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Biochemical ethanol effects affected by a non-steroidal anti-inflammatory drug

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The non-steroidal anti-inflammatory drug, piroxicam, prevents the hepatic increase of triacylglycerols and malondialdehyde resulting from the acute intoxication of rats with ethanol. In addition, in the intoxicated rats, piroxicam consistently produces a decrease in the levels of blood ethanol in comparison with control animals. It is suggested that the anti-inflammatory compound stimulates ethanol oxidation.

Ethyl alcohol; Alcoholic intoxication; Non-steroidal anti-inflammatory agent; Alcoholic hepatitis; Malonates; Piroxicam

1. INTRODUCTION

Piroxicam, a non-steroidal anti-inflammatory drug (NSAID), administered in rats simultaneously with CCl_4, partially prevented the increases in serum activities of two aminotransferases, maintained liver lipoperoxidation and triacylglycerides (TAG) contents at normal values, and attenuated the liver morphological changes caused by the hepatotoxic compound [1]. For these reasons we searched for the putative action of piroxicam preventing the biochemical alterations produced in the whole animal by an extremely common hepatotoxic compound: ethanol.

2. MATERIALS AND METHODS

Male Wistar rats (200–225 g) were fasted 16 h before treatment and divided into four groups: control, receiving isocaloric amounts of glucose (as 40% w/v solution) with regard to the doses of ethanol and equivalent amounts of piroxicam vehicle solution; ethanol, receiving 3 or 5 g of ethanol/kg body weight (as 30% solution) and equivalent amounts of piroxicam vehicle solution; piroxicam, receiving isocaloric amounts of glucose and piroxicam 10 mg/kg body weight (as 7.5 mg/ml in a 25% glycerol/water solution v/v); and ethanol plus piroxicam, receiving ethanol 3 or 5 g/kg and piroxicam 10 mg/kg body weight. The administered compounds were given by orogastric via (OGV) unless otherwise indicated. In most of the experiments rats were killed by decapitation, blood was collected in EDTA and liver samples were taken to evaluate TAG, protein and malondialdehyde (MDA). TAG were determined by the method described by Gottfried et al. [2] directly in liver homogenates prepared in double distilled water (1:9, w/v). MDA was quantified by the barbituric acid method [3], modified as recommended by Hernández-Muñoz et al. [4]. Protein was determined by the Bradford colorimetric method [5]. Blood ethanol was assayed essentially by the method of Bernt and Guttman [6]. Hematocrit was measured with standard clinical laboratory techniques.

In some experiments rats were sacrificed 2 h after treatment, stomachs were excised and weighed, the content of each stomach was carefully recovered with the aid of saline solution in order to measure ethanol gastric content. In another series of experiments, rats were maintained after treatment in individual metabolic cages and urine was collected to determine ethanol concentration.

Reagents were obtained from Sigma Chemical Co. (St. Louis, MO). Ethanol analytical grade was purchased from Baker. Statistical analysis was performed using Student's *t*-test.

Abbreviations: NSAID, non-steroidal anti-inflammatory drug; MDA, malondialdehyde; TAG, triacylglycerides; OGV, orogastric via; IPV, intraperitoneal via.

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3. RESULTS

The well known increase in liver TAG content produced by ethanol was nearly blocked by piroxicam, whereas the NSAID alone exhibited non-significant alteration in these TAG (Fig. 1).

Glucose or piroxicam did not change MDA levels used here as an index of hepatic lipoperoxidation, ethanol treatment showed a significant increase of MDA content between 8 and 12 h after intoxication, being maximal at 8 h; piroxicam produced a statistically significant diminution of MDA values reached with ethanol alone (Fig. 2).

