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INCREASED PARACETAMOL-INDUCED HEPATOTOXICITY AFTER CHRONIC ALCOHOL CONSUMPTION

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Summary

Female rats were pair-fed nutritionally adequate liquid diets containing either ethanol (36 % of total cal.) or isocaloric carbohydrates (controls) for 4 weeks. Compared to controls, chronic alcohol consumption led to slightly increased activities of various hepatic enzymes in the serum. Paracetamol administered 18 hours after ethanol withdrawal resulted within 18 hours in a significant increase of serum GOT and GPT activities, which was much more pronounced in rats fed ethanol chronically than in their pair-fed controls. Thus, chronic alcohol consumption predisposes to increased hepatotoxicity due to paracetamol.

The metabolism of ethanol to acetaldehyde proceeds in the hepatocyte via the alcohol dehydrogenase (ADH) as well as the microsomal ethanol oxidizing system (MEOS) (1 - 3). Analogous to other hepatic microsomal drug metabolizing enzymes (4) MEOS consists of cytochrome P-450, NADPH-cytochrome c reductase and phospholipids (5 - 7). Chronic alcohol consumption increases the content of microsomal P-450 (8, 9) as well as phospholipids (5) and enhances the activity of NADPH-cytochrome P-450 reductase (10), resulting in an induction of activities for MEOS (5, 6, 9, 11, 12) and other microsomal drug metabolizing enzymes (10, 13).

The induction of MEOS activity is associated with an increased production of acetaldehyde (5, 11) which in turn may lead to an increased hepatotoxicity (14, 15). Similarly, as a consequence of the induction of microsomal drug metabolizing enzyme activities an increased hepatotoxicity of carbon tetrachloride was observed following chronic alcohol consumption (16). More recently, it has been suggested that due to micro-

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somal enzyme induction alcoholics may also be more susceptible to commonly prescribed drugs such as paracetamol (17) which is metabolized to toxic metabolites via the cytochrome P-450-dependent mixed function oxidase (18).

In the present experiments the question was studied whether under controlled experimental conditions an increased paracetamol-induced hepatotoxicity can be observed following chronic alcohol consumption.

Material and Methods

Female Sprague-Dawley rats were used in pairs of littermates with a 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 (19). 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 paracetamol at doses of 400, 800 or 1200 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 (20): Glutamate-Oxalacetate-Transaminase (GOT), Glutamate-Pyruvate-Transaminase (GPT), and Glutamate Dehydrogenase (GDH). 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

Compared to animals receiving the control liquid diet, chronic alcohol consumption results a slight increase of serum Glutamate-Oxalacetate-Transaminase (GOT) activities under experimental conditions where no paracetamol was administered (Fig. 1). Moreover, the application of paracetamol to rats pretreated with the control diet for 4 weeks led to a slight increase of GOT activity when the drug was given in increasing amounts up to 800 mg/kg BW (Fig. 1). Similar results were obtained in the alcohol pretreated animals receiving the same acute dose of paracetamol (Fig. 1). Conversely, a striking enhancement of serum GOT activity could be

