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A POSSIBLE PROTECTIVE ROLE FOR SULPHYDRYL COMPOUNDS IN ACUTE ALCOHOLIC LIVER INJURY

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Abstract—The role of various sulphydryl compounds in protective mechanisms against acute ethanol toxicity has been investigated in the mouse. Intraperitoneal administration of varying doses of ethanol (0–6 g/kg as 20 per cent, w/v solutions) produced a linear dose response reduction in hepatic reduced glutathione (GSH) levels. The time scale of this effect suggested that GSH depletion occurred as a consequence of hepatic damage rather than contributing towards it. It was also demonstrated that the sulphydryl compounds β-mercaptoethylamine-HCl, cysteine and methionine significantly increased the survival rate of mice given a lethal dose of ethanol.

Reduced glutathione (GSH) is the major non-protein thiol of the cell. In the liver it is a substrate for the enzyme GSH peroxidase, which is responsible for the further metabolism of lipid peroxides [1, 2], and is important in conjugation reactions with foreign compounds which are then excreted as mercapturic acids [3, 4]. As such its intracellular concentration is important and any diminution could lead to lipid peroxide or other cellular injury.

The significance of this to ethanol induced hepatic injury was indicated when it was found that acute ethanol intoxication resulted in increased lipid peroxide formation [5] and in a reduction in GSH levels in the livers of rats [6]. Unfortunately, administered GSH does not readily enter cells. Recently, however, several reports have been published indicating a protective role for other sulphydryl compounds in various cases of drug induced liver injury including cysteamine in paracetamol poisoning [7] and cysteine in bromobenzene [3], paracetamol [8] and carbon tetrachloride poisonings [9].

The present study was therefore undertaken to further examine the effect of ethanol on GSH levels in the liver and to investigate the effect of the sulphydryl compounds β-mercaptoethylamine-hydrochloride (cysteamine-HCl) and cysteine and the sulphur containing amino acid, methionine, on the toxic action of ethanol and its metabolite acetaldehyde.

MATERIALS AND METHODS

β-Mercaptoethylamine hydrochloride, cysteine and methionine were supplied by British Drug Houses, Poole, Dorset. Titration with iodine and TLC examination [7] confirmed that the purity of β-mercaptoethylamine hydrochloride was greater than 95 per cent.

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1. *The effect of an acute dose of ethanol on hepatic GSH levels in the mouse*

Methods. Male mice weighing approximately 30 g each were divided into seven groups of six animals and starved overnight. Animals in each group were given a single intraperitoneal injection of 6 g ethanol/kg body weight as a 20 per cent w/v solution in saline. Groups were sacrificed at 2-hour intervals from zero time to 10 hr with the exception of one group which was maintained for 24 hr. Livers were removed and hepatic GSH levels measured by a modification of the method described by Jollow *et al.* [3].

Results. Figure 1 shows the depletion of hepatic GSH levels with time in the mouse following an acute dose of ethanol. Up to 4 hr after ethanol administration there was very little difference from zero time levels, but thereafter the level of GSH fell steadily to approximately 45 per cent of normal after 8 hr. This low level of GSH was maintained up to 24 hr after ethanol administration.

2. *Relationship between ethanol dosage and hepatic GSH levels in the mouse*

Methods. The procedure was similar to that above. Seventy mice in groups of ten were given single doses of ethanol varying from 0–6 g ethanol/kg body weight. All animals were sacrificed after 8 hr and their hepatic GSH levels measured.

Results. Figure 2 shows that hepatic GSH levels in the mouse are depleted by increasing ethanol concentrations. The depletion was linear with a correlation coefficient of $r = 0.74$ ($P < 0.001$). The reduction in GSH levels to approximately 45 per cent of normal produced by 6 g ethanol/kg is presumably the maximum response to ethanol since animal mortality dramatically increases above this ethanol dosage (see Table 1).

3. *The effect of β-mercaptoethylamine-HCl, cysteine and methionine on the survival of mice given a lethal dose of ethanol*

Methods. The experiments were designed to determine whether β-mercaptoethylamine-HCl, cysteine

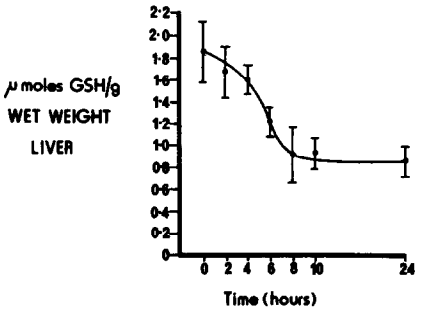

Fig. 1. Changes in the level of GSH in mouse liver following an acute dose of ethanol.

and methionine had any effect on the survival of mice given an LD_{70} dose of ethanol.

Male mice weighing 28–33 g were divided into groups of seventy animals. Control groups received a single intraperitoneal injection of ethanol, while test groups received ethanol followed 10–20 min later by an intraperitoneal injection of one of the test compounds. The dosages used were as follows: ethanol, 6.5 g/kg as a 19 per cent w/v solution in saline, β-mercaptoethylamine-HCl and cysteine, 330 mg/kg as 1 g/10 ml solutions in saline and methionine, 330 mg/kg as a 1 g/20 ml solution in 10 per cent ascorbic acid in saline.

