Inhibition of the Acute Ethanol-Induced Fatty Liver by Pyrazole[1] (34813)

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Previous studies from this laboratory have led to the development of the hypothesis that the mechanism of hepatic cell injury after the administration of such agents as ethanol and carbon tetrachloride is an enhanced peroxidation of unsaturated lipids (1–7). The induced peroxidation was predicated to a free radical attack on unsaturated lipids of specific hepatic subcellular organelles due to decreased lipid antioxidant levels (7). The protection of animals from the hepatotoxic effects of ethanol, as well as carbon tetrachloride-induced hepatic necrosis and cirrhosis, by the administration of antioxidants, was credited to the possible inhibition of lipid peroxidation (1–8).

This concept implied that ethanol-induced hepatic injury results from the metabolism of ethanol rather than ethanol *per se*. Until the present time, the toxicity of ethanol metabolites could not be readily dissociated from possible injurious effects of ethanol. Recently, pyrazole has been demonstrated to profoundly inhibit ethanol metabolism (9–11) through a competitive inhibition of alcohol dehydrogenase (9, 10). Thus, in order to separate the possible toxic effects of ethanol *per se* from the potential toxic effects resulting from ethanol metabolism, studies were undertaken to determine the influence of pyrazole administration on the induction of the acute ethanol-induced fatty liver. Comparative studies were also undertaken to evaluate the effect of pyrazole on the carbon tetrachloride-induced fatty liver to denote the specificity of the pyrazole-induced inhibition of ethanol-induced hepatic injury.

Materials and Methods. Pyrazole in isotonic saline was administered intraperitoneally to male rats in the amount of 36 mg/100 g. Control rats received equivalent volumes of saline. Four hours later the animals were intubated with either saline or ethanol, as a 40% solution, in the amount of 6 g/kg. In view of the induced state of ethanol intoxication, which was particularly pronounced in the pyrazole–ethanol group, all rats were maintained in a controlled temperature environment to prevent the development of hypotheremia, an event which influences fatty liver development (12). The rats were killed either 20 or 44 hr later for the determination of liver and plasma triglycerides (13). Blood ethanol concentrations were determined by the yeast alcohol dehydrogenase (YADH) procedure at these and other time periods to denote the degree and duration of the pyrazole-induced inhibition in ethanol metabolism.

Goldberg and Rydberg (10) reported significantly lower concentrations of ethanol in rats treated with pyrazole when determined by a YADH method as compared to a gas chromatographic method (10). This difference was accredited to partial inhibition by pyrazole of the enzyme employed in the assay procedure. However, Theorell reported pyrazole inhibition to be specific for alcohol dehydrogenase of liver but not of yeast (9). In order to evaluate the validity of the observed blood ethanol levels in our pyrazole-treated rats, a series of recovery determinations were performed in which known amounts of ethanol were added to blood samples obtained at various intervals throughout the experimental period. The recovery studies indicated a mean 94.1 and 93.4% recovery of added ethanol in the saline–ethanol and pyrazole–ethanol groups, respectively, indicating that there was no significant inhibition of the assay enzyme by pyrazole as employed in this study.

In the carbon tetrachloride study, the rats

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TABLE I. Influence of Pyrazole Administration on Blood Ethanol Concentrations.[a]

Group	Time post-ethanol administration (hr)			
	1	2	3	20
Saline–ethanol	239.4 ± 39.0 (5)	243.5 ± 36.5 (5)	227.2 ± 34.7 (5)	27.8 ± 15.4 (10)
Pyrazole–ethanol	201.4 ± 19.3 (6)	224.0 ± 25.8 (6)	206.5 ± 21.3 (6)	192.3 ± 25.8 (18)

[a] Value of blood ethanol in milligrams per 100 ml are expressed as means ± standard error. Figures in parentheses denote the number of animals per group.

received pyrazole or saline as described above; 4 hr later carbon tetrachloride was administered in the dose of 0.2 ml/100 g as a 1:1 mixture of corn oil–carbon tetrachloride. The rats were killed 24 hr later, and liver and plasma triglycerides were determined (13). The data were analyzed by *t* test with 95% confidence limits.

