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ORIGINAL ARTICLE

The Activity of Alcohol Dehydrogenase Isoenzymes and Aldehyde Dehydrogenase in the Sera of Patients with Autoimmune Hepatitis

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SUMMARY

Background: **Autoimmune hepatitis (AIH) is a progressive inflammatory hepatopathy and an important cause of end-stage liver. The liver cells' destruction is reflected by increased activity of different enzymes in the serum. These enzymes include alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH), which play a significant role in the metabolism of many biological substances and exist mainly in the liver. In this study we investigated the activity of alcohol dehydrogenase and its isoenzymes and the total activity of ALDH in the sera of patients with autoimmune hepatitis.**

Methods: **Serum samples were taken for routine biochemical investigation from 32 patients with autoimmune hepatitis and from 40 healthy subjects. Class I and II of ADH and ALDH activity was measured by the spectrofluorometric method. For measurement of class III ADH and total ADH activity we employed the photometric methods.**

Results: **The activity of the class I ADH isoenzyme was significantly higher in the sera of patients with autoimmune hepatitis. The median activity of this isoenzyme in the patients group was approximately 63% (3.94 mU/L) higher than the control level (1.46 mU/L). For this reason, the total ADH activity was also significantly increased. The activities of other ADH isoenzymes and ALDH tested were unchanged.**

Conclusions: **The activity of total ADH and class I isoenzymes in the sera of patients with autoimmune hepatitis is increased, and it seems to be caused by the release of alcohol dehydrogenase from damaged liver cells.**
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INTRODUCTION

Autoimmune hepatitis (AIH) is a severe liver disease affecting all age groups worldwide. AIH is characterized by inflammatory liver histology, elevated transaminase levels, circulating non-organ-specific autoantibodies, and increased levels of immunoglobulin G [1]. The

aetiology and actual prevalence of AIH is unknown. The mechanisms leading to autoimmune liver damage have been a focus of intense investigation over the past three decades. Regardless of the factors triggering the autoimmune process, the pathogenic mechanism leading to liver damage is part of a complex scenario, involving the intervention of both innate and adaptive arms of the immune system [2]. The diagnosis is often difficult but can be facilitated by sequential measurement of relevant autoantibodies, exclusion of other liver disease, ultrasound, and liver histology.

Many studies show that changes in enzyme activity in the liver cells in the course of hepatocyte destruction are reflected by the change of its activity in the serum. Mezey and Cherrick reported total serum alcohol dehydrogenase (ADH) activity in hepatitis [3]. Other studies, presented by Chrostek and Szmitkowski, showed elevated activity of ADH isoenzymes (class I and II) in the serum of patients with acute viral hepatitis B [4]. While various markers have been studied in autoimmune hepatitis, the diagnostic significance of ADH isoenzymes and ALDH activities have not been reported. We hypothesize that the changed activities of ADH and ALDH in damaged hepatocytes in the course of autoimmune hepatitis are reflected in the plasma and perhaps could thus be helpful for diagnosing of this disease. In the current study, we have investigated the effect of liver cell changed by the immune process on the serum activity of alcohol dehydrogenase isoenzymes and aldehyde dehydrogenase.

MATERIALS AND METHODS

Material

The research protocol was approved by the Human Care Committee of the Medical University in Bialystok, Poland (Approval No. R-I-002/180/2014). All patients gave their informed consent for the examination. Serum samples were taken for routine biochemical investigations from 32 patients suffering autoimmune hepatitis (14 males and 18 females, age range 31 - 68 years) hospitalized in the Department of Infectious Diseases and Hepatology University Hospital, Medical University of Bialystok (Poland). The diagnosis of all patients was based on the International Autoimmune Hepatitis Group (IAIHG) revised scoring system and AASLD guidelines [5]. Patients with chronic liver diseases such as drug- or alcohol-induced hepatitis, fatty liver disease, metabolic disorders, genetic disorders, hereditary conditions such as Wilson disease, autoimmune cholangitis, primary biliary cirrhosis, and primary sclerosing cholangitis were excluded. All enrolled patients were seronegative for anti-hepatitis A virus IgM antibody and for HBsAg and for anti-hepatitis A virus IgM antibody, anti-hepatitis C virus IgG antibody, anti-hepatitis D virus IgG antibody, and anti-hepatitis E virus IgM antibody. Alcohol consumption was assessed using a validated questionnaire. Before the examinations, all patients had not consumed alcohol or almost one year.

Serum samples for the control group were taken from 40 volunteers (20 men and 20 women, aged 30 - 65 years). The healthy controls were volunteers and were defined as those with normal results of all physical and blood examinations. Control groups were selected from healthy community residents who attended the hospitals for routine physical check-ups at the Department of Preventive Medicine. Control subjects were recruited from the same geographical location and ethnic populations as the patients and were not from the hospital. All persons of the control group drank alcohol occasionally and self-reported an intake of < 25 g of ethanol per week. None of them consumed any drug.

