

Activity of alcohol dehydrogenase isoenzymes and aldehyde dehydrogenase in sera of patients with hepatitis C

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Abstract

Introduction: The changes of enzyme activity in the hepatocytes in the course of different liver diseases are reflected by increase of the corresponding enzyme activity in the plasma. For example, the activities of alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) correlate with the severity of the condition during cirrhosis. In this study we measured the activity of ADH isoenzymes and ALDH in the sera of patients with hepatitis C.

Material and methods: Serum samples were taken from 60 patients suffering from viral hepatitis C and from 66 control subjects. Total ADH activity and class III and IV isoenzymes were measured by the photometric method and ALDH activity, ADH I and II by the fluorometric method.

Results: The ADH activity was significantly higher in patients with hepatitis C than in healthy ($p < 0.001$). The total activity of ADH was 1284 mU/l in patients, and 745 mU/l (controls). The activity of isoenzymes classes ADH I and ADH II in the hepatitis C group increased respectively 55% (4.24 vs. 1.88 mU/l; $p < 0.001$) and 47% (26.63 vs. 14.11 mU/l; $p < 0.001$) in the comparison to the control. There was significant increase in the activity of ADH I isoenzyme (4.96 vs. 3.81 mU/l; $p < 0.001$) and ADH total (1833 vs. 1105 mU/l; $p < 0.001$) in patients with high viral load in comparison to patients with low viral load.

Conclusions: The activity of class I and II ADH isoenzymes in the sera of patients with hepatitis C is increased, and it seems to be caused by the release of these isoenzymes from damaged liver cells.

Key words: alcohol dehydrogenase isoenzymes, aldehyde dehydrogenase, hepatitis C.

Introduction

Hepatitis C virus (HCV) is an RNA virus classified in the Flaviviridae virus family. Worldwide, one hundred and seventy million individuals are infected with HCV, with an incidence rate of 3%. Hepatitis C virus infections represent a major global public health problem. Hepatitis C virus-related chronic hepatitis are the main causes of cirrhosis and hepatocellular carcinoma (HCC), which are responsible for high rates of morbidity and mortality [1, 2].

It is commonly accepted that cellular injury and necrosis of hepatocytes leads to the release of cytoplasmic enzymes. According to the data obtained by Chrostek and Szmitkowski, the elevated serum activity of alcohol dehydrogenase (ADH) reflects damage of liver parenchymal cells, especially in the course of acute viral hepatitis B and cirrhosis [3, 4]. Their results demonstrate that the activity of ADH isoenzyme can be a useful marker of liver damage in the course of viral hepatitis B. Alcohol dehydrogenase and aldehyde dehydrogenase (ALDH) are principal enzymes catalyzing the conversion of ethanol to acetate. Alcohol dehydrogenase comprises a family of enzymes which has been grouped into several classes. In humans, the first two classes are mainly located in the liver cells. Class I ADH is the classical liver alcohol dehydrogenase, but it is also observed in lesser amounts in the gastrointestinal tract, lungs, and kidneys [5]. Class II isoenzyme of ADH is found only in the liver, whereas class III is present in all examined tissues [6]. Class IV ADH is expressed preferably in the upper part of the digestive tract [7]. In humans, liver aldehyde dehydrogenase exists as several isoenzymes which differ in their subcellular location and kinetic properties. These isoenzymes are usually divided into two broadly defined groups, cytosolic high K_m ALDH and mitochondrial low K_m ALDH, based on their Michaelis constant for acetaldehyde [8].

We hypothesize that the changed activities of ADH and ALDH in damaged hepatocytes in the course of chronic hepatitis C (CHC) are reflected in the plasma. In the present study, we investigated the effect of liver cell inflammation in hepatitis C on the serum activity of alcohol dehydrogenase isoenzymes and aldehyde dehydrogenase.

Material and methods

Patients

The protocol was approved by the Human Care Committee of the Medical University in Białystok, Poland (approval no. R-I-002/572/2013). All patients gave informed consent for the examination.

Serum samples were taken for routine biochemical investigations from 60 patients suffering from viral hepatitis C (38 males and 22 females, mean age 44 years, range: 20–72 years) hospitalized in the Department of Infectious Diseases and Hepatology University Hospital, Medical University of Białystok (Poland). Hepatitis was histopathologically confirmed in each case, with HCV infection diagnosed by molecular biology techniques (quantitative RT-PCR for viral load determination) and sequence-specific RT-PCR with reverse hybrid-

ization for genotype. Disease activity grade and fibrosis stage were quantitatively scored according to the METAVIR classification (grade 1 was found in 21 patients, grade 2 in 15 patients, grade 3 in 12 patients, and grade 4 in 12 patients). For the analysis patients were divided into two groups: 44 patients with genotype 1b and 16 patients with genotype 3a. Patients were stratified according to baseline viral load (31 patients with $\leq 600,000$ IU/ml and 29 patients with $> 600,000$ IU/ml).