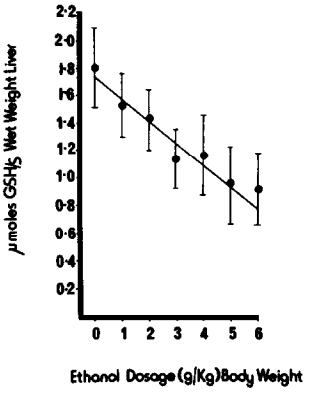

Fig. 2. Dose relationship between ethanol dosage and hepatic GSH levels in the mouse.

In all groups those animals not surviving for a minimum period of 2 hr were excluded from the final assessments, their deaths being attributed to anaesthetic or other non-specific effects of ethanol [10]. The experiments were terminated after 24 hr and the number of animals still surviving in each group counted.

Results. The proportion of fatalities occurring in the first 2 hr following ethanol administration was the same for all groups within each trial (Table 1).

Ethanol administration given alone to control groups produced approximately 70 per cent mortality. The administration of β-mercaptoethylamine-HCl significantly ($P < 0.001$) increased survival as compared with controls (Table 1).

Table 1 also shows that a similar increase in survival was obtained by cysteine ($P < 0.001$), whilst methionine was somewhat less effective ($P < 0.05$).

4. *The effect of cysteine on the survival of mice given a lethal dose of acetaldehyde*

Mice 30–40 g were given cysteine 330 mg/kg 30 min prior to dosage with acetaldehyde solution (0.1 ml) at a dose rate of 15 nmol/kg. The survival of the animals was followed over the subsequent 48 hr.

Results. There was no significant difference in mortality in the experiment between the animals treated with SH compounds and acetaldehyde, and those given acetaldehyde alone (Table 2).

DISCUSSION

It has been shown that the administration of large single doses of ethanol to mice significantly reduces the level of GSH in the liver, a result which is in agreement with our previous observation in rats [6].

Table 1. The effect of β-mercaptoethanolamine-HCl, cysteine and methionine on the survival of mice given lethal doses of ethanol

	Number of animals	Number surviving after 2 hr	Number surviving after 24 hr	% Survival after 24 hr	% Increase in survival
Ethanol control	70	52	20	38%	—
Ethanol + cysteamine-HCl	70	52	37	71%	87%*
Ethanol control	70	65	19	29%	—
Ethanol + cysteine	70	65	39	60%	107%*
Ethanol + methionine	70	65	30	47%	62%†

* $P < 0.001$
† $P < 0.05$

Table 2. Effect of cysteine on mice given a lethal dose of acetaldehyde

	Number of animals	% Survival after 3 hr	24 hr	48 hr
Acetaldehyde control	68	38	15	6
Acetaldehyde + cysteine (330 mg/kg)	68	28	16	13

The maximum depletion appeared to occur 6–8 hr after ethanol administration and was maintained for at least a further 16 hr thereafter. An ethanol dose–response relationship with the level of GSH in the liver was also established. This indicated that GSH levels of 45 per cent of normal were the lowest obtainable with ethanol since further increases in the ethanol dosage resulted in a dramatic increase in animal mortality (Table 1).

Reductions in the GSH content of the liver is a common feature of hepatoxicity. For example, significant reductions in GSH level occur within 5 hr of bromobenzene administration [3] and within 1 hr of paracetamol administration [11]. Associated with this there is an increase in free radical and lipid peroxide formation.

Since GSH inhibits lipid peroxidation as a result of its antioxidant properties [12] and through its involvement with GSH peroxidase [5], a decrease in the GSH content of the liver could well give rise to an increase in both peroxide formation and intracellular injury. However, no depletion of GSH occurred within the first 3 hr following ethanol administration by which time lipid peroxide formation is well established [5]. It therefore appears that the lowering in GSH content is not the cause of the enhanced lipid peroxidation in acute ethanol toxicity but rather may be a consequence of increased peroxidation in the liver cell. The involvement of GSH peroxidation in the detoxification of lipid peroxides could account for a significant proportion of this reduction.

These studies also demonstrate that the sulphydryl compounds β-mercaptoethylamine-HCl, cysteine and methionine, significantly increased the survival of mice given a lethal dose of ethanol. No such effect was observed with ethanol's primary metabolite, acetaldehyde, although such a diminution has been reported previously [13]. This may in part be due to the very much greater toxicity of acetaldehyde.

To what extent this effect of these sulphydryl compounds was due to their ability to prevent liver injury has not been established. However, it has previously been shown that these agents prevent liver damage by other hepatoxic agents by providing an extra source of SH groups for use in conjugation reactions with toxic metabolites [3, 7–9]. That such a mechanism may also operate in ethanol toxicity is suggested by increased excretion of [^{14}C]metabolites in the urine following the administration of [^{14}C]cysteine to rats acutely intoxicated with ethanol [14]. Further, the increase in excretion of these metabolites occurred between 3 and 24 hr after ethanol administration so coinciding with the depletion of GSH in the liver. Although the identity of these metabolites has not yet been established, similar excretion patterns have been observed after administration of other hepatoxic agents. In such instances they have been identified as mercapturic acids [4].

It is also possible that these sulphydryl compounds may serve as alternative substrates for GSH peroxidase. Although the substrate specificities are lower than for GSH [1] their contribution could increase when GSH levels were low. Their potential use may be worthy of consideration in the human situation where mortality from acute alcoholic hepatic failure and hepatic coma remains high [15].

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