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Ethanol Metabolizing Enzymes in Cholestatic Rats with Chronic Ethanol Intoxication

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=Abstract=

Hepatic alcohol dehydrogenase (ADH), microsomal ethanol oxidizing system (MEOS), and catalase activities were measured in rats with ethanol intoxication combined with extrahepatic cholestasis induced by common bile duct (CBD) ligation to investigate the effects of alcohol ingestion on the alcohol metabolism in the liver under the hepatobiliary disease. The activities of ADH and catalase were decreased in the CBD ligated group. The activity of MEOS was increased in the CBD ligated group. When chronic ethanol intoxication was combined with extrahepatic cholestasis, only the activity of ADH among the three ethanol metabolizing enzymes was increased to catalyze the ethanol. These results indicate that the ethanol metabolism under the hepatobiliary disease is maintained by induction of MEOS instead of decreased ADH and catalase activities. ADH plays the major role under the hepatobiliary disease combined with chronic ethanol intoxication.

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

Introduction

In human beings cholestasis, in the liver occurs 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 certain drugs (Eddleston, 1994). In rats, cholestasis can be induced by common bile duct (CBD) ligation. CBD ligation in rats causes known biochemical and morphological abnormalities in the liver such as inflammation, necrosis, fatty change, biliary hyperplasia, fibrosis, and cirrhosis (Kountouras *et al*, 1984; Chang *et al*, 1987; Kim *et al*., 1989).

Most of ethanol absorbed in the body is metabolized in the liver (Murray, 1996, Chun, 1998), and it is oxidized to acetaldehyde and excreted or next to acetate in the liver (Murray, 1996, Chun, 1998). In the process of ethanol oxidation in the liver, it is well known that 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) play the role of catalysts (Murray, 1996, Seitz and Poschl,

1997, Porodenko, 1997, Chun, 1998). The activities of these alcohol metabolizing enzymes will be changed with the alteration of ethanol metabolism in extrahepatic cholestasis induced by CBD ligation. And if alcohol drinking is superimposed with hepatic disease, the alteration in the metabolism of ethanol including the activities of alcohol metabolizing enzymes is expected.

The activities of ADH, MEOS, and catalase were measured in rats with CBD ligation after chronic ethanol intoxication to investigate the effects of alcohol ingestion on the alcohol metabolism in the liver under the hepatobiliary disease.

Materials and Methods

Animals

Normal male rats of the Sprague-Dawley strain, weighing between 280 and 320 grams, were used in this experiment. All the experimental groups, with 5 rats in each group, were divided as follows: One sham operation group (Group 1): The rats were sacrificed at the 14th day after sham operation. One CBD ligation group (Group 2): The rats were sacrificed at 14th day after CBD ligation. One sham operation after chronic ethanol intoxication group (Group 3): The rats were provided with 5% (v/v) ethanol instead of water for sixty days according to method of Eagon *et al* (1987). They were continuously supplied with ethanol and sham operation was done. Rats were sacrificed at the 14th day after sham operation. One CBD ligation after chronic ethanol intoxication group (Group 4): The rats were provided with 5% (v/v) ethanol instead of water for sixty days according to method of Eagon *et al* (1987). They were continuously supplied with ethanol and their CBDs were ligated. Rats were sacrificed at the 14th day after CBD ligation. All animals were maintained on commercial pellets purchased from Sam Yang Food Co.

The rats were anesthetized lightly with ether for surgery or being sacrificed and they were fasted for 12 h prior to sacrifice. The CBD was exposed through a middle line incision. After double ligation of CBD, the mid point was cut. The sham operation was performed in the same manner without CBD ligation and cutting.

Chemicals

-Nicotinamide adenine dinucleotide (-NAD$^+$,from yeast, grade III), -nicotinamide adenine dinucleotide reduced form (-NADH, from yeast, grade III, disodium salt), N,N-dimethyl-p-nitrosoaniline, glycine, n-butanol, alcohol dehydrogenase (from equine liver, A6128), -nicotinamide adenine dinucleotide phosphate (NADP$^+$, from yeast, Sigma grade, monosodium salt), nicotinamide, glucose-6-phosphate, semicarbazide-HCl, catalase (from bovine liver, C3155) and bovine albumin were purchased from Sigma Chemical Co. (U.S. A.). Ethanol (99%-100%) was purchased from E Merk Co. All other chemicals were of the highest purity commercially available.

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. Serum and liver were obtained. The liver obtained was

rinsed in 0.25 M sucrose. The surface then wipe dry. Enzyme samples were obtained by centrifugation on density gradient made of sucrose (Kwak and Kwak, 1986).

All the procedures (described above) were performed at 2-4℃.

Enzyme assays

The assay of cytosolic ADH activity was carried out according to the method of Koivula *et al.* (1975) with ethanol and NAD+ as substrates. The amount of NADH produced was estimated spectrophotometrically. Results are expressed as nmol of NADH produced per minute per *mg* of protein.

