

Activities of Hepatic Enzymes for Ethanol Metabolism in Cholestatic Rats with Acute Ethanol Intoxication

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: , Alcohol dehydrogenase, Catalase, Microsomal ethanol oxidizing system

Introduction

In human beings, cholestasis is observed in various diseases such as late viral hepatitis, carcinoma of

the bile duct, gallstones in the bile duct, primary biliary cirrhosis, sclerosing cholangitis, biliary atresia, alcoholic hepatitis, and drug-induced liver disease.¹ In rats, cholestasis can be induced by common bile duct (CBD) ligation which causes biochemical and morphological abnormalities in the liver such as inflammation, necrosis, fatty change, biliary hyperplasia, fibrosis, and cirrhosis.²⁴

Most of ethanol absorbed in the body is metabolized in the liver;⁵⁶ it is oxidized to acetaldehyde and

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then, metabolized further to acetate or excreted.⁵⁶ The process of ethanol oxidation in the liver is mediated by alcohol dehydrogenase (alcohol: NAD⁺ oxidoreductase, EC 1.1.1.1, ADH), microsomal ethanol oxidizing system (MEOS) and catalase (hydrogen peroxide oxidoreductase, EC 1.11.1.6).^{57,8} The activities of these alcohol-metabolizing enzymes can be changed in case of extrahepatic cholestasis. Therefore, alcohol drinking under cholestasis can induce alteration in the activities of alcohol metabolizing enzymes.

To investigate the effects of alcohol ingestion on the alcohol-metabolizing enzymes in cholestatic liver, the activities of ADH, MEOS, and catalase were measured in rats by means of acute ethanol intoxication after CBD ligation.

Subject and Methods

1. Animals

Thirty normal male rats of the Sprague-Dawley strain, weighing between 280 and 320 grams, were used in this experiment. All 30 rats were equally divided into 6 groups (group 1, group 2, group 3A and 3B, group 4A and 4B). The group 1 were normal controls. The group 2 was CBD ligation group. The rats in this group were sacrificed at 14th day after CBD ligation. In group 3A and 3B, ethanol intoxication was performed. The rats were intoxicated with 4 g of ethanol per kg of body weight according to the method of Liu et al.⁹ Then, they were sacrificed 1.5 hours (Group 3A) and 24 hours (Group 3B) after acute ethanol intoxication. In the remaining two groups (group 4A and 4B), both CBD ligation and ethanol intoxication were carried out. At the 14th day after CBD ligation, the rats were intoxicated with 4 g of ethanol per kg of body weight according to the method of Liu et al.⁹ Then, they were sacrificed 1.5 hours (Group 4A) and 24 hours (Group 4B) after acute ethanol intoxication. All animals were main-

tained on commercial pellets purchased from Sam Yang Food Co.

The rats were fasted for 12 hours prior to sacrifice and they were anesthetized lightly with ether for surgery or being sacrificed. The CBD was exposed through a middle line incision. After double ligation of CBD, the mid point was cut.

2. Chemicals

β -Nicotinamide adenine dinucleotide (β -NAD⁺, from yeast, grade III), β -nicotinamide adenine dinucleotide reduced form (β -NADH, from yeast, grade III), N,N-dimethyl-p-nitrosoaniline, glycine, n-butanol, alcohol dehydrogenase of equine liver, β -nicotinamide adenine dinucleotide phosphate (NADP⁺, from yeast, Sigma grade), nicotinamide, glucose-6-phosphate, semicarbazide-HCl, catalase of bovine liver, and bovine albumin were purchased from Sigma Chemical Co. (U.S.A.). Ethanol (99-100%) was purchased from E Merck Co. All other chemicals were of the highest purity available commercially.

3. Enzyme sample preparations

The rats were anesthetized lightly with ether, blood was collected from abdominal aorta, and liver was perfused through the portal vein with 0.25 M sucrose. The liver obtained was rinsed in 0.25 M sucrose. Then, the surface was wiped and dried. Enzyme samples were obtained by centrifugation using density gradient made of sucrose.¹⁰

All the procedures described above were performed at 2-4 °C.

4. Enzyme assays

The assay of cytosolic ADH activity was carried out with ethanol and NAD⁺ as substrates according to the method of Koivula et al.¹¹ Cytosolic ADH activity was expressed as nmol of NADH produced per minute per mg of protein.

The assay of serum ADH activity was carried out

using n-butanol and N, N-dimethyl-p-nitrosoaniline as substrates according to the method of Skursky et al.¹² Serum ADH activity was expressed as μmol of butyraldehyde produced per minute per mg of protein.

The assay of MEOS activity was carried out according to the method of Lieber and DeCarli.¹³ MEOS activity was expressed as nmol of acetaldehyde produced per minute per mg of protein.

The assay of catalase activity was carried out using hydrogen peroxide as a substrate according to the method of Nelson and Kiesow.¹⁴ Catalase activity was expressed as μmol of hydrogen peroxide reduced per minute per mg of protein.

5. Statistical analysis

The values were expressed as mean $\pm$ S.D. Statistical evaluation of significant difference between mean values was performed with the Student's t-test.

P values less than 0.05 were considered significant. The PC-SAS version 6.04 program was used.

Results

Hepatic ADH activity was lower in group 2 than in group 1 (Fig. 1). It was higher in groups 3A and 3B than in group 1, and also higher in group 4B than in group 2 (Fig. 1).

Hepatic MEOS activity was higher in group 2 than in group 1 (Fig. 2). There was no significant difference in the activities between group 4A, group 4B and group 2 (Fig. 2).