Methods

Determination of total ADH activity

Total ADH activity was estimated by the photometric method with p-nitrosodimethylaniline (NDMA) as a substrate [6]. The reaction mixture (2 mL) contained 0.1 mL of serum and 1.8 mL of a 26 μM solution of substrate in 0.1 M of sodium phosphate buffer, pH 8.5 and 0.1 mL of a 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 (Shimadzu Europa GmbH, Duisburg, Germany).

Determination of total ALDH activity

ALDH activity was measured using the fluorogenic method based on the oxidation of 6-methoxy-2-naphtaldehyde to the fluorescent 6-methoxy-2 naphtoate [7]. The reaction mixture contained 60 μL of serum, 60 μL of substrate, 20 μL of 11.4 mM NAD, and 2.8 mL of 50 mM of sodium phosphate buffer, pH 8.5. The mixture contained also 50 μL of a 12 mM solution of 4-methylpyrazole as a specific inhibitor of ADH activity. The fluorescence was read at excitation wavelength 310 and emission wavelength 360 nm.

Determination of class I and II ADH isoenzymes

Class I and II alcohol dehydrogenase isoenzyme activity were measured using fluorogenic substrates (4-methoxy-1-naphthaldehyde for class I and 6-methoxy-2-naphthaldehyde for class II) in reduction reaction according to Jelski W et al. [6]. The assays were performed in a reaction mixture containing a serum (60 μL), substrate (150 μL of 300 μM), NADH (100 μL of 1 mM), and 0.1 M sodium phosphate buffer, pH 7.6 (2.69 mL) in conditions previously described [8]. The measurements were performed on a Shimadzu RF-5301 spectrofluorophotometer (Shimadzu Europa GmbH, Duisburg, Germany) at excitation wavelength 316 nm for both substrates and emission of 370 nm for class I and 360 nm for class II isoenzymes.

Determination of class III ADH isoenzyme
The assay mixture for class III of alcohol dehydrogenase activity contained a serum (100 µL), n-octanol as a substrate (31 µL of 1 mM), NAD (240 µL of 1.2 mM) in 0.1 M NaOH-glycine buffer pH of 9.6 [6]. The reduction of NAD was monitored at 340 nm and 25°C on a Shimadzu UV/VIS 1202 spectrophotometer.

Determination of class IV ADH isoenzyme
The assay mixture for class IV of alcohol dehydrogenase activity contained a serum (50 µL), m-nitrobenzaldehyde as a substrate (132 µL of 80 µM), NADH (172 µL of 86 µM) in 0.1 M sodium phosphate buffer pH 7.5 [9]. The oxidation of NADH was monitored at 340 nm and 25°C on a Shimadzu UV/VIS 1202 spectrophotometer.

Statistical analysis
A preliminary statistical analysis (Chi-square test) revealed that the distribution of ADH and ALDH activities did not follow a normal distribution. Consequently, the Wilcoxon test was used for statistical analysis. Statistically significant differences were defined as comparisons resulting in $p < 0.05$.

RESULTS

The activities of alcohol dehydrogenase, its isoenzymes and aldehyde dehydrogenase in the sera are presented in Table 1. The total activity of ADH was significantly higher (about 42.7%) in patients with autoimmune hepatitis than in healthy subjects ($p < 0.05$). The median total activity of ADH was 1,195 mIU/L in the sera of patients with AIH, and 685 mIU/L in healthy subjects. We have also observed that ALDH activity did not indicate significant differences between tested groups.
The comparison of ADH isoenzyme activities showed that only the activity of ADH I was significantly higher in the serum of patients in contrast to healthy subjects. The median activity of this class of ADH was 3.94 mIU/L in the tested patients and 1.46 mIU/L in the control group. The other tested classes of ADH isoenzymes had higher activities in the serum of patients with autoimmune hepatitis but differences were not statistically significant ($p > 0.05$).

