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CHOLESTASIS FOLLOWING CHRONIC ALCOHOL CONSUMPTION: ENHANCEMENT AFTER AN ACUTE DOSE OF CHLORPROMAZINE

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Summary

Sprague-Dawley rats were pair-fed nutritionally adequate liquid diets containing either 36 % of total calories as ethanol or isocaloric carbohydrates (controls) for 4 weeks. Compared to controls, chronic alcohol consumption leads to slightly increased serum activities of various hepatic enzymes including the alkaline phosphatase (AP). Chlorpromazine administered as a single dose 18 hours after ethanol withdrawal resulted within 18 hours in a significant increase of serum AP activity in rats fed ethanol chronically but not in their pair-fed controls. It is concluded that chronic alcohol consumption predisposes to cholestasis due to chlorpromazine.

Ethanol is oxidized to acetaldehyde in the hepatocytes by cytosolic alcohol dehydrogenase (ADH) and the microsomal ethanol oxidizing system (MEOS) (1 - 5). MEOS consists of cytochrome P-450, NADPH-cytochrome c reductase and phospholipids (2, 6 - 8) and therefore resembles other microsomal drug metabolizing enzymes (9). Prolonged alcohol intake increases the content of microsomal cytochrome P-450 (10 - 12) as well as phospholipids (13) and enhances the activity of NADPH-cytochrome P-450 reductase (14). These alterations result in an induced activity of MEOS (1, 4, 6, 13, 15) and other microsomal drug metabolizing enzymes (14, 16).

The microsomal enzyme induction observed following prolonged alcohol consumption leads to an increased oxidation of ethanol to the hepatotoxic acetaldehyde (1, 17). Similarly, an enhanced metabolism of carbon tetrachloride to toxic intermediates can be observed under these experimental conditions (18). Recent studies have also shown that chronic pretreatment with alcohol predisposes to paracetamol-induced hepatotoxicity, most probably as a consequence of increased formation of toxic metabolites (19).

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Psychotropic drugs including phenothiazines are widely used and may cause cholestasis due to microsomal metabolism of the parent compound to toxic intermediates (20). Since the use of phenothiazine derivatives such as chlorpromazine is not uncommon in connection with alcohol consumption, the question arose whether chronic alcohol consumption may predispose to chlorpromazine-induced cholestasis.

Material and Methods

Female Sprague-Dawley rats were used in pairs of littermates with a starting body weight of 120 - 150 g. The animals were pair-fed nutritionally adequate liquid diets containing either ethanol (36 % of total calories) or the same diet in which ethanol had been replaced isocalorically by carbohydrates (control diet) for 4 weeks (21). To study the effect of acute drug administration after chronic ethanol consumption, rats fed either the ethanol-containing diet or the control diet were given the control diet during the 18 h preceding the drug administration to allow complete clearing of ethanol from the blood. The diets were then replaced by tap water, and the animals (alcohol and control rats) received chlorpromazine at doses of 15, 30 or 45 mg/kg BW dissolved in physiological saline solution by i. p. injection. Some animals (alcohol and control rats) received physiological saline solution only.

The animals were killed by decapitation 18 h after drug administration. Blood was collected from the neck vessels, and serum activities of the following enzymes were determined: Alkaline Phosphatase (AP) (22), Glutamate-Pyruvate-Transaminase (GPT) (23), Glutamate-Oxalacetate-Transaminase (GOT) (23), and Glutamate Dehydrogenase (GDH) (23). Each measurement was carried out in duplicate. The means ($\pm$ SEM) and individual differences were calculated, and their significances were assessed by the Student's t-test.

