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# MECHANISM OF THE PROTECTIVE ACTION OF THIOL COMPOUNDS IN ETHANOL-INDUCED LIVER INJURY

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**Abstract**—The protective action of cysteine or mercaptopropionylglycine (MPG) in acute ethanol-induced liver injury has been investigated in the rat. Cysteine accelerated clearance of ethanol and acetaldehyde from blood and liver and prevented an increase in hepatic content of triglyceride and serum ornithine carbamoyl transferase activity. MPG accelerated clearance of ethanol and acetaldehyde less efficiently but prevented an increase in these variables to the same degree. The mode of action of thiol compounds in acute ethanol-induced liver injury has been discussed.

Thiol compounds, such as cysteine, are known to increase the survival rate of animals given a lethal dose of ethanol [1], to reduce ethanol-induced sleeping time [2], and to prevent ethanol-induced fatty liver [3]. The protective action of thiol compounds in acute ethanol intoxication may be interpreted as a replacement for a decrease in reduced glutathione and/or lipid peroxidation, by its antioxidant properties. Since, however, acute ethanol intoxication does not cause significant depletion of either glutathione or lipid peroxidation, the protective mechanism of thiol compounds in acute ethanol intoxication or in acute ethanol-induced liver injury remains to be clarified.

Recently, acetaldehyde (AcH), a metabolite produced during ethanol oxidation, has been shown to inhibit several hepatic functions [4, 5]. Thiol compounds, such as cysteine, *in vitro*, afford almost complete relief from AcH-induced mitochondrial damage [6]. Cysteine probably exerts its protective effects, at least partly, by forming an adduct with AcH, e.g. thiazolidine, thereby preventing AcH from interacting with mitochondria. The present study was undertaken to distinguish the protective action of thiol compounds in acute ethanol-induced liver injury from the interaction of thiol compounds with ethanol metabolism.

## MATERIALS AND METHODS

Male Wistar strain rats weighing from 200 to 220 g were starved overnight and divided into four groups. Animals in three ethanol groups received a single oral administration of 5 g ethanol/kg of body weight as a 40% solution in saline by a metal gastric tube. Forty minutes prior to ethanol administration, the animals in the two thiol groups received, intraperitoneally, 1.25 mmoles of either L-cysteine (Kishida Chem. Co., Osaka) per kg of body weight as a 3% aqueous solution or 2-mercaptopropionylglycine (MPG, Santen Pharm. Co., Osaka) per kg body weight as a 4% aqueous solution. The animals were anesthetized with 30 mg pentobarbital (injected i.p. as a 1% solution per kg body weight) and, then, were subjected to experimental manipulation. Blood (0.5 ml) was obtained by heart puncture, sequentially 1, 2 and 4 hr after ethanol administration. The blood samples were used for ethanol and AcH determinations. In another animal group, 8 hr after ethanol administration, the animals were killed by exsanguination from the abdominal aorta. The animals in the control group were given isocaloric 40% glucose solution and killed similarly after 8 hr. Blood and liver were subjected to chemical analysis.

For ethanol and AcH determinations, 1 g of liver was immediately frozen *in situ* by means of aluminium clamps precooled in liquid nitrogen [7, 8]. The frozen liver samples were homogenized in 9 ml of ice-cold 0.35 M perchloric acid containing 0.83 mM thiourea (Wako Pure Chem. Ind., Osaka), to prevent non-enzymatic formation of AcH, and 0.55 mM *n*-propanol (Wako Pure Chem. Ind.), as an internal standard for gas chromatography. Peripheral blood (0.5 to 1 ml) was pipetted into 9 vol. of the same solution. The precipitates were centrifuged at 2000 *g* for 10 min at 4°. The supernatant fractions were analysed for ethanol and AcH by gas chromatography with a Shimazu model GC-4LM gas chromatograph (column: 25% polyethylene glycol on Shimolite, and column oven temperature: 90°) using a flame ionization detector.

Hepatic lipids were extracted by the method of Folch *et al.* [9]. Hepatic and serum triglycerides were determined by the method of Sardesai and Manning [10]. Serum ornithine carbamoyl transferase (OCT) activity was measured by the method of Ohshita *et al.* [11], expressed as I.U.

