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Serum Class I and II Alcohol Dehydrogenase Activity During the Course of Viral Hepatitis

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Summary: We examined the activities of class I and II alcohol dehydrogenase isoenzymes in the sera of patients with viral hepatitis using the fluorogenic substrates 4-methoxy-1-naphthaldehyde for class I and 6-methoxy-2-naphthaldehyde for class II. It was found that serum activities of class I and II alcohol dehydrogenase isoenzymes over the course of five weeks of hospitalisation were higher than those of controls. The greatest increase in activities was found at the onset of disease, exceeding the mean control value by about 30 fold for class I and 4 fold for class II. These activities were lower than that of aminotransferase but higher than those of lactate dehydrogenase, alkaline phosphatase and γ-glutamyltransferase. Thereafter, the activity of alcohol dehydrogenase isoenzymes gradually decreased, but did not reach the values of the control groups in the last period of the study. Activities of class I and II alcohol dehydrogenase isoenzymes correlated well with those of alanine and aspartate aminotransferases and lactate dehydrogenase in the first weeks of illness. These results clearly demonstrate that especially the activity of class I alcohol dehydrogenase isoenzyme measured by a fluorimetric method can be a useful marker of liver cell damage in the course of viral hepatitis.

Introduction

Human alcohol dehydrogenase[1]) (alcohol : NAD^+ oxidoreductase, EC 1.1.1.1) exists in multiple molecular forms that have been grouped into four classes (1). Alcohol dehydrogenase classes exhibit important differences in both substrate specificity and tissue distribution. Isoenzymes of class I exist mainly in the liver (up to 95% of total activity in this organ) (2), and have been termed "classical" alcohol dehydrogenase. Class II in humans is found only in the liver (3), whereas class III is present in all tissues examined (4). The newly discovered gastric alcohol dehydrogenase has been termed class IV (5).

The liver alcohol dehydrogenase isoenzymes have a broad substrate specificity that includes a variety of alcohols and aldehydes of both aliphatic and aromatic character, such as primary and secondary alcohols, diols, and aromatic alcohols (6, 7), some hydroxy and keto steroids (8, 9), ω-hydroxy fatty acids, retinoids and cytotoxic aldehydes generated in lipid peroxidation (10).

Two fluorogenic substrates for human alcohol dehydrogenase were described by *Wierzchowski* et al. (11), and classes I and II of this enzyme exhibit different preferences for these substrates. Class I alcohol dehydrogenase can be easily detected by the reduction of 4-methoxy-1-naphthaldehyde to the strongly fluorescent 4-methoxy-1-naphthalenemethanol, and class II by the reduction of 6-methoxy-2-naphthaldehyde to 6-methoxy-2-naphthalenemethanol. Fluorometric assays of alcohol dehydrogenase isoenzyme activities are more sensitive and specific than the previously used classical method based on NADH absorbance and measurement of the total activity of this enzyme (12).

[1]) Enzymes:
Alcohol dehydrogenase (EC 1.1.1.1)
Alanine aminotransferase (EC 2.6.1.2.)
Aspartate aminotransferase (EC 2.6.1.1)
Alkaline phosphatase (EC 3.1.3.2)
γ-Glutamyltransferase (EC 2.3.2.2)
Lactate dehydrogenase (EC 1.1.1.27)

This fluorimetric method permits the investigation of alcohol dehydrogenase isoenzyme activity especially in liver cells damaged by cytotoxic or cholestatic agents. In this study, we have investigated the activities of class I and II isoenzymes of alcohol dehydrogenase in the sera of patients with viral hepatitis. These results were also compared with total alcohol dehydrogenase activity and the activities of enzymes which are commonly accepted as liver cell injury markers.

Patients and Methods

Patients

Serum of blood from 77 patients (men − 38, women − 39, aged − 17−81 years) suffering from viral hepatitis B were collected at the onset of disease in the first week of hospitalisation (sample I) and 4 times thereafter at intervals of 7 to 9 days (sample II, III, IV and V). Clinical diagnosis of illness was made on the basis of serological examinations (HBsAg, anti-HBs and Anti-HBc). Serum samples were also obtained from 59 healthy adults aged 20−60 years (men − 32, women − 27).

