paracetamol was given intraperitoneally and DMSO by stomach tube. Values represent means and their standard errors of 12 mice each.

Paracetamol (g kg^{-1}, orally)	DMSO g kg^{-1}, i.p.	GOT mU ml^{-1}	GPT mU ml^{-1}	SDH mU ml^{-1}
	—	73 ± 9	39 ± 5	14·9 ± 4·6
—	2·0	98 ± 16	43 ± 5	11·1 ± 2·9
0·5	—	1748 ± 812	2073 ± 1187	694 ± 375
0·5	0·25	358* ± 123	301 ± 216*	17·4* ± 1·6
0·5	0·5	235* ± 34	104* ± 46	40·6* ± 15·0
0·5	1·0	134* ± 18	67* ± 9	23·5* ± 9·8
1·0	—	1537 ± 115	4017 ± 462	586 ± 115
1·0	1·0	180* ± 21	135* ± 17	15·3* ± 1·1
0·5	—	687 ± 467	936 ± 782	236 ± 197
0·5	1·0†	150* ± 21	122* ± 16	13·6* ± 3·1
0·5, i.p.	—	865 ± 507	1251 ± 596	351 ± 180
0·5, i.p.	1·0 orally	240* ± 26	237* ± 152	42* ± 29

* Significant difference to the corresponding value without DMSO-treatment. (P <0·05; rank test of Wilcoxon-Mann-Whitney).
† 1 h later.

toprotective activity of DMSO was again evident (Table 1).

The results in Table 2 show that DMSO was also active against the hepatotoxic effects of bromobenzene and thioacetamide. Thus, the elevations in serum enzyme activities normally induced by these compounds were completely suppressed by simultaneous treatment with DMSO (1·0 g kg^{-1}, i.p.).

Liver glutathione

The hepatotoxic metabolites of paracetamol and bromobenzene are partially inactivated by glutathione (GSH) and excreted as mercapturic acid conjugates. This results in a significant depletion of liver GSH stores following high dosage with these toxins (Mitchell, Jollow & others, 1973; Jollow, Mitchell & others, 1974; Siegers, Schütt & Strubelt, 1977). In the present work, paracetamol (0·5 g kg^{-1}, orally depleted liver GSH by approximately 94% and simultaneous treatment with DMSO (0·25 kg^{-1}, i.p.) reduced this depletion to 73%. Simultaneous treatment with DMSO 1 g kg^{-1} (i.p.) reduced the depletion still further to 41% (Fig. 1). Bromobenzene (0·25 ml kg^{-1}, orally) caused a 60% depletion of liver GSH which was completely prevented by simultaneous injection of DMSO (1 g kg^{-1}, i.p.) (Fig. 1).

Table 2. *Serum enzyme activities 24 h after poisoning with bromobenzene, carbon tetrachloride, (CCl_4) thioacetamide and simultaneous treatment with dimethyl sulphoxide (DMSO) in mice.* Values represent means and their standard errors of 12 mice each.

Hepatotoxin Doses kg^{-1} orally	DMSO g kg^{-1}, i.p.	GOT mU ml^{-1}	GPT mU ml^{-1}	SDH mU ml^{-1}
Bromobenzene				
0·25 ml	—	957 ± 528	1160 ± 701	229 ± 54
0·25 ml	1·0	85 ± 9*	47 ± 2*	14·1 ± 2·0*
Thioacetamide				
0·05 g	—	691 ± 245	1213 ± 262	279 ± 23
0·05 g	1·0	84 ± 11*	57 ± 6*	11·2 ± 2·4*
CCl_4				
0·1 ml	—	1313 ± 237	2591 ± 271	1533 ± 217
0·1 ml	1·0	1920 ± 592	3026 ± 1067	2280 ± 370
0·1 ml	2·0	1687 234	3727 701	2092 ± 289

* Significant difference to the corresponding value without DMSO-treatment. (P <0·05; rank test of Wilcoxon-Mann-Whitney).

DISCUSSION

The use of DMSO as a solvent in experiments with hepatotoxic agents must clearly be avoided since this compound may exert a protective influence on

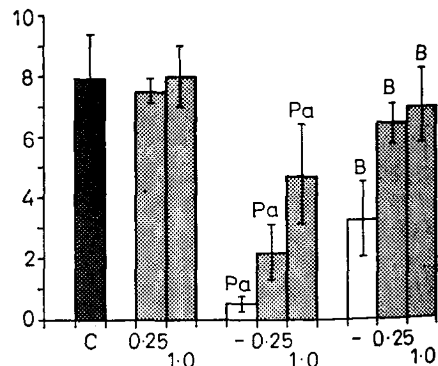

Fig. 1. Hepatic glutathione (G-SH) in mice after oral treatment with paracetamol or bromobenzene and simultaneous intraperitoneal injection of DMSO. Values represent means and their confidence limits for $P = 0·95$ (n = 6). C = control; Pa: paracetamol; B: bromobenzene. Ordinate: μmol g^{-1} liver. Abscissa: g kg^{-1}.

the liver. The toxic effects of paracetamol, bromobenzene and thioacetamide are thought to arise from their oxidation by the hepatic mixed-function oxidase system to chemically reactive alkylating agents (Mitchell & others, 1973; Jollow & others, 1974; Ammon, Berninger & others, 1967). The antidotal properties of DMSO could therefore be due to inhibition of microsomal oxidation, a theory recently advanced to explain the protective properties of dithiocarbamate (Siegers, Strubelt & others, 1976). This hypothesis fails to explain the inability of DMSO to protect mice against carbon tetrachloride hepatotoxicity, however, since this compound is also activated by the mixed-function oxidase system. With mice, DMSO (1 g kg^{-1}, i.p. 1 h before death) had no effect on aniline hydroxylation in a 9 000*g* liver homogenate supernatant (Siegers, unpublished work). This contrasts with the findings of Stock & Fouts (1971) who reported a stimulatory effect of DMSO on microsomal hydroxylation of aniline after *in vivo* injection into rats or *in vitro* addition to rat liver microsomes. Nevertheless, the reduction in paracetamol and bromobenzene-induced liver GSH depletion brought about by DMSO does indicate a curb on the formation of conjugates between glutathione and hepatotoxic metabolites. This might be explained by (1) a decreased metabolic conversion of paracetamol and bromobenzene; (2) inactivation of the toxic metabolites via direct combination with DMSO; (3) a DMSO-mediated change in the distribution, renal or biliary excretion of the unchanged or metabolized compounds; (4) decreased covalent binding of the toxic metabolites in the presence of DMSO.

Thus, the mechanism of the antihepatotoxic actions of DMSO remains obscure.

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