The assay of serum ADH activity was carried out according to the method of Skursky *et al* (1979) with n-butanol and N, N-dimethyl-p-nitrosoaniline as substrates. The amount of N,N-dimethyl-p-nitroso-aniline reduced was estimated spectrophotometrically. Results are 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 (1968). The amount of acetalde-hyde- semicarbazide complex produced was estimated spectrophotometrically. Results are expressed as nmol of acetaldehyde produced per minute per *mg* of protein.

The assay of catalase activity was car-ried out according to the method of Nelson and Kiesow (1972) with hydrogen perox-ide as a substrate. The amount of hydro-gen peroxide reduced was estimated spectrophotometrically. Results are ex-pressed as μmol of hydrogen peroxide reduced per minute per *mg* of protein.

Statistical analysis

Values were expressed as mean ± S.D. Statistical evaluation of significant dif-ference between means was performed with the Student's t-test. P values of ≤ 0.05 were considered significant.

Results

Hepatic ADH activity was lower in group 2 (28.5±5.9 nmol NADH/*mg* protein/min) than in group 1 (77.6±12.2, $P<0.001$, Fig-ure 1). It was lower in group 4 (38.7±5.0) than in group 3 (88.3±13.5, $P<0.001$), and higher in group 4 than in group 2 ($P<0.05$, Figure 1).

Hepatic MEOS activity was higher in group 2 (7.7±1.5 nmol acetaldehyde/*mg* protein/min) than in group 1 (5.4±0.8, $P<0.05$, Figure 2). There was no significant difference in the activity between group 4 (7.7±1.3) and group 2 (6.6±1.3, Figure 2).

Hepatic catalase activity was lower in group 2 (23.8±4.2 μmol H2O2 reduced/*mg* protein/min) than in group 1 (33.1±3.6, $P<0.01$), and lower in group 4 (23.0±3.2)than in group 3 (31.9±3.3, $P<0.05$). However, there was no difference in the extents of de-crease between group 4 and group 2 (Figure 3).

Serum ADH activity was higher in group 2 (44.6±16.1 nmol butyraldehyde/*mg* pro-tein/min) than in group 1 (4.9±1.4, $P<0.001$), and showed a greater increase in group 4 (80.9±20.3) than in group 2 ($P<0.01$) and 3 (5.7±1.1, $P<0.001$), respectively (Figure 4).

Discussion

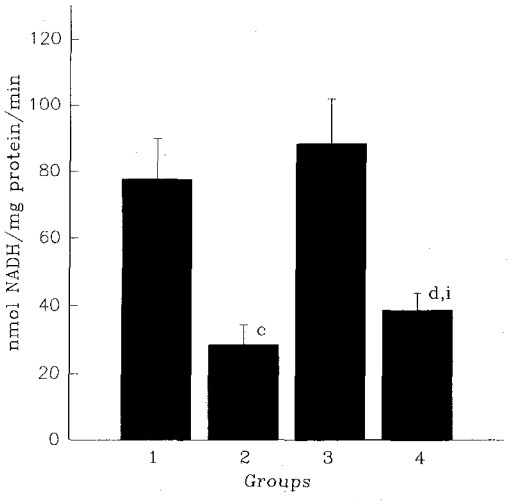

Figure 1. Hepatic ADH activity Values are means ± S.D. with 5 rats in each group. Animal groups are described in text.
c; P<0.001 vs group 1, d; P<0.05 vs. group 2,
i; P<0.001 vs group 3.

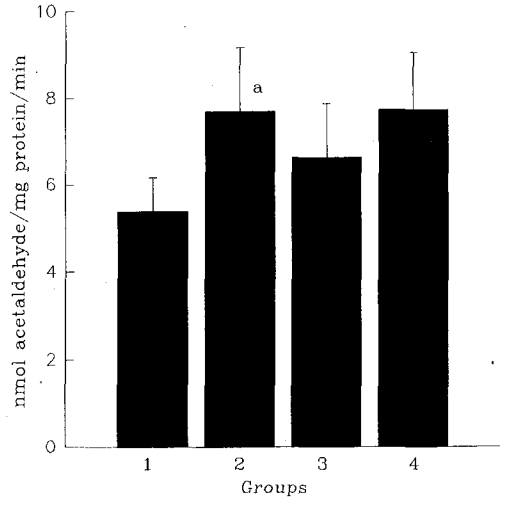

Figure 2. Hepatic MEOS activity Values are means ± S.D. with 5 rats in each group. Animal groups are described in text.
a; P<0.05 vs group 1