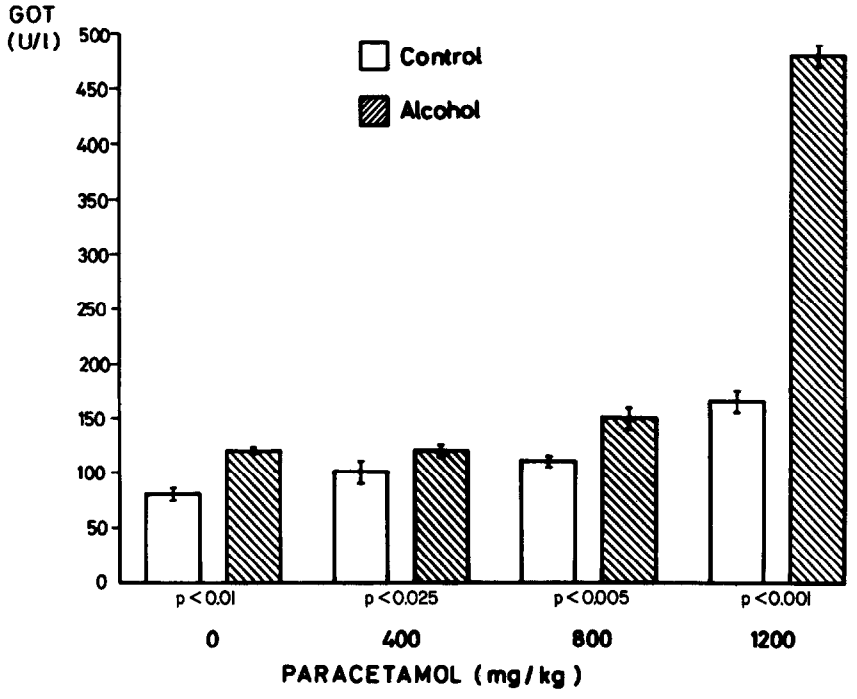

Fig. 1: Effect of an acute dose of paracetamol on serum GOT activity following chronic alcohol consumption. Rats were pair-fed for 4 weeks containing ethanol, whereas the control diet contained additional carbohydrates instead of ethanol. 18 hours after ethanol withdrawal paracetamol was administered i. p. at a dose as indicated, and serum GOT activity was determined 18 h thereafter. Each experimental group consisted of 6 animals.

demonstrated following a high dose of paracetamol (1200 mg/kg), and this increase was much more pronounced in the alcohol fed animals compared to their pair-fed littermates receiving the control diet (Fig. 1).

Since GOT may originate from hepatic mitochondria and/or hepatic cytoplasm, the activity of other enzymes was determined to assess the localization of the hepatocellular damage due to the administration of paracetamol. Therefore, the activity of Glutamate Dehydrogenase was determined an enzyme exclusively confined to hepatic mitochondria.Compared to controls, rats fed the alcohol containing diet for 4 weeks exhibited a slight increase of GDH activity in the serum, irrespective whether a single low dose of paracetamol (400 mg/kg BW) or physiological saline solution was injected (Fig. 2). A striking enhancement of serum GDH activity could be observed,

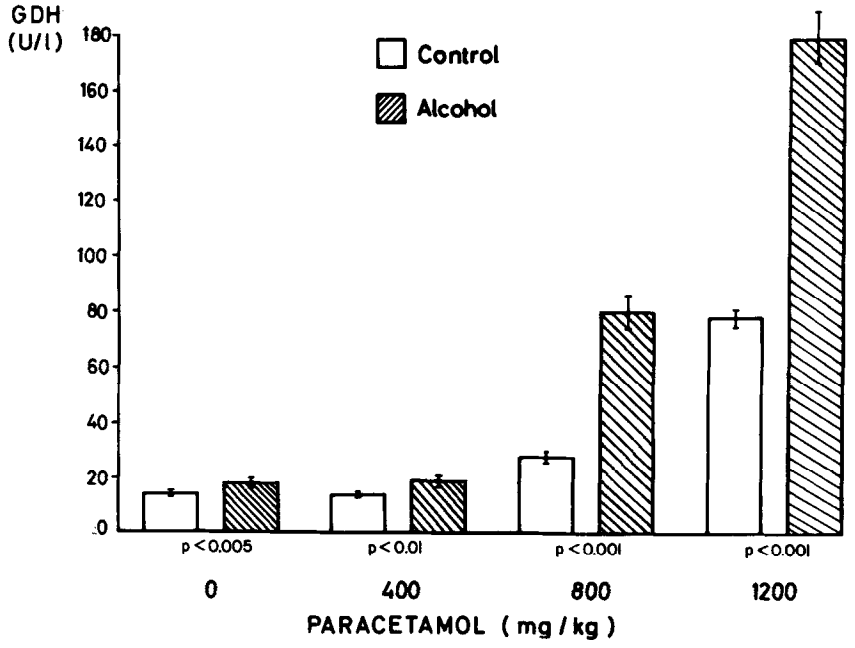

Fig. 2: Effect of an acute dose of paracetamol on serum GDH activity following chronic alcohol consumption. The experimental conditions are given in the legend of Fig. 1.

however, when intermediate or high doses of paracetamol (800 or 1200 mg/kg BW) were administered (Fig. 2). In accordance with the data for GOT (Fig. 1), the increase of GDH activities after 800 or 1200 mg paracetamol/kg BW was much more striking in the alcohol pretreated rats than in their pair-fed controls, receiving the same amount of paracetamol (Fig. 2). Moreover, further studies at high doses of paracetamol failed to show a similar increase of activity for cytoplasmic GPT (Fig. 3), clearly indicating that hepatocellular injury due to paracetamol can be confined primarily to hepatic mitochondria.

