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Gender-Related Differences in Hepatic Activity of Alcohol Dehydrogenase Isoenzymes and Aldehyde Dehydrogenase in Humans

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Alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH), which are most abundant in the liver, are the main enzymes involved in ethanol metabolism in humans. Gender-related differences in total liver ADH and ALDH activity among different animal species have been observed in many studies. We measured total ADH and ALDH activity, and the activity of class I–IV ADH in the livers of male and female patients. Total ADH and class I and II ADH activities were significantly higher in males than in females ($P = 0.0052$, $P = 0.0074$, $P = 0.020$, respectively). Class III and IV ADH and total ALDH activities were not significantly different between the genders ($P = 0.2917$, $P = 0.0590$, $P = 0.2940$, respectively). The results of our study clearly show that there is a difference in enzymatic activity between male and female patients for those isoenzymes that actively participate in ethanol oxidation in the liver (class I and II ADH), although the main form of ADH in this organ is class III ADH. J. Clin. Lab. Anal. 17: 93–96, 2003.

Key words: alcohol dehydrogenase isoenzymes; aldehyde dehydrogenase; liver; gender

INTRODUCTION

It is generally accepted that the first steps in the metabolism of ethanol in liver are catalyzed by alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH). In the human liver, ADH is grouped into three classes (ADH I, ADH II, and ADH III), and ALDH is divided into two main forms (ALDH 1 and ALDH 2). The distribution of ALDH in the liver parenchyma has not yet been determined (1,2). ADH I and II, and ALDH 1 and 2 all play major roles in the oxidation of ethanol to acetate in the liver. Many studies have demonstrated gender differences in alcohol pharmacokinetic parameters in different animal species and in humans (3,4). These differences were mainly attributed to the lower gastric ADH activity (5) and higher ADH liver activity in women compared to men (6). Maly and Sasse (6) showed a 1.5 times higher activity of total ADH in the perivenous zone in women of any age compared to that in young men; however, ALDH activity exhibited only minor variations between men and women. In agreement with previous studies (8), a study by Lai et al. (7) in Han Chinese demonstrated that gender does not significantly influence the activities of ADH and ALDH in the human stomach. A recent study by Lee et al. (9) on alcohol-naïve hamsters showed that liver ALDH activity was higher in males, whereas the activity of ADH did not show any gender difference. Thus, the effect of gender on ADH and ALDH activity in the stomach and liver remains a matter of controversy.

The relative contribution of ADH and ALDH to the oxidation of ingested alcohol depends on the amount of these enzymes in the liver. The aim of this study was to assess the activity of class I–IV ADH izoenzymes and ALDH in liver homogenates, and to determine whether this activity exhibits gender-related differences.

MATERIALS AND METHODS

Tissue Samples

Liver samples were taken surgically during partial hepatectomy from 50 patients (30 males and 20 females, 29–63 years old) suffering from liver carcinoma. The patients had no history of heavy alcohol abuse, and

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self-reported an intake of <60 g of ethanol per week. The tissue samples used for assay were histologically normal. The samples were weighed and immediately homogenized in Eppendorf vials containing 0.1 M of potassium phosphate buffer (1:4 ratio, w/v) for 20 sec, pH 7.4, using the ultrasonificator (Sonoplus HD 70; Bandolin, Berlin, Germany). The sonicates were centrifuged at 14,000 rpm for 30 min at 4°C. The supernatants were used for the assay of ADH and ALDH activity. The protein content in the supernatant fraction was measured according to the method of Lowry et al. (10). This study was approved by the Ethics Committee of the Medical Academy of Bialystok, Bialystok, Poland.

Determination of Class I and II ADH Isoenzymes

The activity of class I ADH isoenzymes was measured using a fluorometric method based on fluorogenic substrates, as described by Wierzchowski et al. (11). The assays were performed in a reaction mixture containing 60 μL of supernatant, 150 μL of a 300-μmol/L solution of 4-methoxy-1-naphthaldehyde (Aldrich Chemical Company, Inc., Milwaukee, WI), 100 μL of 1 mmol/L NADH (Sigma Diagnostics, St. Louis, MO), and 2.69 mL of 0.1 mol/L sodium phosphate buffer, pH 7.6. The reaction was started by the addition of supernatant. The fluorescence changes were recorded for up to 10 min on an RF-5301 spectrofluorophotometer (Shimadzu, Duisburg, Germany) at an excitation wavelength of 316 nm and an emission wavelength of 370 nm. Then 60 μL of a 200-μmol/L solution of 4-methoxy-1-naphthalenemethanol (product) was added to provide an internal standard. For the evaluation of ADH activity, two assays were performed: one with substrate alone, and one with substrate and 50 μL of a 12-mmol/L solution of 4-methylpyrazole as a specific inhibitor of the enzyme (Aldrich Chemical Company, Inc.).

Determination of Class III ADH Isoenzyme

The activity of class III ADH was measured by the photometric method, with n-octanol as a substrate, by monitoring the increase of NADH at 340 nm (12). The assays were performed at 25°C in 0.1 M glycine buffer (pH 9.6), using 1.2 mM of NAD, 1 mM of octanol, and 50 μL of the supernatant in the final volume of 2 mL.

Determination of Class IV ADH Isoenzyme

The activity of class IV ADH was measured by the photometric method, with m-nitrobenzaldehyde as a substrate, by monitoring the decrease of NADH at 340 nm (13). The analysis was performed at 25°C in 0.1 M sodium phosphate buffer (pH 7.5), using 86 μM NADH, 80 μM m-nitrobenzaldehyde, and 100 μL of the supernatant in the final volume of 2 mL.