Results. In agreement with the observations of Lester *et al.* (11) and Goldberg and Rydberg (10), the administration of ethanol to pyrazole-treated rats was associated with a profound inhibition of ethanol metabolism as reflected by the sustained blood levels of ethanol (Table I). The pyrazole–ethanol- and saline–ethanol-treated groups attained peak blood alcohol concentration at 1–3 hr after oral intubation. The blood alcohol in the control group showed a mean 88% decrease from the 3-hr value at the 20-hr period, while sustained elevations of blood alcohol concentrations were observed in the pyrazole–ethanol group. Indeed, the blood ethanol concentration in the pyrazole-treated group at 20 hr was unaltered from that manifested during the 1- to 3-hr period after ethanol administration.

In agreement with previous observations (1, 4) the administration of ethanol was associated with a mean 5-fold increase in liver triglyceride concentrations at the 20-hr period (Table II). In marked contrast, the pyrazole-treated group which received ethanol manifested unaltered liver triglyceride concentrations. Liver triglyceride concentrations were not significantly altered in the pyrazole-treated group which received saline.

Plasma triglyceride concentrations were not significantly altered in any of the experimental groups (Tables II and III). In studies conducted 44 hr after ethanol administration, the saline–ethanol group was characterized by normal liver triglyceride values, demonstrating the reversible nature of the ethanol-induced liver triglyceride alterations. The pyrazole-treated group which received ethanol also had normal liver triglyceride concentrations at this latter time period indicating that pyrazole did not merely delay the onset of liver triglyceride alterations, but effectively prevented the development of the acute fatty liver. Blood ethanol levels were still significantly elevated at this time period in the pyrazole group (Table III).

The administration of carbon tetrachloride to pyrazole-treated rats resulted in liver

TABLE II. Prevention of the Acute Ethanol-Induced Fatty Liver by Pyrazole.[a]

Group	Body wt (g)	Liver wt (g)	Blood ethanol (mg/100 ml)	Triglyceride	
				Liver (mg/g)	Plasma (mg/100 ml)
Saline–saline (8)	280 ± 3.3	8.2 ± 0.26	1.55 ± 0.25	4.3 ± 1.86	39.6 ± 7.5
Pyrazole–saline (8)	244 ± 10.4	7.5 ± 0.03	1.48 ± 0.37	2.6 ± 0.55	29.3 ± 4.3
Saline–ethanol (7)	232 ± 12.5	8.2 ± 0.16	0.88 ± 0.30	20.7 ± 3.96	53.5 ± 11.3
Pyrazole–ethanol (10)	232 ± 11.3	7.8 ± 0.25	143.8 ± 6.5	4.8 ± 0.49	59.9 ± 13.2

[a] Values are expressed as means ± standard error and were determined 20 hr after the oral administration of ethanol 6 g/kg. Pyrazole was administered intraperitoneally 4 hr preceding the oral administration of ethanol.

TABLE III. Plasma and Liver Triglyceride Concentrations and Blood Ethanol Levels in Pyrazole-Treated Rats 44 hr after Ethanol Administration.[a]

Group	Liver wt (g)	Blood ethanol (mg/100 ml)	Triglyceride	
			Liver (mg/g)	Plasma (mg/100 ml)
Saline–ethanol	8.0 ± 0.27	1.86 ± 0.05	4.7 ± 0.51	46.1 ± 5.2
Pyrazole–ethanol	9.6 ± 0.20	139.1 ± 29.8	4.0 ± 0.47	53.1 ± 11.5

[a] Values are expressed as mean ± standard error and are derived from five rats/group.