Hepatic catalase activity was lower in group 2 than in group 1 (Fig. 3). Hepatic catalase activity was higher in groups 4A and 4B than in group 2 (Fig. 3).

Serum ADH activity was higher in group 2 than in

Fig. 1. Hepatic ADH activity. Values are mean $\pm$ S.D. of 5 rats in each group. group 1, Normal rats; group 2, CBD ligated rats; groups 3A and 3B, The rats were sacrificed at the 1.5 hours (group 3A) and 24 hours (group 3B) after acute ethanol intoxication; group 4A and 4B, The rats were sacrificed at the 1.5 hours (group 4A) and 24 hours (group 4B) after acute ethanol intoxication with CBD ligation.

* $p < 0.001$ compared to group 1.

** $p < 0.05$ compared to group 2.

Fig. 2. Hepatic MEOS activity. Values are mean $\pm$ S.D. of 5 rats in each group. group 1, Normal rats; group 2, CBD ligated rats; groups 3A and 3B; The rats were sacrificed at the 1.5 hours (group 3A) and 24 hours (group 3B) after acute ethanol intoxication; group 4A and 4B, The rats were sacrificed at the 1.5 hours (group 4A) and 24 hours (group 4B) after acute ethanol intoxication with CBD ligation.

* $p < 0.05$ compared to group 1.

group 1 (Fig. 4). It was higher in groups 4A and 4B than in group 2 (Fig. 4).

Discussion

It is well known that 98 percent of ethanol absorbed in normal human body is metabolized in the liver and alcohol dehydrogenase plays a role in this process.⁵⁶ In our experiment, only the activity of ADH among the three ethanol metabolizing enzymes was increased after the acute ethanol intoxication in normal rats (groups 3A and 3B, Fig. 1). This result indicates that ethanol is metabolized mainly by ADH which is the major catalyst in the process of ethanol oxidation⁵⁶ in case of the normal subject who drinks alcoholic liquors.

CBD ligation in rats causes morphological abnormalities in the liver such as inflammation, necrosis,

fatty change, biliary hyperplasia, fibrosis, cirrhosis,²⁴ and also causes biochemical changes including enzyme activities.¹⁵ In this experiment, the activity of MEOS among the three ethanol metabolizing enzymes was increased in the CBD ligated group (group 2, Fig. 2), but the activities of ADH and catalase were decreased (Fig. 1, 3). These results suggest that the homeostasis of ethanol metabolism under cholestasis is maintained by induction of MEOS despite of decreased activities of ADH and catalase.

In the case of cholestasis combined with acute ethanol intoxication (groups 4A and 4B), the activities of ADH and catalase were higher than in cholestasis group without ethanol intoxication (Fig. 1, 3). However, cytosolic ADH activity in groups 4A and 4B was still lower than that in group 1. These results suggest that if the subject with cholestasis is exposed to a great quantities of alcoholic liquors, both ADH

Fig. 3. Hepatic catalase activity. Values are mean $\pm$ S.D. of 5 rats in each group. group 1, Normal rats; group 2, CBD ligated rats; groups 3A and 3B, The rats were sacrificed at the 1.5 hours (group 3A) and 24 hours (group 3B) after acute ethanol intoxication; group 4A and 4B, The rats were sacrificed at the 1.5 hours (group 4A) and 24 hours (group 4B) after acute ethanol intoxication with CBD ligation.

* $p < 0.01$ compared to group 1.

** $p < 0.05$ compared to group 2.

Fig. 4. Serum ADH activity. Values are mean $\pm$ S.D. of 5 rats in each group. group 1, Normal rats; group 2, CBD ligated rats; groups 3A and 3B, The rats were sacrificed at the 1.5 hours (group 3A) and 24 hours (group 3B) after acute ethanol intoxication; group 4A and 4B, The rats were sacrificed at the 1.5 hours (group 4A) and 24 hours (group 4B) after acute ethanol intoxication with CBD ligation.

* $p < 0.001$ compared to group 1.

** $p < 0.05$ compared to group 2.

and catalase play roles in the process of ethanol oxidation. However, ADH can not metabolize all the alcoholic liquors absorbed under cholestasis, thus catalase is activated.

Increase of serum ADH activity was greater in group 4A and 4B than in group 2 (Fig. 4). These results suggest that large amount of ADH in the liver flows into the blood. Such flow is caused by increased damage to the liver under the alcohol intoxication combined with cholestasis.

Summary

Background/Aims: The effects of alcohol ingestion on the alcohol metabolizing enzymes of the liver under the cholestasis were investigated. **Methods:** The activities of hepatic alcohol dehydrogenase (ADH), microsomal ethanol oxidizing system (MEOS), and catalase were measured in rats with extrahepatic cholestasis induced by common bile duct (CBD) ligation combined with acute ethanol intoxication. **Results:** The activity of ADH was increased under the acute ethanol intoxication in normal rats. In the CBD ligated group, the activity of MEOS was increased. When acute ethanol intoxication was combined with cholestasis, the activities of ADH and catalase were increased. **Conclusions:** These results suggest that if subjects with cholestasis drink a large quantity of ethanol, not only ADH but also catalase is activated because induction of MEOS activity is limited and ADH level is lower under the acute ethanol intoxication combined with cholestasis than under normal condition.

Key Words: Alcohol intoxication, Extrahepatic cholestasis, Alcohol dehydrogenase, Catalase, Microsomal ethanol oxidizing system

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