Results

When compared to animals receiving the control liquid diet, prolonged alcohol administration for 4 weeks resulted in a significant rise of alkaline phosphatase (AP) activity in the serum under experimental conditions where no chlorpromazine was administered (Fig. 1). Moreover, the administration of chlorpromazine in increasing amounts up to 45 mg/kg BW failed to enhance serum AP activity in rats fed the control diet when compared to those control animals receiving no chlorpromazine. Conversely, in rats chronically pretreated with alcohol chlorpromazine in increasing amounts up to 45 mg/kg BW caused a correspondent enhancement of serum AP activity. In particular,

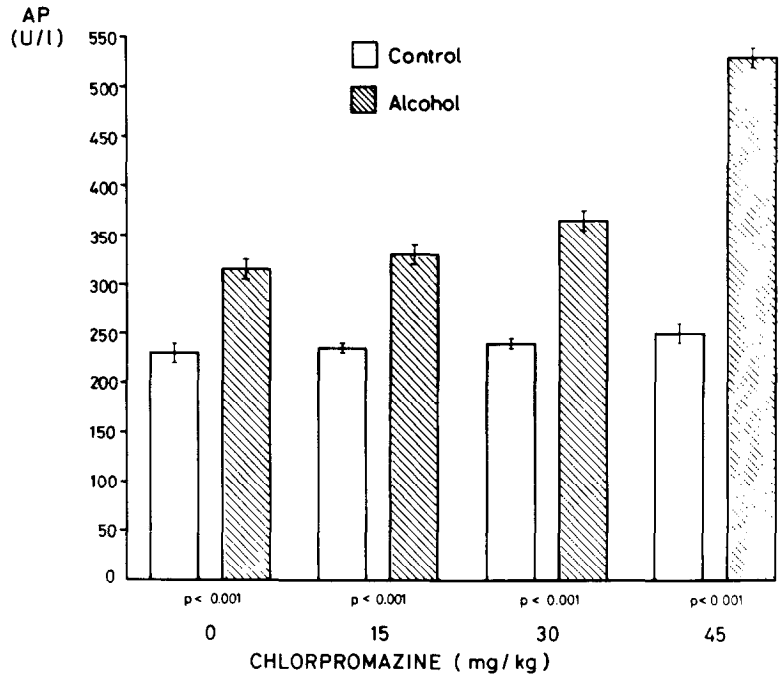

Fig. 1: Effect of an acute dose of chlorpromazine on serum alkaline phosphatase (AP) activity following chronic alcohol consumption. Rats were pair-fed for 4 weeks diets containing ethanol (36 % of total calories), whereas the control diet contained additional carbohydrates instead of ethanol. 18 hours after ethanol withdrawal chlorpromazine was administered i. p. at doses as indicated, and serum AP activity was determined 18 hours thereafter. Each experimental group consisted of 6 animals.

the serum AP activity observed in the alcohol animals following the largest dose of chlorpromazine (45 mg/kg BW) was significantly higher than in the alcohol-pretreated animals receiving no chlorpromazine (Fig. 1). Since increases of serum alkaline phosphatase activities are suggestive for cholestasis, it is concluded from these experiments that chronic alcohol pretreatment may predispose to chlorpromazine-induced cholestasis.

Compared to control animals, chronic alcohol consumption per se led to a significant increase of serum glutamate-pyruvate-transaminase (GPT) activity (Fig. 2), an enzyme confined to the cytosol of the hepatocyte. The administration of chlorpromazine in increasing amounts resulted in a steadily rise of serum GPT activity which was somewhat more pronounced in the alcohol-pretreated animals than in the corresponding control

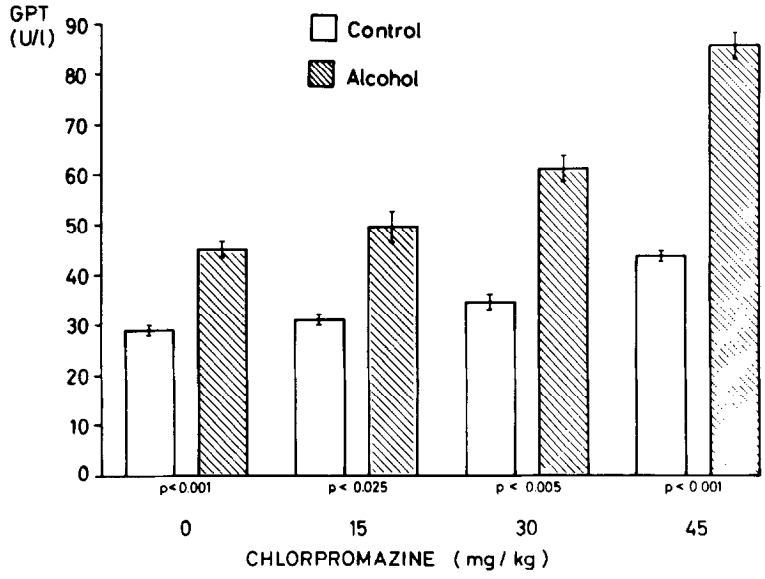

Fig. 2: Effect of an acute dose of chlorpromazine on serum glutamate-pyruvate-transaminase (GPT) activity following chronic alcohol consumption. The experimental conditions are given in the legend of Fig. 1.