TABLE IV. Inability of Pyrazole Administration to Modify Carbon Tetrachloride-Induced Fatty Liver.[a]

Group and treatment	CCl_4	Triglyceride	
		Plasma (mg/100 ml)	Liver (mg/g)
Saline	—	25.0 ± 0.86	6.3 ± 1.05
Saline	+	13.2 ± 2.1	24.7 ± 1.57
Pyrazole	+	10.5 ± 1.7	26.6 ± 1.7

[a] Values expressed as mean ± standard error and derived from eight rats/group. Rats were killed 24 hr after intubation with 0.4 ml/100 g of a 1:1 mixture of corn oil–carbon tetrachloride solution.

triglyceride elevations which were comparable to the saline-treated carbon tetrachloride group (Table IV). The pronounced plasma hypotriglyceridemia which is manifested in carbon tetrachloride-treated animals was also not significantly altered in the pyrazole-treated group denoting, that in contrast to the findings with ethanol, the toxic effects of carbon tetrachloride are not modified by pyrazole administration.

Discussion. Pyrazole, a potent inhibitor of alcohol dehydrogenase and, therefore, of ethanol metabolism, is clearly a unique agent to separate the direct effects of ethanol *per se* from the metabolic effects of ethanol. The administration of pyrazole effectively inhibited ethanol metabolism as reflected by the prolonged period of elevated blood ethanol levels, as well as the observed pronounced state of intoxication that developed in association with the elevated blood levels of ethanol. Assuming that the microsomal ethanol-oxidizing system (MEOS) (14) is not inhibited by pyrazole, then on the basis of the sustained blood levels in pyrazole-treated rats, it would appear that the MEOS is not of major significance in promoting the oxidation of ethanol in animals which have not previously received ethanol. The absence of inhibition of pyrazole on a variety of enzyme systems has recently been demonstrated (15).

In spite of the prolonged and sustained blood ethanol concentrations and resulting intoxication, fatty liver, a consistent feature of ethanol administration (1–4, 6) did not develop in pyrazole-treated rats. These findings demonstrate that accumulation of triglyceride in liver is related to ethanol metabolites rather than to ethanol *per se*. The failure of fatty liver to develop in pyrazole-treated rats also negated a contributing role of ethanol-induced intoxication, with all its central nervous system manifestations, on fatty liver development.

The fact that pyrazole did not influence the development of carbon tetrachloride fatty liver also limits the possibility that pyrazole exerted its action by an effect on some, as yet undefined, aspect of lipid metabolism, a possibility that is also negated in part by the finding of unaltered liver and plasma triglyceride levels in pyrazole-treated rats which received saline. A decrease in plasma free fatty acids and a lowering of plasma triglycerides has been observed in rats treated with 3, 5-dimethyl pyrazole (16). Since Bizzi *et al.* (16) reported that the lowering of plasma free fatty acids always produced a decrease in plasma triglyceride concentration, it is unlikely that pyrazole exerts its protective effect by influencing free fatty acid mobilization since plasma triglycerides were unaltered in the pyrazole-treated groups.

Lelbach studied the effect of prolonged ethanol intake on liver histology in the presence of pyrazole administration (17). A definitive hepatotoxic effect of pyrazole–ethanol administration was manifested. Lelbach (17) proposed that pyrazole-induced inhibition of ethanol metabolism increased ethanol toxicity. This hypothesis is not supported by the present findings of the absence of fatty infiltration in acute ethanol-treated rats which received pyrazole. It appears that in the chronic model, ethanol may well be potentiating the demonstrated hepatotoxic effects of pyrazole (17) which are manifested when pyrazole is given in high doses.

The finding that ethanol must be metabolized before fatty liver develops, directs attention to either (1) the toxic effect of acetaldehyde (18) or (2) an alteration of the redox state of the hepatic cell due to the increased generation of hydrogen equivalents and the resulting alteration in the NAD/NADH (19–22) ratio. Since antioxidants, which prevent the development of ethanol- and carbon tetrachloride-induced injury (1–8), also act as a free radical trap and have been demonstrated to maintain NAD/NADH ratios in carbon tetrachloride-treated rats (23), the locus of the molecular basis of ethanol-induced hepatic injury may well reside with the hydrogen-generating capacity of ethanol.