Patients were excluded if they had coinfection with human immunodeficiency virus or hepatitis B, or any other cause of liver disease. Alcohol consumption was assessed using a validated questionnaire. Before the examinations all patients had not consumed alcohol for almost 1 year.

The control group comprised 66 volunteers (36 men and 30 women, aged 25–70 years). The healthy controls were recruited from the same geographical location and ethnic populations as the patients and were not from the hospital. Control groups were selected from healthy community residents who attend the hospitals for routine physical check-ups at the Department of Preventive Medicine. Control subjects were volunteers and were defined as those with normal results of all physical and blood examinations. 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.

Biochemical assays

Determination of total ADH activity

Total ADH activity was estimated by the photometric method with p-nitrosodimethylaniline (NDMA) as a substrate [9]. The reaction mixture (2 ml) contained 0.1 ml of serum and 1.8 ml of a 26 μ M 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-naphthaldehyde to the fluorescent 6-methoxy-2-naphthoate [10]. The reaction mixture contained 60 μ l of serum, 60 μ l of substrate, 20 μ l of 11.4 mM NAD and 2.8 ml of 50 mM sodium phosphate buffer, pH 8.5. The mixture also contained 50 μ l of a 12 mM solution of 4-methylpyrazole as a specific inhibitor of ADH activity. The fluorescence was read at an excitation wavelength of 310 nm and emission wavelength of 360 nm.

Determination of class I and II ADH isoenzymes

Class I and II alcohol dehydrogenase isoenzyme activity was measured using fluorogenic substrates (4-methoxy-1-naphthaldehyde for class I and 6-methoxy-2-naphthaldehyde for class II) in a reduction reaction according to Wierchowski *et al.* [11]. The assays were performed in a reaction mixture containing serum (60 µl), substrate (150 µl of 300 µM), NADH (100 µl of 1 mM) and 0.1 M of sodium phosphate buffer, pH 7.6 (2.69 ml) in conditions previously described [12]. The measurements were performed on a Shimadzu RF-5301 spectrofluorophotometer (Shimadzu Europa GmbH, Duisburg, Germany) at an excitation wavelength of 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 alcohol dehydrogenase activity contained 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 9.6 [13]. The reduction of NAD was monitored at 340 nm and at 25°C on a Shimadzu UV/VIS 1202 spectrophotometer.

Determination of class IV ADH isoenzyme

The assay mixture for class IV alcohol dehydrogenase activity contained serum (50 µl), *m*-nitrobenzaldehyde as a substrate of (132 µl of 80 µM), NADH (172 µl of 86 µM) in 0.1 M sodium phosphate buffer, pH 7.5 [14, 15]. The oxidation of NADH was monitored at 340 nm and at 25°C on a Shimadzu UV/VIS 1202 spectrophotometer.

Statistical analysis

A preliminary statistical analysis (χ^2 test) revealed that the distribution of ADH and ALDH activities did not follow a normal distribution. Con-

sequently, Wilcoxon's test was used for statistical analysis. Data are presented as median, range and mean values. Statistically significant differences were defined as comparisons resulting in $p < 0.05$.

Results

The activities of alcohol dehydrogenase, aldehyde dehydrogenase and isoenzymes of alcohol dehydrogenase in the sera are presented in Table I. The total activity of alcohol dehydrogenase was significantly higher in patients with hepatitis C than in healthy subjects (about 72%). The median total activity of ADH was 1284 mU/l in patients, and 745 mU/l in controls. The analysis of ALDH activity did not reveal a significant difference between the tested group and healthy controls.

The comparison of ADH isoenzyme activities showed a large difference for class I and II ADH. The median activity of these classes of isoenzymes in the hepatitis C group increased by about 125% (4.24 vs. 1.88 mU/l) and 89% (26.63 vs. 14.11 mU/l) respectively in the comparison to the control level. This increase was statistically significant ($p < 0.001$). The other tested classes of ADH isoenzymes had higher activities in the serum of patients with hepatitis C, but the differences were not statistically significant in all patient groups ($p > 0.05$).

The analysis of ADH, ALDH and ADH isoenzyme activities in the serum did not reveal significant differences between patients with hepatitis C virus genotype 1b and 3a (Table II).