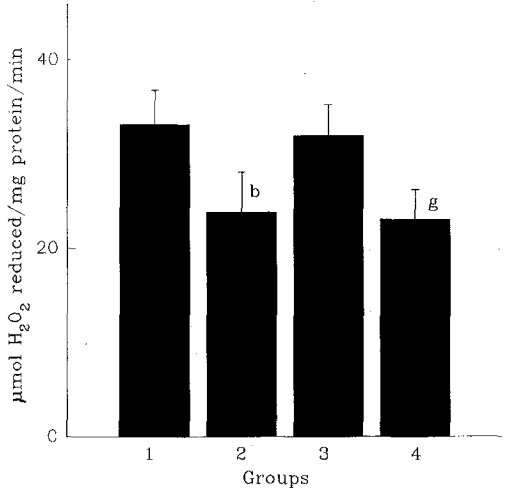

Figure 3. Hepatic catalase activity Values are means ± S.D. with 5 rats in each group. Animal groups are described in text. b; P<0.01 vs group 1, g; P<0.05 vs. group 3.

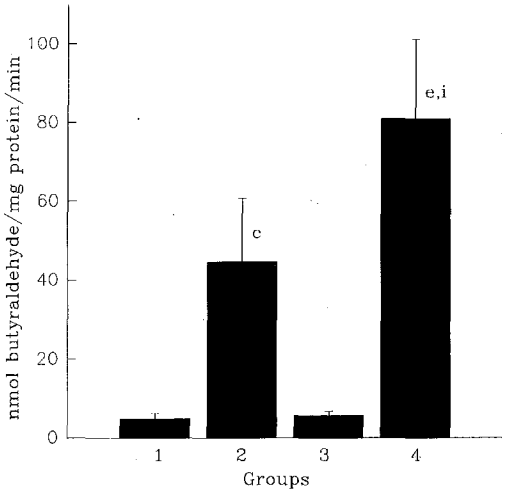

Figure 4. Hepatic ADH activity Values are means ± S.D. with 5 rats in each group. Animal groups are described in text.
c; P<0.001 vs group 1, e; P<0.05 vs. group 2, i; P<0.001 vs group 3.

It is well known that 98 percent of ethanol absorbed in normal human body is metabolized in the liver (Murray, 1996, Chun, 1998). In the process of ethanol oxidation in the liver, alcohol dehydrogenase is the first and major route of ethanol metabolism (Murray, 1996, Chun, 1998). The alcohol metabolism under the cholestasis combined with chronic alcohol intoxication is not clear. In this experiment, the activities of three alcohol metabolizing enzymes such as ADH, MEOS, and catalase were measured in rats with CBD ligation after chronic ethanol intoxication to investigate the effects of alcohol ingestion on the alcohol metabolism in the liver under the hepatobiliary disease.

CBD ligation in rats causes morphological abnormalities in the liver such as inflammation, necrosis, fatty change, biliary hyperplasia, fibrosis, cirrhosis (Kountouras *et al*, 1984; Chang *et al*, 1987; Kim *et al*, 1989), and also causes biochemical changes including enzyme activities (Mun and Kwak, 1997). In this experiment, the activity of MEOS among the three ethanol metabolizing enzymes was increased in the CBD ligated group (Group 2, Figure 2), but the activities of ADH and catalase were decreased (Figure. 1 and 3). These results suggest that the homeostasis of ethanol metabolism under the hepatobiliary disease is maintained by induction of MEOS to replace the decreased ADH and catalase activities.

When chronic ethanol intoxication was combined with cholestasis (Group 4), only the activity of ADH among the three ethanol metabolizing enzymes was higher at the 14th day in the group 4 than in the group 2 (Figure 1). This result indicates that only ADH plays the major role in the process of ethanol oxidation under the hepatobiliary disease combined with chronic ethanol intoxication.

Serum ADH activity showed a greater increase in group 4 than in group 2 (Figure 4). This result suggests that there is more severe damages under the alcohol intoxication combined with cholestasis than under cholestasis alone, and ADH in the liver flows into the blood in large quantities due to increased damage to the liver under the alcohol intoxication combined with cholestasis.

Summary

Hepatic alcohol dehydrogenase (ADH), microsomal ethanol oxidizing system (MEOS), and catalase activities were measured in rats with ethanol intoxication combined with extrahepatic cholestasis induced by common bile duct (CBD) ligation to investigate the effects of alcohol ingestion on the alcohol metabolism in the liver under the hepatobiliary disease. All the experimental groups, with 5 rats in each group, were divided as follows: Sham operation group, CBD ligation group, sham operation after chronic ethanol intoxication group, and CBD ligation after chronic ethanol intoxication group. The activities of ADH and catalase were decreased in the CBD ligated group. However, the activity of MEOS was increased in the CBD ligated group. When chronic ethanol intoxication was combined with extrahepatic cholestasis, only the activity of ADH among the three ethanol metabolizing enzymes was

increased to catalyze the ethanol. These results indicate that the ethanol metabolism under the hepatobiliary disease is maintained by induction of MEOS instead of decreased ADH and catalase activities. However, only ADH plays the major role under the hepatobiliary disease combined with chronic ethanol intoxication.

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