Methods

Fluorimetric assays

Reaction mixture (3 ml) contained 150 µl of 300 µmol/l solution of 4-methoxy-1-naphthaldehyde (substrate for class I) (Aldrich Chemicals Company, Inc. Milwaukee, WI) or 6-methoxy-2-naphthaldehyde (substrate for class II synthesised by Dr. *J. Wierzchowski*, Department of Physical Chemistry, Medical School, Warsaw, Poland), 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. After equilibration for 5 min, the reaction was initiated by the addition of 60 µl of serum. The changes in fluorescence were recorded for up to 10 min on an Aminco Bowman spectrofluorimeter (excitation wavelength 316 nm, emission wavelength 370 nm for class I and 360 nm for class II). Subsequently, 60 µl of 200 µmol/l 4-methoxy-1-naphthalenemethanol (for measurement of class I) or 6-methoxy-2-naphthalenemethanol (for measurement of class II), synthesised by Dr. *J. Wierzchowski* was added to provide an internal standard and its fluorescence intensity was then measured. For the evaluation of alcohol dehydrogenase activity, two assays were carried out: one with substrate alone and the second with substrate and 50 µl of a 12 mmol/l solution of 4-methylpyrazole as a specific inhibitor of alcohol dehydrogenase activity (Aldrich Chemicals Company, Inc. Milwaukee, WI). The rate of reaction (initial velocities) was calculated as mU/l (11).

Spectrophotometric assays

Serum total alcohol dehydrogenase activity was estimated at pH 9.2 (13) according to the method of *Bonnichsen & Brink* (14) using the Technicon RA-1000 analyzer. The enzyme was assayed at 340 nm and 37 °C for 3 minutes in the reaction mixture (362 µl) which contained 18 µl of serum and 344 µl of 0.1 mol/l glycine buffer. In this volume the final NAD (Sigma Diagnostics, St. Louis, MO) and ethanol (Aldrich Chemicals Company, Inc. Milwaukee, WI) concentrations were 0.44 mmol/l and 28 mmol/l, respectively. The control tubes contained the reaction mixture without alcohol. Using the specific inhibitor of alcohol dehydrogenase 4-methylpyrazole (Sigma Diagnostics, St. Louis, MO) at a final concentration of 12 mmol/l, non-specific activity was subtracted.

Determination of other enzymes

The activity of alanine and aspartate aminotransferases, lactate dehydrogenase, γ-glutamyltransferase and alkaline phosphatase were measured in an Express Plus biochemical analyzer using diagnostic kits from bioMerieux.

Statistical analysis

Statistical analysis was performed using the *Wilcoxon*'s test for paired samples and *Pearson*'s correlation coefficient. The mean value and standard deviation (SD) of results were calculated for all tested groups and samples.

Results

As presented in figure 1, we found that the serum activity of class I alcohol dehydrogenase isoenzymes was elevated in each period of the course of viral hepatitis, and was highest at the onset of disease (sample I, 14.59 ± 15.88 mU/l), exceeding by 30 fold the mean value of the control group. After some time, this isoenzyme activity gradually decreased and in the following samples reached the following values: 10.11 ± 10.33 mU/l, 4.79 ± 5.35 mU/l, 4.42 ± 4.52 mU/l and 4.78 ± 3.69 mU/l. However, in the last period of examination, the activities of class I alcohol dehydrogenase isoenzymes did not return to the values of the control group. The multiples of the mean control value were about 19, 9, 8 and 9 for samples II, III, IV and V, respectively. These were higher than the multiple of lactate dehydrogenase[1]) (mean 1.13), alkaline phosphatase[1]) (2.55) and γ-glutamyltransferase[1]) (8.29) but significantly lower than aspartate[1]) (20.7) and alanine aminotransferases[1]) (16.76) (tab. 1). During the whole observation period we observed statistically significant differences in activities of class I alcohol dehydrogenase isoenzymes between the patients and the control group.