## RESULTS

Table 1 and Fig. 1 show blood levels of ethanol and AcH after ethanol administration. A minute amount (2 ± 1 nmoles/ml) of an AcH-like substance

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Table 1. Effects of cysteine and mercaptopropionylglycine on concentrations of ethanol and acetaldehyde in blood, and on concentrations of triglycerides and ornithine carbamoyl transferase in serum 8 hr after ethanol feeding*

| Group | No. of rats | Blood ethanol (μmoles/ml) | Blood acetaldehyde (nmoles/ml) | Serum triglycerides (mg/ml) | Serum OCT (units) |
|---|---|---|---|---|---|
| Glucose | 9 | 0 | 2 ± 1 | 0.39 ± 0.04 | 2.8 ± 0.4 |
| Ethanol | 11 | 61.3 ± 6.2† | 110 ± 11† | 0.49 ± 0.06 | 9.1 ± 1.2† |
| Ethanol + cysteine | 9 | 0‡ | 8 ± 1†‡ | 0.65 ± 0.04† | 4.1 ± 1.2§ |
| Ethanol + MPG | 9 | 35.6 ± 2.9†§ | 60 ± 6†‡ | 0.61 ± 0.06‖ | 5.2 ± 0.8§‖ |

* Results are expressed as means ± S.E.M.
† $P < 0.01$, compared to the glucose group.
‡ $P < 0.01$, compared to the ethanol group.
§ $P < 0.05$, compared to the ethanol group.
‖ $P < 0.05$, compared to the glucose group.

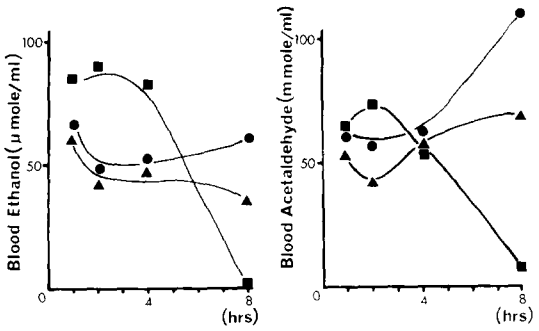


Fig. 1. Effects of cysteine and mercaptopropionylglycine on blood levels of ethanol and acetaldehyde after ethanol feeding. Each point at 1, 2, and 4 hr represents the mean of four animals, and each point at 8 hr represents the mean of nine or more animals. Key: ethanol (●); ethanol + cysteine (■); and ethanol + MPG (▲).

was detected in control blood. Although the nature of the AcH in the control bloods is not known, it seems that the substance was an artifact rather than true AcH, thereby requiring clarification by further study. The administration of ethanol produced marked increases in the blood concentrations of ethanol, AcH and OCT. Eight hours after ethanol administration, blood ethanol was the same as at 1, 2 and 4 hr and blood AcH reached its highest value, probably as a result of the absorption of a large amount of ethanol. These results indicate that at 8 hr ethanol oxidation attained a maximum rate, whereas AcH oxidation was limited.

Ethanol (EtOH) + cysteine treatment increased, at 1–4 hr, the blood levels of ethanol, above those with ethanol alone; 8 hr later the level was reduced to zero. The effect of EtOH + cysteine on AcH was like that of EtOH alone, at 1, 2 and 4 hr, but at 8 hr after EtOH + cysteine the level of AcH had fallen almost to zero. The results suggest that cysteine accelerated absorption and metabolism of ethanol. At 8 hr MPG + EtOH treatment caused a significant lowering of blood levels of ethanol and AcH, and cysteine + EtOH and MPG + EtOH treatments significantly reduced the serum OCT activity.

Table 2 shows the hepatic contents of ethanol, AcH and triglycerides in the experimental groups 8 hr after ethanol administration. In the control rat group, hepatic ethanol was not detected, but small amounts of AcH were. The ethanol administration increased triglycerides in the liver at 8 hr. Treatment with cysteine + EtOH or MPG + EtOH caused a significant reduction of ethanol, AcH and triglycerides; cysteine + EtOH blocked almost completely the increase in ethanol and AcH. The relationships between hepatic and blood levels of ethanol and AcH after 8 hr of ethanol feeding are shown in Fig. 2. There was a highly significant correlation, with a correlation coefficient of +0.992 ($P < 0.001$)

Table 2. Effects of cysteine and mercaptopropionylglycine on hepatic contents of ethanol, acetaldehyde and triglycerides 8 hr after ethanol feeding*

| Group | No. of rats | Ethanol (μmoles/g) | Acetaldehyde (nmoles/g) | Triglycerides (mg/g) |
|---|---|---|---|---|
| Glucose | 9 | 0 | 21 ± 3 | 3.7 ± 0.5 |
| Ethanol | 11 | 55.0 ± 4.5† | 135 ± 11† | 14.8 ± 1.9† |
| Ethanol + cysteine | 9 | 0‡ | 34 ± 2†‡ | 8.0 ± 0.2†§ |
| Ethanol + MPG | 9 | 31.2 ± 2.1†‡ | 95 ± 7†§ | 9.2 ± 0.7†§ |

* Results are expressed as means ± S.E.M.
† $P < 0.01$, compared to the glucose group.
‡ $P < 0.01$, compared to the ethanol group.
§ $P < 0.05$, compared to the ethanol group.