Patients with a high baseline viral load ($> 600\,000$ IU/ml) had a significantly higher total activity of alcohol dehydrogenase than patients with lower viraemia (Table III). Total ADH activity significantly differed between these groups and the control group. In contrast, the analysis of ALDH activity did not show a significant difference between patients with high viraemia, low viraemia and controls. Significantly higher class I ADH activity was found in the sera of patients with a high viral load (4.96 mU/l) in comparison to patients

Table I. Alcohol dehydrogenase and aldehyde dehydrogenase activity in sera of patients with hepatitis C

Tested group	ADH I	ADH II	ADH III	ADH IV	ADH total	ALDH total
	Median Range Mean	Median Range Mean	Median Range Mean	Median Range Mean	Median Range Mean	Median Range Mean
Hepatitis C (n = 60)	4.24 1.14–0.53 4.03	26.63 10.25–41.47 26.40	11.52 7.05–18.68 11.27	5.61 2.86–11.98 5.33	1284 392–2704 1233	3.18 1.62–6.85 2.99
Control (n = 66)	1.88 0.91–3.57 1.71	14.11 5.44–21.05 13.72	11.15 6.84–18.16 10.88	5.22 2.49–11.48 5.08	745 318–2246 716	2.93 1.46–6.2 2.84
	$p < 0.001^*$	$p < 0.001^*$	$p = 0.327$	$p = 0.473$	$p < 0.001^*$	$p = 0.527$

ADH – alcohol dehydrogenase, ALDH – aldehyde dehydrogenase. Data are expressed as mU/l; *statistically significant differences between groups, p – hepatitis C vs. control.

Table II. Alcohol dehydrogenase and aldehyde dehydrogenase activity in sera of patients with hepatitis C virus genotype 1b and 3a

Tested group	ADH I	ADH II	ADH III	ADH IV	ADH total	ALDH total
	Median Range Mean	Median Range Mean	Median Range Mean	Median Range Mean	Median Range Mean	Median Range Mean
Genotype 1b (n = 44)	4.31 1.32–6.53 4.12	26.86 10.62–41.47 26.61	11.77 7.41–18.68 11.38	5.74 2.97–11.98 5.53	1306 454–2704 1283	3.29 1.78–6.85 3.14
Genotype 3a (n = 16)	4.18 1.14–6.04 4.12	26.44 10.25–40.54 26.31	11.43 7.05–18.05 11.06	5.48 2.86–11.60 5.31	1275 392–2611 1248	3.07 1.62–6.22 2.81
	<i>p</i> = 0.448	<i>p</i> = 0.548	<i>p</i> = 0.385	<i>p</i> = 0.426	<i>p</i> = 0.404	<i>p</i> = 0.545

ADH – alcohol dehydrogenase, ALDH – aldehyde dehydrogenase. Data are expressed as mU/l; *p* – genotype 1b vs. genotype 3a.

Table III. Alcohol dehydrogenase and aldehyde dehydrogenase activity in sera of patients with hepatitis C depending on viral load

Tested group	ADH I	ADH II	ADH III	ADH IV	ADH total	ALDH total
	Median Range Mean	Median Range Mean	Median Range Mean	Median Range Mean	Median Range Mean	Median Range Mean
Viral load > 600 000 IU/ml (n = 29)	4.96 1.65–6.53 4.68	27.08 10.96–41.47 26.77	11.88 7.46–18.68 11.60	5.79 2.92–11.98 5.68	1833 675–2704 1746	3.35 1.83–6.85 3.16
Viral load ≤ 600 000 IU/ml (n = 31)	3.81 1.14–6.08 3.72	26.35 10.25–41.01 26.416	11.36 7.05–18.11 11.14	5.50 2.86–11.35 5.28	1105 392–2466 1044	3.11 1.62–6.40 2.85
Control (n = 66)	1.88 0.91–3.57 1.71	14.11 5.44–21.05 13.72	11.15 6.84–18.16 10.88	5.22 2.49–11.48 5.08	745 318–2246 716	2.93 1.46–6.2 2.84
	<i>p</i> ^a < 0.001* <i>p</i> ^b < 0.001* <i>p</i> ^c < 0.001*	<i>p</i> ^a < 0.001* <i>p</i> ^b < 0.001* <i>p</i> ^c = 0.285	<i>p</i> ^a = 0.513 <i>p</i> ^b = 0.365 <i>p</i> ^c = 0.416	<i>p</i> ^a = 0.438 <i>p</i> ^b = 0.387 <i>p</i> ^c = 0.422	<i>p</i> ^a < 0.001* <i>p</i> ^b < 0.001* <i>p</i> ^c < 0.001*	<i>p</i> ^a = 0.512 <i>p</i> ^b = 0.546 <i>p</i> ^c = 0.453