Determination of Total ADH Activity

Total ADH activity was estimated by the photometric method, with p-nitrosodimethylaniline (NDMA) as a substrate (14). The reaction mixture (2 mL) contained 1.9 mL of a 26-μM solution of substrate in 0.1 M Na-phosphate buffer, pH 8.5, and 0.1 mL of mixture containing 0.25 M n-butanol and 5 mM NAD. The reduction of NDMA was monitored at 440 nm on a Shimadzu UV/VIS 1202 spectrophotometer.

Determination of Total ALDH Activity

ALDH activity was measured using the fluorogenic method based on the oxidation of 6-methoxy-2-naphthaldehyde to the fluororescent 6-methoxy-2-naphthoate (15). The reaction mixture contained 60 μL of substrate, 20 μL of 11.4 mM NAD, and 2.8 mL of 50 mM Na-pyrophosphate buffer, pH 8.5. The fluorescence was read at an excitation wavelength of 310 nm and an emission wavelength of 360 nm.

Statistical Analysis

The results were expressed as the mean $\pm$ standard deviation. The statistical analysis was performed with the χ^2 test and Student's t-test for independent samples. Differences were considered significant at $P<0.05$.

RESULTS

The activities of class I and II ADH in the liver homogenates were significantly higher in men than in women ($P<0.05$) (Table 1). These activities differed by

TABLE 1. The activity of hepatic ADH isoenzymes and ALDH in male and female patients

Gender	ADH I	ADH II	ADH III	ADH IV	ADH total	ALDH total
Men	0.662±0.105	0.135±0.019	6.211±0.815	0.024±0.004	9.186±1.034	0.127±0.016
Women	0.559±0.103	0.120±0.015	5.706±0.618	0.023±0.003	8.249±0.648	0.121±0.014
	$P<0.05$	$P<0.05$			$P<0.05$	

Data are expressed as nmol/min/mg of protein. Mean±standard deviation.
$P<0.05$ men vs. women.

about 18% for class I and 12% for class II ADH. In contrast, the activities of class III and IV ADH were not significantly different between both genders ($P > 0.05$) (Table 1). Although we did not find significant changes in all of the tested ADH isoenzymes, the total ADH activity was significantly higher in men than in women (about 11%). In a comparison of total ALDH activities in the liver of men and women, no significant difference was found ($P > 0.05$). We found that among the tested isoenzymes, class III ADH had the highest activity. The activity of class I ADH was about 10 times lower, and the activity of class II about 40 times lower than that of class III ADH. All samples from both men and women exhibited a barely detectable activity of class IV ADH.

DISCUSSION

The main ethanol-metabolizing isoenzymes in the liver are class I and II ADH. Class I ADH has its highest activity with relatively low ethanol concentration (Km about 1–2 mM) but class II with high concentration (Km about 34 mM) (16). Ethanol is the best substrate for this class. Class III ADH does not participate significantly in the hepatic ethanol oxidation under physiological conditions. Class IV ADH, with its high Km value, plays a major role in extrahepatic ethanol metabolism, mainly in the stomach. In our study, differences in enzymatic activity between male and female patients were shown for class I and II ADH–isoenzymes that actively participate in the degradation of ethanol in the liver. Class III and IV isoenzymes do not play a role in ethanol metabolism in the liver, and the differences in these isoenzyme activities were not significant. In contrast with previous results, we observed higher total ADH activity in males than in females (6). Baraona et al. (4) mainly attributed gender differences in blood alcohol levels to a slower gastric metabolism in females. They also found a faster rate of hepatic ethanol metabolism in women than in men. This difference could explain the enhanced generation of acetaldehyde observed in women, but does not account for the higher blood alcohol level. The present findings indicate that the activities of enzymes involved mainly in hepatic ethanol metabolism are higher in men than in women. Thus, our results could help explain the association between the rate of ethanol oxidation in the liver, and blood alcohol levels in women and men who have ingested the same amount of alcohol.

The results of the present study show that class III ADH is the principal form of ADH in the human liver, and the class I named classical liver ADH has a low activity. Class I is found up to 95% of total activity in the liver. These results are in agreement with data reported by Danielsson and Jörnvall (17), and show that a higher protein content in class III ADH compared to class I, and a higher enzymatic activity with glutathione/formaldehyde (the specific substrate for class III ADH) compared to that with ethanol (the specific substrate for class I) are found in the human liver.

Our study demonstrates that the activity of class IV ADH in the liver is barely detectable in both genders. This trace of activity may derive from the blood vessels, because ADH activity was detected in all human arteries and veins (18). Class I isoenzymes were responsible for most of the ADH activity in the blood vessels. Class III ADH was present in all vessels, but in some individuals class IV izoenzyme was also detected.

It is generally accepted that chronic alcohol consumption is associated with an increased risk for digestive tract cancer, including cancer of the liver (19). One of the major risk factors for hepatocellular carcinoma is male sex (20). In this study we have shown that hepatic ADH activity (class I and II) is higher in males than in females, which may promote an increased production of carcinogenic acetaldehyde in men, compared to women, after they have consumed the same dose of ethanol. There was no difference between genders in the activity of ALDH, which is the enzyme involved in acetaldehyde disappearance.

In conclusion, the activity of class I and II ADH isoenzymes is lower in women than in men. These isoenzymes play an active role in ethanol metabolism in the liver. This difference could help explain the fact that after men and women ingest the same dose of ethanol, women have higher blood alcohol levels than men.

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