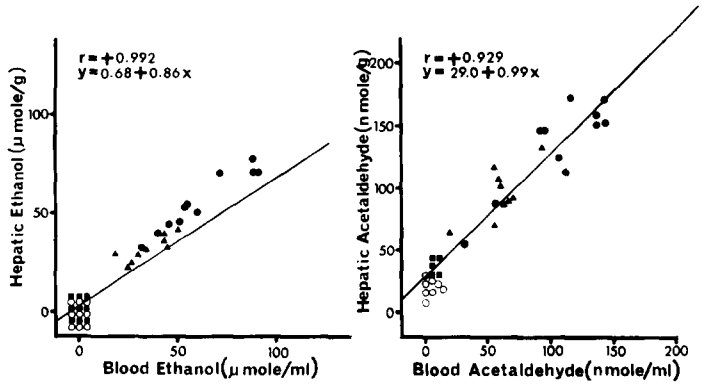


Fig. 2. Relationship between hepatic level and blood concentration of ethanol and of acetaldehyde. Key: control (○); ethanol (●); ethanol + cysteine (■); and ethanol + MPG (▲).

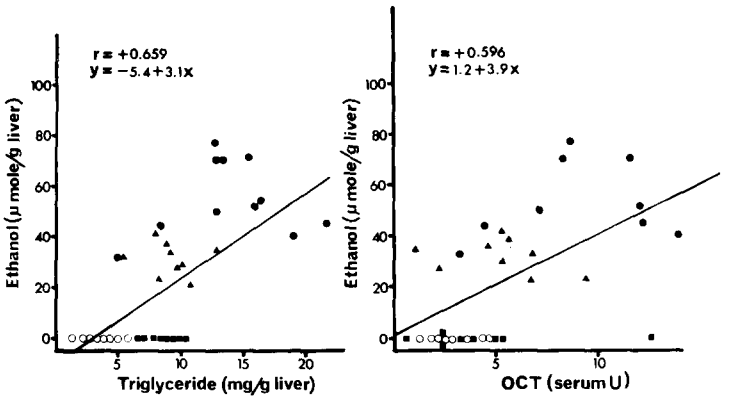


Fig. 3. Correlation of hepatic ethanol levels to hepatic triglyceride levels and to serum ornithine carbamoyl transferase activities. Key: control (○); ethanol (●); ethanol + cysteine (■); and ethanol + MPG (▲).

between the ethanol concentrations in the liver and the blood and of +0.929 ($P < 0.001$) between the AcH concentrations in the liver and the blood respectively.

To reveal the relationships of hepatic ethanol and AcH to hepatic injury, correlations are presented in Figs. 3 and 4. There was a significant correlation of hepatic ethanol concentration to hepatic triglyceride level, with a coefficient of +0.659 ($P < 0.001$), and to serum OCT with a coefficient of +0.596 ($P < 0.001$) respectively. Similarly, there was a significant correlation of hepatic AcH concentration to hepatic triglyceride level, with a coefficient of +0.756 ($P < 0.001$), and to serum OCT with a coefficient of +0.640 ($P < 0.001$) respectively. Finally, to clarify the relationship of hepatic triglyceride accumulation to hepatic mitochondrial damage, a correlation is shown in Fig. 5. There was a significant correlation between hepatic triglyceride concentration and serum OCT with a coefficient of +0.715 ($P < 0.001$).

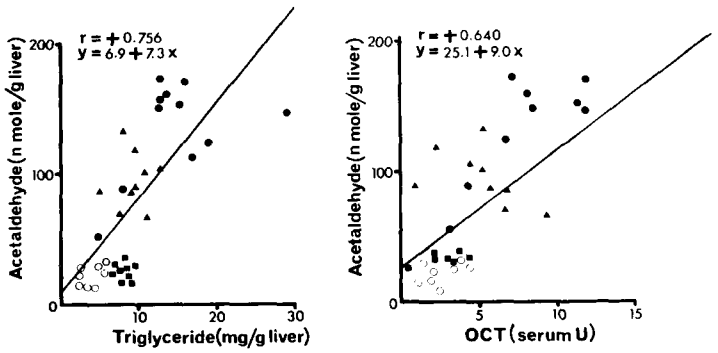


Fig. 4. Correlation of hepatic acetaldehyde levels to hepatic triglyceride levels and to serum ornithine carbamoyl transferase activity. Key: control (○); ethanol (●); ethanol + cysteine (■); ethanol + MPG (▲).