ADH – alcohol dehydrogenase, ALDH – aldehyde dehydrogenase. Data are expressed as mU/l. *Statistically significant differences between groups, *p*^a – viral load > 600 000 IU/ml vs. control, *p*^b – viral load ≤ 600 000 IU/ml vs. control, *p*^c – viral load > 600 000 IU/ml vs. viral load ≤ 600 000 IU/ml.

with a low load (3.81 mU/l). The ADH I and ADH II activity was significantly higher regardless of the viraemia compared with the control. The analysis of ADH III and IV activity did not show a significant difference between patients with high viraemia, low viraemia and control.

Having analyzed activity of particular ADH isoenzymes depending on the progression stage of the fibrosis (Table IV) significantly higher the ADH class I and II activities were found in the hepatitis C patients regardless of stage in comparison to the control group (*p* < 0.05). Significantly higher ADH class I and II activities were found in the hepatitis C patients regardless of stage in comparison to the control group (*p* < 0.05). The other isoenzymes did not exhibit any marked changes of activity among patients at various advancing stages of fibrosis. The serum level of total ADH activity was significantly higher in the tested group (each stage) than the control group. ALDH shows no significant difference in activity depending on the degree of liver damage.

Discussion

In the last few years, considerable progress has been made in the knowledge of epidemiology and factors influencing the course of hepatitis C. Still, important efforts are needed for screening for early diagnosis in order to improve the treatment of patients with chronic hepatitis C [1]. Diagnostics of CHC in the early stage is usually difficult as the disease is normally asymptomatic. The majority of HCV-infected patients have no hepatic symptoms, whereas the extrahepatic manifestations may be the reason that they seek medical care and thus have a chance to be investigated, diagnosed and finally properly treated [16]. Therefore it is very important to find markers that detect hepatocellular damage as soon as possible, and for a long time there have been attempts to achieve this. Some authors have studied various substances as markers of hepatitis C. For example, Ciesla *et al.* reported that Met-enkephalin concentration measurement in the liver tissue seems to be a useful method for differentiation of stage 2 from stages 3 and 4

Table IV. Alcohol dehydrogenase and aldehyde dehydrogenase activity in sera of patients with hepatitis C depending on fibrosis stage

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
Grade 1 (n = 21)	4.01 1.14–5.74 3.92	26.13 10.25–39.84 25.77	11.28 7.05–18.02 11.01	5.39 2.86–11.17 5.05	1204 392–2526 1178	3.02 1.62–6.25 2.84
Grade 2 (n = 15)	4.22 1.55–6.08 4.16	26.39 10.84–40.08 26.18	11.46 7.48–18.30 11.22	5.52 3.09–11.46 5.44	1256 467–2612 1240	3.15 1.81–6.64 3.06
Grade 3 (n = 12)	4.35 1.82–6.41 4.28	26.65 11.21–41.13 26.47	11.60 7.66–18.47 11.46	5.75 3.40–11.69 5.62	1291 488–2674 1265	3.33 1.90–6.68 3.20
Grade 4 (n = 12)	4.46 1.95–6.53 4.37	26.89 11.66–41.47 26.74	11.81 8.12–18.68 11.76	5.83 3.99–11.98 5.71	1315 544–2704 1296	3.36 1.94–6.85 3.28
Control (n = 66)	1.88 0.91–3.57 1.71	14.11 5.44–21.05 13.72	11.15 6.84–18.16 10.88	5.22 2.49–11.48 5.08	745 318–2246 716	2.93 1.46–6.2 2.84
	$p^a < 0.001^*$ $p^b < 0.001^*$ $p^c < 0.001^*$ $p^d < 0.001^*$	$p^a < 0.001^*$ $p^b < 0.001^*$ $p^c < 0.001^*$ $p^d < 0.001^*$	$p^a = 0.426$ $p^b = 0.387$ $p^c = 0.312$ $p^d = 0.285$	$p^a = 0.417$ $p^b = 0.343$ $p^c = 0.465$ $p^d = 0.335$	$p^a < 0.001^*$ $p^b < 0.001^*$ $p^c < 0.001^*$ $p^d < 0.001^*$	$p^a = 0.558$ $p^b = 0.514$ $p^c = 0.443$ $p^d = 0.422$

ADH – alcohol dehydrogenase, ALDH – aldehyde dehydrogenase. Data are expressed as mU/l, *statistically significant differences between groups, p^a – stage 1 vs. control, p^b – stage 2 vs. control, p^c – stage 3 vs. control, p^d – stage 4 vs. control.