Since alcohol oxidation is required to augment the lipoperoxidation index in liver [7] and since piroxicam treatment avoids the increase in MDA promoted by ethyl alcohol (Figs. 1 and 2), an inhibition in ethanol oxidation and a consequent rise in its blood levels due to piroxicam administration could be expected. Nevertheless, in rats intoxicated with ethanol by OGV, the

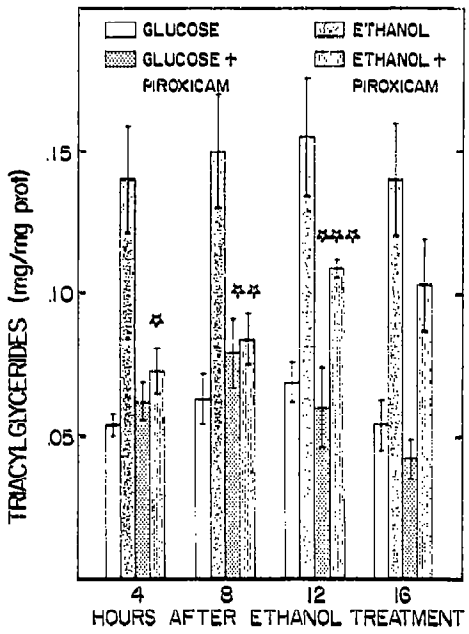

Fig. 1. Effect of piroxicam on ethanol-promoted lipid accumulation in liver. Levels of TAG in the liver of rats at different times after treatment. The dose of ethanol was 5 g/kg of body weight by OGV. Piroxicam was administered consecutively to glucose or ethanol and by the same route. Range of individual assays (n) from 4 to 15; P values comparing ethanol group vs ethanol + piroxicam group; $^{*}P < 0.001$, $^{**}P < 0.01$ and $^{***}P < 0.05$.

curve of blood ethanol as a function of its administration was lowered with piroxicam, either used by OGV or intraperitoneal via (IPV) (Fig. 3). This action of piroxicam was also observed when ethyl alcohol, 3 g/kg, was provided by IPV (data not shown).

Ethanol content in the stomachs of rats receiving 5 g of ethanol per kg of body weight by OGV 2 h before was 4.62 ± 1.14 mmol/g of stomach (n=4), when ethanol was given alone, and it was 6.22 ± 1.51 (n=4) when ethanol was supplemented with piroxicam; the difference was without statistical significance ($P < 0.2$). Similarly, the treatment with piroxicam produced no difference in the amount of ethanol eliminated by urine from rats treated with 3 g of ethanol per kg of body weight by IPV; i.e. 6 nmol/h and 5 nmol/h with and without piroxicam, respectively. Furthermore, the hematocrit value was the same in both groups of animals.

4. DISCUSSION

Administration of the NSAID impaired the hepatic increase in TAG content and in MDA generation promoted by ethanol treatment (Figs. 1 and 2). These 'protective' actions of piroxicam toward alcohol intoxication coincided temporarily with its capacity to decrease blood ethanol levels (Fig. 3), which cannot be attributed to a delay in alcohol absorption from the gastrointestinal tube since the effect was present in animals receiving the toxic compound by IPV, and piroxicam did not modify the rate of absorption of ethanol by the stomach. In addition, the NSAID neither stimulated the elimination of ethanol by the kidney, nor originated hemodilution. Therefore, it appears that piroxicam caused an increase in ethanol oxidation probably due to an induction in the synthesis of nonspecific oxidases, as has been reported, for example, with barbiturates [8]. The activation in ethanol disappearance caused by piroxicam contrasted with the reported effect of aspirin, the prototype of NSAIDs, decreasing the activity of gastric alcoholic dehydrogenase in human subjects and in rat models at low ethanol doses [9].

DiLuzio and Stege clearly showed that peroxidative lipid degradation products formed after ethanol consumption were markedly reduced when ethanol metabolism was blocked by pyrazole, which diminishes acetaldehyde formation, and were augmented by disulfiram, an inhibitor that promotes acetaldehyde accumulation [7]. Thus, the increase in ethanol oxidation promoted by piroxicam should be accompanied by a similar or higher increase in acetaldehyde oxidation (there is a report on the induction of cytoplasmic aldehyde dehydrogenase by drugs [8]) in order to discourage a rise in lipoperoxidation and in turn in MDA levels.