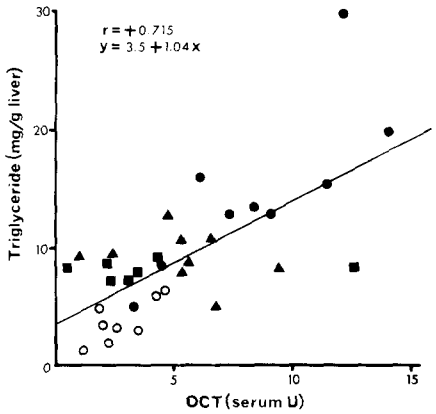


Fig. 5. Correlation of hepatic triglycerides to serum OCT activity. Key: control (○); ethanol (●); cysteine (■); and MPG (▲).

## DISCUSSION

It is well known that a large dose of ethanol causes a reversible fatty liver, characterized mainly by an accumulation of triglycerides. Both decreased lipid oxidation and/or enhanced lipogenesis can be linked to ethanol oxidation but not to ethanol *per se*. For instance, 4-methylpyrazole, which inhibits ethanol oxidation by forming a complex with alcohol dehydrogenase, lessens ethanol-induced fatty liver [12]. The primary mechanism of ethanol oxidation is a reaction with alcohol dehydrogenase, the cytoplasmic enzyme, with NAD as a cofactor. The reaction leads to the formation of AcH and the reduction of NAD to NADH. AcH is converted to acetate by acetaldehyde dehydrogenase, primarily in mitochondria, also with NAD as a cofactor. As a consequence of ethanol oxidation, the increased NADH/NAD ratio may decrease lipid oxidation and increase lipogenesis [4, 5]. Further, AcH has deleterious effects on cell organelles, especially the mitochondria, which participate in fatty acid oxidation. In fact, the present study indicates that triglyceride accumulation in the liver correlates well with serum OCT activity, which is regarded as a reliable index of mitochondrial damage induced by ethanol [13].

It is generally accepted that the most important factor responsible for fatty liver induced by ethanol is probably an increase in the NADH/NAD ratio or a generation of AcH. Our previous study, however, indicated that a restoration of the NADH/NAD ratio by tocopherol lacton did not inhibit ethanol-induced fatty liver [14]. More recently, methylene blue or pyruvate, which corrected the disordered redox state by ethanol, did not reverse *in vitro* the inhibition by ethanol of hepatic glycoprotein synthesis and secretion [15]. Thus, the role of AcH in the actions of ethanol has recently become a subject of much interest. In fact, concerning the fatty liver induced by one large dose of ethanol, attention has been focused on AcH-induced hepatocyte damage, including mitochondrial damage [16–18]. If AcH impairs mitochondrial function, this derangement could further reduce the oxidation of AcH, leading to "circulus vitiosus".

Hepatic AcH levels during ethanol metabolism have been reported from studies *in vivo* and from perfusion studies. The major portion of AcH is metabolized within the liver, and a small amount leaves the liver [7, 19]. Hepatic AcH metabolism achieves a steady state within 15 min of the administration of ethanol. In the present study, the blood AcH concentration correlated well with the corresponding hepatic AcH concentration. The present investigation has shown that cysteine or MPG efficiently removed ethanol and AcH that was produced by the metabolism of a large dose of ethanol. A possible explanation for these observations could be the interaction of thiol compounds with ethanol metabolism.

First, active alcohol dehydrogenase probably consists of two similar polypeptide chains, each containing a reactive SH group in a cysteine residue [20]. The SH group appears to be involved in binding substrate to enzyme in each of the active centers within the enzyme molecule [21]. Because alcohol dehydrogenase is inhibited at an ethanol concentration above about 8 mM [22], which is a much lower concentration than in the present experiments, thiol compounds could accelerate ethanol metabolism through the activation of alcohol dehydrogenase. Second, and a more plausible explanation, thiol compounds, which are capable of forming an adduct with AcH [6], may thereby remove AcH generated in ethanol metabolism. Because the affinity of AcH for alcohol dehydrogenase is high, and the equilibrium constant favors the reduction of AcH rather than the oxidation of ethanol [21], the removal of AcH can accelerate ethanol oxidation. Furthermore, thiol compounds such as cysteine prevent the AcH-induced inhibition of mitochondrial functions.

It has been reported that MPG, like glutathione, is able to protect against ethanol-induced fatty liver [3]. According to the present study, MPG is capable of removing ethanol and AcH, probably through the formation of an adduct, such as thioacetal with AcH. Unlike cysteine, however, MPG could not efficiently remove ethanol and AcH in the liver and the blood, suggesting that MPG interacts with AcH to a lesser degree. On the other hand, MPG prevented fat accumulation and mitochondrial injury after acute ethanol administration as efficiently as cysteine did. One possible mode of action of MPG is the stabilization of cell constituents against AcH, as is observed in the prevention of paracetamol-induced hepatic necrosis in the rat [23]. In fact, MPG has been found to produce an increase of reactive SH groups in the mitochondrial membrane, because the SH group in the 2 position of the molecule is easily available for metabolic needs under conditions of lowered SH content [24].

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