rats (Fig. 2). Similar results are obtained for the serum activities of glutamate-oxalacetate-transaminase (GOT) (Fig. 3), an enzyme of cytosolic as well as mitochondrial origin of the hepatocyte.

In addition, chronic alcohol consumption led to slightly increases of serum glutamate dehydrogenase (GDH) activities compared to animals fed the control diet (Fig. 4). After administration of chlorpromazine serum GDH activity increased in both animal groups fed either the control or the alcohol diet. However, with the largest dose of chlorpromazine (45 mg/kg BW) the difference in GDH activity between the alcohol and control animals was much more striking than with lower doses of chlorpromazine (Fig. 4). Since GDH activity is primarily localized in liver mitochondria, these results suggest that chronic alcohol pretreatment predisposes not only to chlorpromazine-induced cholestasis as evidenced by increased activities of serum AP (Fig. 1) but also to chlorpromazine-mediated hepatocellular damage as shown by increased serum GDH activities (Fig. 4).

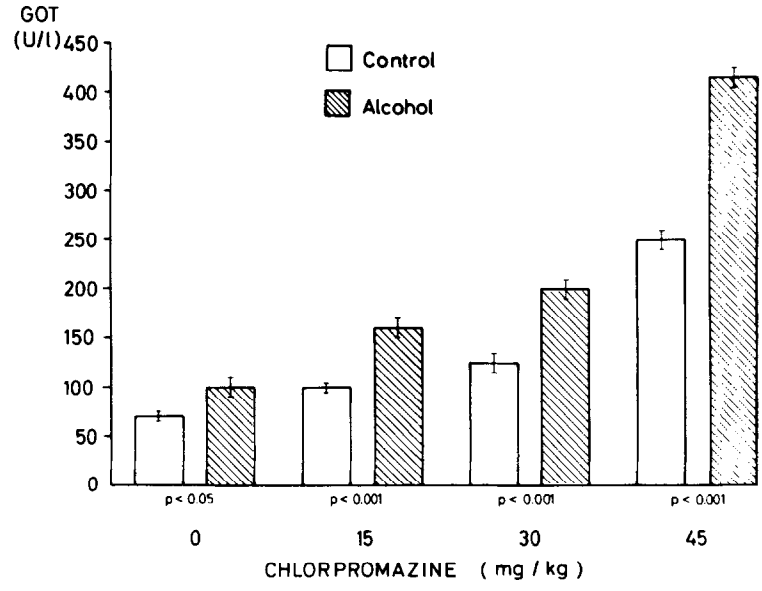

Fig. 3: Effect of an acute dose of chlorpromazine on serum glutamate-oxalacetate-transaminase (GOT) activity following chronic alcohol consumption. The experimental conditions are given in the legend of Fig. 1.