of severe liver fibrosis [17]. The changes in enzyme activity in the damaged hepatocytes during the course of different liver diseases are reflected by an increase in the corresponding enzyme activity in the plasma. In this study we found that the serum total alcohol dehydrogenase activity changed in the course of hepatitis C. The increase of total ADH activity was positively correlated with ADH I and ADH II, so the cause of the increase of total ADH in the course of this disease is an elevation of class I and II 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. Class II ADH is detected only in the hepatocytes. So, the elevated activity of ADH I and ADH II seems to be caused by the isoenzymes released from inflamed altered liver cells. The changes in activities of other ADH isoenzymes were not significant in the serum of patients with hepatitis C. Aldehyde dehydrogenase is present in the liver, although the activity of ALDH seems to be disproportionately low compared to ADH activity. The serum levels of aldehyde dehydrogenase were not significantly higher in patients with HCV infection in comparison to the healthy group. The reason for this difference in expression of ADH and ALDH in CHC may be disproportionately low ALDH activity in the liver compared to the activity of ADH and ALDH with subcellular localization in the mitochondria. The half-lives of ADH and ALDH in the serum are about 26 and 22 h respectively [18].

A very interesting issue is the possible influence of viral factors such as genotype and baseline vi-

ral load on the activity of enzymes in the serum. In eastern and southern Europe, mainly type 1b occurs. In Poland, according to a study in groups of adult patients and asymptomatic carriers, the most commonly detected genotype is 1b, 3a is detected rarely, and other genotypes of the virus are detected in individual cases [19]. In this study the genotype of the virus did not have an effect on the activity of tested enzymes. We found that the activity of ADH and ALDH in serum of patients with genotype 1b did not differ from that of patients with genotype 3a, while the viral load had a significant effect on the activity of alcohol dehydrogenase. High viraemia is defined differently by various authors, with the most widely applied consensus being a plasma virus load of > 600 000 IU/ml [20, 21]. In our study we also adopted this level of viral load as the cut-off point. In our analysis, we found that the baseline viral load significantly affects the ADH I activity and total activity of alcohol dehydrogenase. ADH II activity did not change depending on the level of viraemia, although it was significantly higher in patients with HCV infection compared to the control group. Further multiplication of the virus does not cause an increase in the activity of this isoenzyme. This may indicate that ADH II is released faster and earlier than class I. Moreover, HCV is not only a hepatotropic pathogen; thus in the serum of patients with a high viral load ADH I may be derived from other organs [22]. Another explanation may be the fact that viruses can stimulate modification of occurring proteins

or production of completely new enzymatic proteins similar to ADH I [23].

All noxious agents that chronically act on the liver and damage hepatic cells may eventually cause chronic hepatitis, which may in turn lead to progression of fibrosis. HCV infection is also a cause of progressive fibrosis of liver tissue. Viral hepatitis C accompanied by cellular injury and necrosis leads to the release of cytoplasmic enzymes such as ADH. This study showed that the activity of class I and II alcohol dehydrogenase in CHC increased in parallel with the development of the disease. We have observed a tendency towards an increase of the total ADH activity together with the progression of the hepatitis C. The other markers of liver lesions (bilirubin concentration, activities of aminotransferases) were also significantly elevated. The median concentration of total bilirubin was 3.95 mg/dl in patients, and 0.65 mg/dl in controls. The median activity of alanine transaminase and aspartate transaminase was respectively 360 U/l and 610 U/l in the serum of patients with hepatitis C and 26 U/l and 20 U/l in healthy controls. The activity of both transaminases and alcohol dehydrogenase correlate well with the severity of the conditions, and regular monitoring can be a diagnostic marker of hepatic cell damage during chronic hepatitis C.

The results in the present paper are similar to other studies performed in various liver diseases. For example, Chrostek and Szmittkowski found much higher ADH I and II isoenzyme activity in the sera of patients with acute viral hepatitis B [3]. They also observed that only serum activity of class I alcohol dehydrogenase was significantly higher in chronic hepatitis B than in controls [24]. 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 [4, 25]. In our previous study we found that activity of class I alcohol dehydrogenase isoenzymes and total ADH activity was elevated in the serum of patients with metastatic liver cancer. ADH I was significantly higher in the sera of patients with metastatic tumours than in those with primary cancers [26].

In conclusion, our data indicate that the increase of the activity of class I and II alcohol dehydrogenase isoenzyme in the sera of patients with HCV infection seems to be caused by the release of this isoenzyme from damaged liver cells.

Conflict of interest

The authors declare no conflict of interest.

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