Some consequences of a piroxicam-mediated lower blood ethanol level might include the diminution of liver

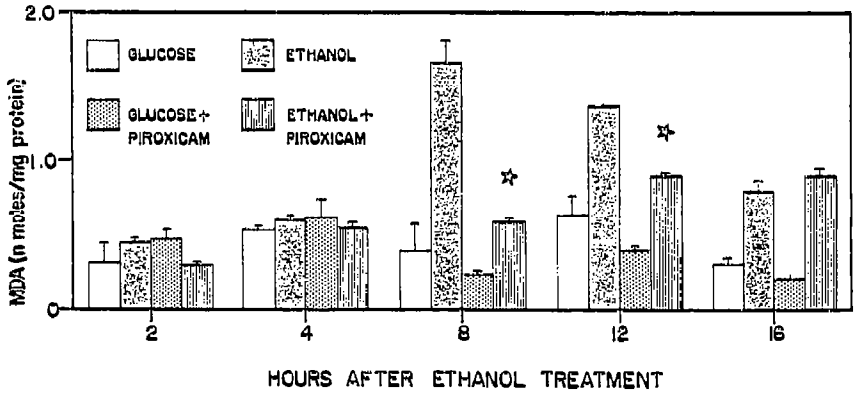

Fig. 2. Effect of piroxicam on ethanol-promoted liver peroxidation. Levels of MDA in the liver of rats at different times after receiving the treatment indicated in the figure. Other indications as in Fig. 1. n from 3 to 7. $^{*}P < 0.001$ comparing ethanol vs. ethanol plus peroxicam.

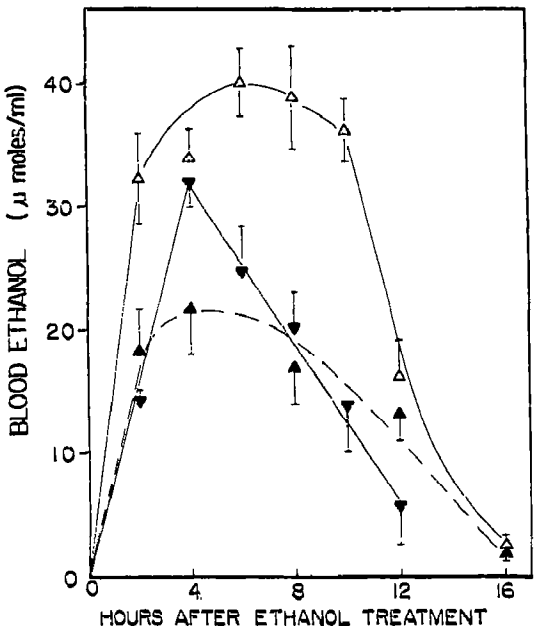

Fig. 3. Role of piroxicam on time course ethanol levels in blood. All animals received 5 g/kg ethanol by OGV plus: (△–△), 0.1 ml saline by OGV; (▲-----▲), 0.1 ml 20 mg/ml piroxicam by OGV; and 20 mg/ml piroxicam by IPV, (▼.....▼). For 2 h n=8, and n=5 in other data. Other indications as in Fig. 1. Values of P are reported comparing results of ethanol vs. ethanol + piroxicam at each of the different times: $P <$ 0.001, 8 h OGV, 2 h and 10 h IPV; $P < 0.01$, 6 h and 8 h IPV; $P <$ 0.05, 2 and 4 h OGV, 12 h IPV.

TAG content (Fig. 1), a minor cellular transformation to hydroxyethyl free radical [10,11] and therefore no activation of lipoperoxidation, and also the reported decrease in the duration of hypnosis [12]. Finally, the action of piroxicam limitating lipoperoxidation and MDA production (Fig. 2) might also be related to the reported action of NSAIDs to scavenge, or to inhibit, the generation of free radicals [13,14].

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