Ethanol-Induced Fatty Liver in Rats: Effects of Pyrazole and Glucose (35823)

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A single acute dose of ethanol (6 mg/kg) has been shown in rats to result in the production of fatty liver characterized by the accumulation of triglycerides (1, 2). Pyrazole, an inhibitor of liver alcohol dehydrogenase and the microsomal ethanol oxidizing system (MEOS) (3–5), has been used to inhibit ethanol metabolism in experiments attempting to determine the mechanism behind hepatic triglyceride accumulation (6, 7). These experiments led to somewhat conflicting results. Thus, Morgan and DiLuzio (7) reported that male rats were protected from fatty liver by an intraperitoneal injection of pyrazole 4 hr prior to the administration of ethanol by gastric gavage. On the other hand, Bustos *et al.* (6) showed that female rats injected with pyrazole 10 min prior to gavage with ethanol showed no significant decrease in hepatic triglyceride accumulation.

The experiments reported below attempt to determine whether these observed differences in the protection by pyrazole against ethanol-induced fatty liver in rats result from a difference in sex or a difference in elapsed time between the injection of pyrazole and the administration of ethanol.

Materials and Methods. Male and female rats (averaging 200 g, Sprague-Dawley strain) were obtained from Charles River Breeding Laboratories, North Wilmington, Massachusetts. The rats were fed Purina chow *ad libitum* and then fasted 7 hr prior to the onset of the experiments.

Pyrazole (4 mmoles/kg) was given by intraperitoneal injection from a solution containing 0.25 moles/liter. Ethanol (6 g/kg) was administered by stomach tube with glucose or saline in a volume of 28 ml/kg either 4 hr or 10 min after pyrazole. Glucose doses were isocaloric with the dose size of ethanol for the particular rat. In control experiments, physiological saline replaced either the glucose, ethanol, or pyrazole in a volume appropriate to the size of the animal. Approximately 16 hr after administration of ethanol the rats were decapitated, the blood was collected, and the livers were removed. The livers were then homogenized in 0.066 *M* phosphate buffer, pH 7.0, and triglycerides were extracted. Liver and blood triglycerides were then measured colorimetrically (8, 9). MEOS activity was measured according to a modification of the method described by Lieber and DeCarli (10) in which the oxidation of ^{14}C-ethanol was measured by determining the radioactivity of the ^{14}C-acetaldehyde semicarbazone. Blood ethanol was measured spectrophotometrically by the observed change in absorbance at 340 mμ in a buffered incubation mixture containing NAD^+, yeast alcohol dehydrogenase, and a sample of a perchloric acid extract of serum (11). Endogenous hepatic NAD^+ and NADH were determined in homogenates by a fluorometric assay (12).

Results. The data reported in Table I show that a single acute dose of ethanol (6 mg/kg) results in a threefold increase in liver triglycerides in male rats, and that pyrazole effectively lowers this accumulation to the level obtained with control animals. The results are similar whether pyrazole was injected 4 hr or 10 min before ethanol administration.

Table II shows that in female rats there is also an accumulation of triglycerides after acute ethanol administration. However, in comparison with the results obtained with

TABLE I. Blood Ethanol and Hepatic Triglyceride Concentrations after the Administration of Ethanol and/or Glucose and/or Pyrazole to Male Rats.

Pyrazole injection prior to ethanol admin (min)	Hepatic triglycerides (mg/g of wet wt)	Blood ethanol (mg/100 ml)
	Ethanol	
10[a] (5)[b]	20.44 ± 3.60[c]	0
240[d] (5)	23.60 ± 3.31	0
	Ethanol + pyrazole	
10 (5)	8.76 ± 1.80	287.2 ± 13.70
240 (5)	8.53 ± 2.60	292.0 ± 35.00
	Ethanol + glucose	
10[a] (5)	14.50 ± 3.30	0
240[d] (4)	13.20 ± 3.00	0
	Ethanol + glucose + pyrazole	
10 (4)	12.40 ± 1.87	249.0 ± 17.14
240 (5)	11.45 ± 2.86	260.0 ± 23.00
	Glucose	
Control (6)	10.44 ± 2.40	—
	Saline	
Control (6)	8.00 ± 1.10	—

[a] Although this group was not given pyrazole, it was treated with ethanol at the same time as the group treated with pyrazole.