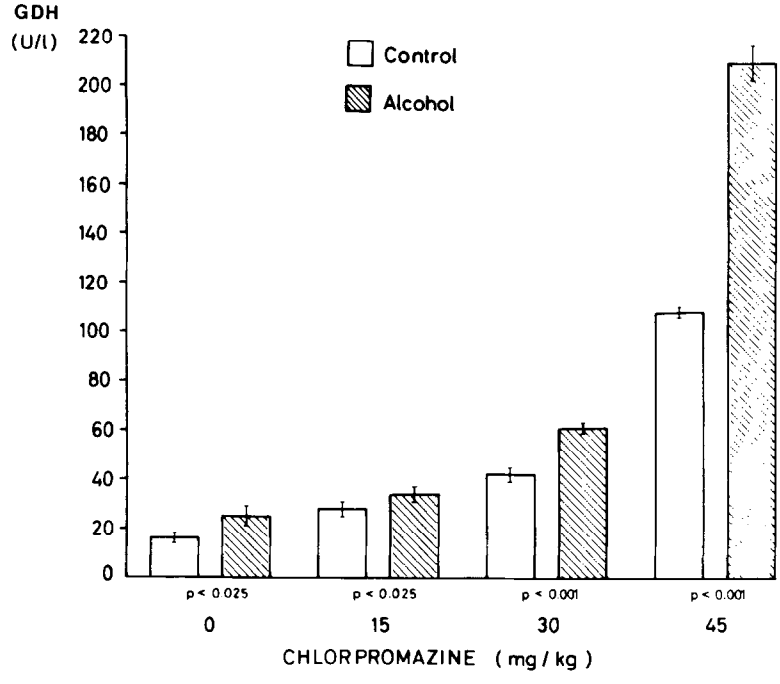

Fig. 4: Effect of an acute dose of chlorpromazine on serum glutamate dehydrogenase (GDH) activity following chronic alcohol consumption. The experimental conditions are given in the legend of Fig. 1.

Discussion

The present study shows that the acute administration of high doses of chlorpromazine leads to a striking dose-related rise of serum AP activity in alcohol pretreated animals (Fig. 1). Of particular interest was the finding that in the rat fed chronically the control diet serum AP activity remained virtually unchanged irrespective whether chlorpromazine was acutely administered or not (Fig. 1). Since elevations of serum AP activities are commonly associated with cholestasis, these findings suggest that chronic alcohol consumption may predispose to chlorpromazine-induced cholestasis. In addition, chlorpromazine causes increased serum activities of GPT (Fig. 2), GOT (Fig. 3) and GDH (Fig. 4) which were more pronounced following chronic alcohol pretreatment. Since GPT, GOT and GDH are of cytosolic and/or mitochondrial origin of the hepatocyte, chronic alcohol pretreatment also predisposes to hepatocellular damage as a consequence of an acute administration of chlorpromazine.

The view is generally held that chlorpromazine is extensively metabolized by liver microsomes (24). In this reaction the methyl group is being hydroxylated (25), and cytochrome P-450 may function as the terminal oxidase (26). In addition, liver microsomes are capable of generating chlorpromazine free radicals from the parent compound chlorpromazine (20, 27). The chlorpromazine free radicals may then react with oxygen to form stable chlorpromazine sulfoxide metabolites (20, 28). It has been postulated that the chlorpromazine free radical irreversibly inhibits a variety of enzymes including Na^+, K^+-ATPase via an interaction with the enzyme's free sulfhydryl groups, and this inhibition was partially prevented by reduced glutathione (20). Chlorpromazine or one of its metabolites may therefore conceivably induce cholestasis by a direct toxic effect on the bile secretory mechanism of the liver through an interaction with canalicular membrane ATPases. Moreover, the degree of cholestasis may be influenced by the extent of microsomal conversion of chlorpromazine to more active metabolites in form of free radicals or to minimally active metabolites such as chlorpromazine sulfoxide (20).

Since chronic alcohol consumption leads to increased activities of various hepatic microsomal drug metabolizing enzymes (14, 16) and to an enhanced content of cytochrome P-450 (10 - 12), it is conceivable that under these experimental conditions chlorpromazine is metabolized more rapidly to toxic free radicals. The latter may in turn promote cholestasis as evidenced by increased serum AP activities (Fig. 1) which is associated with increased hepatocellular damage as demonstrated by the enhanced activities of various serum enzymes originating from the liver (Fig. 2, 3, 4). In addition, prolonged treatment with alcohol has been reported to result in a decreased liver content of reduced glutathione (29) which in turn could further promote the development of cholestasis due to a decreased capacity of the liver to scavanger the extensively formed chlorpromazine free radicals.

In conclusion, the data of the present study show that prolonged intake of alcohol predisposes to cholestasis induced by an acute dose of chlorpromazine. This effect might be ascribed primarily to an increased formation of toxic radicals which are more rapidly formed due to microsomal enzyme induction.

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