Discussion

The present study shows that the acute administration of paracetamol resulted in a significant increase of serum GOT and GDH activities which was much more pronounced in rats fed alcohol chronically than in their pair-fed controls (Fig. 1 and 2). These findings suggest that chronic alcohol

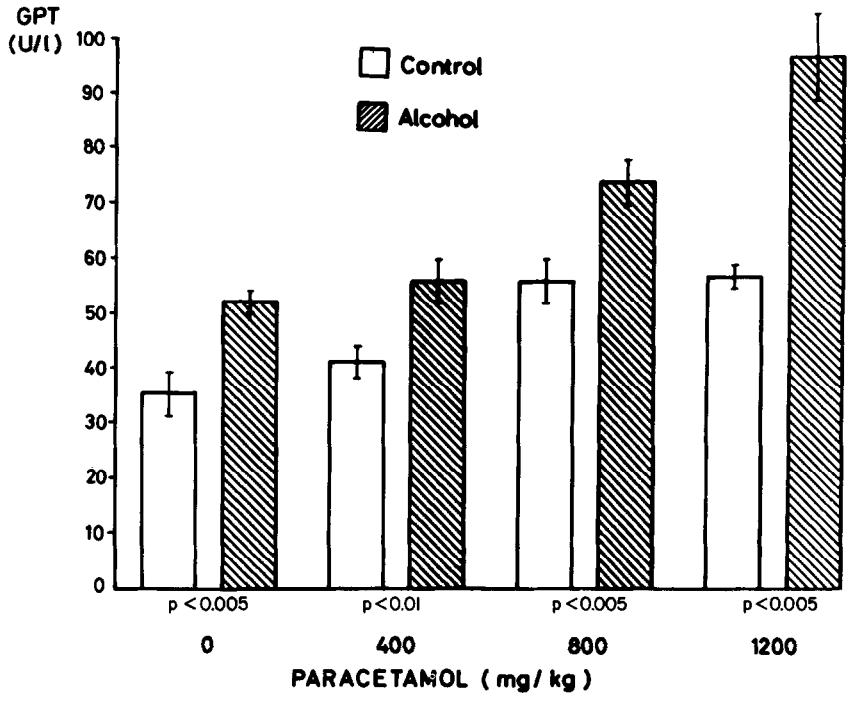

Fig. 3: Effect of an acute dose of paracetamol on serum GPT activity following chronic alcohol consumption. The experimental conditions are given in the legend of Fig. 1.

consumption predisposes to increased paracetamol induced hepatotoxicity which can be confined primarily to hepatic mitochondria.

It is generally accepted that paracetamol is excreted to a major extent as sulfate or glucuronide (18). On the other hand, paracetamol metabolism also proceeds via the cytochrome P-450 dependent microsomal mixed function oxidase which may result in the formation of toxic intermediates. Glutathione normally combines with the metabolite and forms a readily excreted mercapturic acid, preventing a combination of the toxic intermediate with liver macromolecules essential for a regular function of the hepatocyte (18). Since chronic alcohol consumption results in an induction of various microsomal enzyme activities including the mixed function oxidase consisting of cytochrome P-450 and NADPH-cytochrome P-450 reductase (8 - 13), it is conceivable that the metabolism of paracetamol increases following chronic alcohol consumption like that other drugs. This will result

in an increased formation of hepatotoxic metabolites which in turn may lead to an increased paracetamol induced hepatotoxicity following chronic alcohol consumption due to depletion of glutathione. Thus, the clinical observation of an increased paracetamol-induced hepatotoxicity following chronic alcohol consumption (17) could be substantiated in the present study under controlled experimental conditions.

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