While additional studies will be essential to define the molecular basis of ethanol-induced hepatic injury, the finding that ethanol, *per se*, is not hepatotoxic lends further support to the lipid peroxidation-antioxidant concept of ethanol-induced cell injury and stresses the importance of the dehydrogenation of ethanol to the induction of the acute ethanol-induced fatty liver.

The future employment of selective and specific enzyme inhibitors, such as pyrazole, to dissect the relative phases of ethanol metabolism should be of significant value in defining the molecular basis of ethanol-induced hepatic cell injury as well as contributing to defining the biochemical aspects of alcoholism.

Summary. Since pyrazole specifically inhibits liver alcohol dehydrogenase and impairs the metabolism of ethanol, studies were undertaken in rats to evaluate the influence of inhibition of ethanol metabolism on the development of an acute ethanol-induced fatty liver. In agreement with previous observations, 20 hr after ethanol administration a 5-fold increase in hepatic triglyceride concentration occurred. In marked contrast, the pyrazole- and ethanol-treated group showed complete inhibition of ethanol-induced hepatic triglyceride accumulation. The prevention of the ethanol-induced hepatic triglyceride accumulation occurred in the presence of enhanced blood ethanol concentrations in the pyrazole group. The carbon tetrachloride-induced fatty liver and plasma hypotriglyceridemia was not modified by pyrazole, denoting specificity in the pyrazole inhibition of ethanol-induced hepatic injury. These findings demonstrate that pyrazole can completely prevent the development of the ethanol-induced fatty liver in the presence of elevated concentrations of blood ethanol. Thus, ethanol, *per se,* is not the causative factor in fatty liver development.

1. Di Luzio, N. R., Life Sci. **3**, 113 (1964).

2. Di Luzio, N. R., and Costales, F., Exp. Mol. Pathol. **4**, 141 (1965).

3. Di Luzio, N. R., Lab. Invest. **15**, 50 (1966).

4. Comporti, M., Hartman, A. D., and Di Luzio, N. R., Lab. Invest. **16**, 616 (1967).

5. Di Luzio, N. R., Progr. Biochem. Pharmacol. **3**, 324 (1967).

6. Di Luzio, N. R., and Hartman, A. D., Fed. Proc. **26**, 1436 (1967).

7. Di Luzio, N. R., and Hartman, A. D., Exp. Mol. Pathol. **11**, 38 (1969).

8. Hartman, A. D., Di Luzio, N. R., and Trumbull, M. L., Exp. Mol. Pathol. **9**, 349 (1969).

9. Theorell, H., Experientia **21**, 553 (1965).

10. Goldberg, L., and Rydberg, U., Biochem. Pharmacol. **18**, 1749 (1969).

11. Lester, D., Leokosky, W. A., and Felzenberg, F., Quart. J. Stud. Alc. **29**, 449 (1968).

12. Larson, R. E., and Plaa, G. L., J. Pharmacol. Exp. Ther. **147**, 103 (1965).

13. Van Handel, E., and Zilversmit, D. B., J. Lab. Clin. Med. **50**, 152 (1957).

14. Lieber, C. S., and De Carli, L. M., Science **162**, 917 (1968).

15. Reynier, M., Acta Chem. Scand. **23**, 1119 (1969).

16. Bizzi, A., Veneroni, E., and Garattini, S. J. Pharm. Pharmacol. **18**, 611 (1966).

17. Lelbach, W. K., Experientia **25**, 816 (1969).

18. Truitt, E. B., Duritz, G., Markowitz, S., and Twardowicz, A. H., Fed. Proc. **25**, 657 (1966).

19. Lieber, C. S., Ann. Rev. Med. **18**, 35 (1967).

20. Williamson, J. R., Scholz, R., Browning, E. T., Thurman, R. G., and Fukami, M. H., J. Biol. Chem. **224**, 5044 (1969).

21. Reboucas, G., and Isselbacher, K. J., J. Clin. Invest. **40**, 1355 (1964).

22. Raiha, N. C., and Oura, E., Proc. Soc. Exp. Biol. Med. **109**, 908 (1962).

23. Gallagher, C. H., Aust. J. Exp. Biol. **40**, 241 (1962).

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