[b] The numbers in parentheses indicate the number of animals used in each experimental group.

[c] Values are expressed as the mean ± standard deviation.

[d] Same as [a] but treated with the group given pyrazole 4 hr prior to ethanol.

male rats (Table I), the accumulation of triglycerides is higher and pyrazole is less effective in lowering this triglyceride accumulation to control levels. Again, there was no significant difference between the results obtained either 10 min or 4 hr after injection by pyrazole.

It is also apparent from the data in Tables I and II that in the absence of pyrazole, glucose markedly lowers the accumulation of triglycerides following the administration of ethanol in both male and female rats. However, only a slight corresponding lowering of the blood ethanol levels was observed in the presence of pyrazole. Acute ethanol administration in animals not receiving pyrazole resulted in a lower hepatic NAD/NADH ratio than in those treated with pyrazole.

The MEOS was assayed from liver of female rats. The activity for a group of five rats receiving no ethanol was 1.69 ± 0.21 mμmoles/min/mg. The corresponding activity for another group of six rats receiving ethanol was 1.69 ± 0.16 mμmoles/min/mg. The activity for a third group of four rats receiving ethanol after pyrazole was 1.49 ± 0.06 mμmoles/min/mg. Therefore, the MEOS showed no significant change in activity associated with acute ethanol administration with or without pyrazole injection.

Although the results show that the absolute lowering of hepatic triglycerides by pyrazole is the same in male rats as in female rats, the present inhibition of acute ethanol-induced fatty liver by pyrazole is greater in male than in female rats whether pyrazole is administered 4 hr or 10 min prior to the ethanol. The effect of pyrazole on blood alcohol levels is the same in both sexes. There-

TABLE II. Blood Ethanol and Hepatic Triglyceride Concentrations after the Administration of Ethanol and/or Glucose and/or Pyrazole to Female Rats.[e]

Pyrazole injection prior to ethanol admin (min)	Hepatic triglycerides (mg/g of wet wt)	Blood ethanol (mg/100 ml)
	Ethanol	
10[a] (5)[b]	34.38 ± 4.80[c]	0
240[d] (3)	33.80 ± 0.75	0
	Ethanol + pyrazole	
10 (5)	21.90 ± 2.90	280.60 ± 18.90
240 (3)	21.17 ± 2.00	—
	Ethanol + glucose	
10[a] (5)	13.00 ± 2.80	0
240[d]	—	—
	Ethanol + pyrazole + glucose	
10 (4)	15.81 ± 2.67	236.00 ± 8.12
240	—	—
	Glucose	
Control (6)	11.00 ± 2.38	—
	Saline	
Control (6)	9.90 ± 1.60	—

[e] Conditions and footnotes are the same as those described in Table I.

fore, the difference in the effect of pyrazole on hepatic triglyceride appears to be related to sex rather than to drug absorption or drug action time. The failure of acute ethanol administration to increase MEOS activity in the absence of pyrazole and the persistence of elevated blood ethanol levels and hepatic triglycerides in the presence of pyrazole in female rats indicated that in female rats, at least, metabolism of ethanol in the liver is not necessary for triglyceride accumulation. A plausible explanation for the accumulation of hepatic triglycerides might involve lipolysis and the mobilization of fatty acids from adipose tissue (13), as well as alterations in hepatic fatty acid metabolism (14).

Regarding effects of glucose on ethanol metabolism, studies reported by Lowenstein *et al.* (15) show that fructose given by infusion significantly increases ethanol metabolism, whereas glucose does not. Therefore, the data showing that glucose given simultaneously with ethanol lowers hepatic triglyceride accumulation without significantly changing blood ethanol concentrations suggest that glucose protects against fatty liver by some effect other than increasing ethanol metabolism.

Summary. The acute administration of ethanol causes the accumulation of hepatic triglycerides in male rats and to a greater extent in female rats. Pyrazole, injected prior to ethanol administration, completely prevents accumulation of hepatic triglycerides in male rats, whereas in female rats the same treatment with pyrazole only partially lowers accumulation of hepatic triglycerides. Glucose administration simultaneously with ethanol decreases the concentration of hepatic triglycerides equally in both sexes.

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