DISCUSSION

Autoimmune hepatitis is a chronic immune-mediated liver disorder characterized by female preponderance, elevated transaminase and immunoglobulin G levels, and seropositive for autoantibodies and interface hepatitis. Its etiology remains unknown, though both genetic and environmental factors are involved in its development. The major mechanism of autoimmune liver damage involves immune reactions against host liver antigens [10]. The criteria for the diagnosis of autoimmune hepatitis have been established and revised by the International Autoimmune Hepatitis Group. Autoantibodies play a crucial role in the diagnosis and monitoring of autoimmune hepatitis. They should be marked as early as possible with the first suspicion of the disease. The most commonly detected autoantibodies are anti-nuclear antibodies (ANA), anti-smooth muscle antibodies (SMA), anti-actin antibodies, anti-mitochondrial antibodies (AMA) and anti-liver-kidney-microsomal type 1 antibodies (anti-LKM1). They are also a differentiation criterion between AIH type 1 and type 2 [11]. Due to the desire to improve diagnostic accuracy, further marker antibodies are still being sought. The diagnostic system, which includes positive and negative scores, grades clinical, histology and laboratory features of AIH, including response to treatment [5]. Although AIH in most patients has an insidious onset with symptoms of chronic liver disease, some 20 - 30% of patients present with an acute icteric hepatitis. AIH is characterized by elevated levels of transaminases. In general, the increase in aspartate aminotransferase and alanine aminotransferase levels is much more striking than that of bilirubin and alkaline phosphatase [12]. Another laboratory feature typical of autoimmune hepatitis, albeit not always present, is a generalized elevation of serum globulins and particularly gamma globulins mainly due to an increase in the IgG fraction [13]. There is no single laboratory test for autoimmune hepatitis. The presence of other causes of liver disease must be excluded, and it is very important to find markers which would detect a liver failure [14]. The diagnostic of AIH should be considered during the workup of any patient with increased liver enzyme levels.
In this study, we have found that the serum total alcohol dehydrogenase activity changed in the course of autoimmune hepatitis. The increase of total ADH activity was positively correlated with ADH I so the cause for the increase of total ADH in the course of this disease is an elevation of class I ADH isoenzymes. ADH I is also present in the gastrointestinal tract, kidneys, and lungs, but up to 95% of this activity is found in the liver. So, the elevated activity of ADH I seems to be caused by the isoenzymes released from damaged liver cells. The changes of other ADH isoenzyme activities were not significant in the serum of patients with AIH. Aldehyde dehydrogenase is present in the liver although the activity of ALDH seems to be disproportionately low to ADH activity. The serum levels of aldehyde dehydrogenase were not significantly higher in patients with AIH in comparison to the healthy group. The reason for this difference in expression of ADH and ALDH in AIH could be the disproportionately low ALDH activity in the liver compared to the activity of ADH and ALDH subcellular localization in the mitochondria. The link between alcohol consumption and liver disease is not direct and several factors including autoimmunity to hepatocyte components have been implicated. Ma and co-workers identified alcohol dehydrogenase as an autoantigen in autoimmune liver disease and in a proportion

Table 1. The comparison of ADH isoenzymes and ALDH activities in the sera of patients with autoimmune hepatitis.

Tested group	ADH I median range mean	ADH II median range mean	ADH III median range mean	ADH IV median range mean	ADH Total median range mean	ALDH Total median range mean
Autoimmune hepatitis (n = 32)	3.94 1.14 - 6.02 3.46	16.06 6.26 - 29.86 15.72	11.49 5.87 - 18.44 11.14	5.04 2.13 - 11.45 4.86	1,195 364 – 2,478 1,104	2.76 1.32 - 6.17 2.50
Control (n = 40)	1.46 0.65 - 3.11 1.28	14.94 5.04 - 24.15 14.33	10.85 5.36 - 17.68 10.52	4.96 2.08 - 11.14 4.60	685 257 – 2,126 626	2.61 1.13 - 6.01 2.42
	$p < 0.05$ *	p = 0.621	p = 0.536	p = 0.482	$p < 0.05$ *	p = 0.505

Data are expressed as mU/L, * - statistically significant differences between suitable groups, p - autoimmune hepatitis vs. control.

of patients with alcoholic liver disease. Their findings suggest that anti-ADH antibodies may be triggered by alcohol consumption and act as a disease activity marker in alcoholic liver disease [15]. It is not in agreement with data reported by Ngu and colleagues. They have shown that antibiotics are an independent risk factor for the development of AIH, whereas alcohol consumption and living in a childhood home with wood heating are independent protective factors against the later development of AIH [16].

The results in the current paper are similar to other studies performed in the various liver diseases. In our previous investigation we have found that the activity of class I and II ADH isoenzymes were significantly higher in the serum of patients with hepatitis C than in healthy subjects [6]. We observed also that total ADH activity and ADH I was elevated in the serum of patients with metastatic liver cancer. Moreover, ADH I was significantly higher in the sera of patients with metastatic tumors than with primary cancers [17]. Chrostek and Szmitkowski found significantly higher activity of ADH I and ADH II isoenzymes in the sera of patients with acute viral hepatitis B [4]. They reported also that activity of class I alcohol dehydrogenase was significantly higher in the serum of patients with chronic hepatitis B than in healthy [18]. In addition, the comparison of total alcohol dehydrogenase activity in the sera of alcoholics and non-alcoholic cirrhotic patients indicates similar changes in both cases, with an evident elevation of class I and total enzyme activity [19].

CONCLUSION

We can state that the increase of the activity of total ADH and class I alcohol dehydrogenase isoenzyme in the sera of patients with autoimmune hepatitis seems to be caused by release of this isoenzyme from damaged liver cells to blood. Probably the total ADH and ADH I determination in serum could be helpful for diagnostic of AIH.

Declaration of Interest:
The authors declare that they have no conflict of interest related to the publication of this manuscript.

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