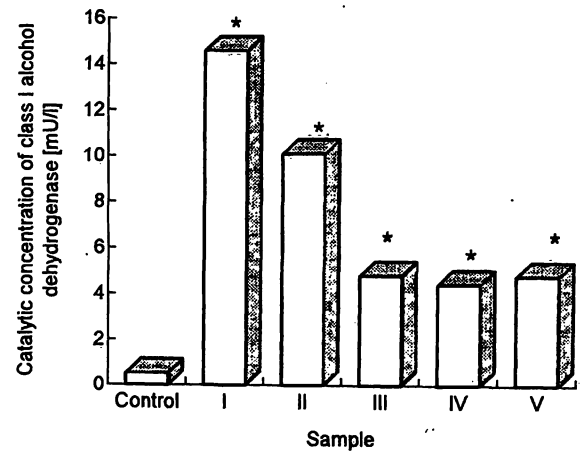

Fig. 1 Catalytic concentration of class I alcohol dehydrogenase in the sera of patients with viral hepatitis.
I−V = samples in the course of viral hepatitis
* $p < 0.001$, compared with control

Tab. 1 The activity of liver injury enzymatic markers in the course of viral hepatitis.

Group	Alanine aminotransferase U/l	Aspartate aminotransferase U/l	Lactate dehydrogenase U/l	γ-Glutamyl-transferase U/l	Alkaline phosphatase U/l
Control	18 ± 7	16 ± 5	172 ± 31	17 ± 9	23 ± 7
Sample I	627 ± 407	725 ± 522	254 ± 118	145 ± 118	58 ± 24
Sample II	390 ± 347	445 ± 464	209 ± 102	131 ± 107	55 ± 27
Sample III	239 ± 226	234 ± 257	193 ± 85	133 ± 131	51 ± 28
Sample IV	144 ± 146	158 ± 168	183 ± 87	139 ± 103	52 ± 25
Sample V	110 ± 131	97 ± 114	138 ± 43	157 ± 153	78 ± 63

Tab. 2 Correlation coefficient between activity of alcohol dehydrogenase isoenzymes and of other enzymes.

Alcohol dehydrogenase		Alanine aminotrans-ferase		Aspartate aminotrans-ferase		Lactate dehydrogenase		γ-Glutamyl-transferase		Alkaline phosphatase		Total alcohol dehydro-genase	
Isoenzymes	week	r	α	r	α	r	α	r	α	r	α	r	α
Class I	I	0.40	0.00*	0.37	0.00*	0.31	0.00*	−0.08	0.46	0.02	0.86	−0.09	0.42
	II	0.41	0.00*	0.26	0.03*	0.07	0.55	−0.03	0.82	0.14	0.23	−0.12	0.30
	III	0.34	0.01*	0.38	0.00*	0.16	0.24	−0.08	0.57	0.30	0.29	−0.00	0.97
	IV	0.18	0.33	0.20	0.27	−0.28	0.13	0.18	0.34	−0.00	0.96	−0.08	0.67
	V	0.01	0.96	0.04	0.89	−0.04	0.89	−0.01	0.97	−0.14	0.63	0.12	0.70
Class II	I	0.42	0.00*	0.52	0.00*	0.59	0.00*	−0.03	0.79	−0.06	0.59	0.33	0.00*
	II	0.35	0.00*	0.33	0.00*	0.30	0.01*	−0.03	0.83	0.14	0.23	−0.00	0.99
	III	0.03	0.81	0.19	0.17	0.12	0.39	−0.12	0.38	0.04	0.77	−0.11	0.45
	IV	0.25	0.17	0.13	0.49	−0.21	0.27	−0.22	0.24	−0.17	0.37	−0.36	0.05
	V	0.64	0.02*	0.54	0.05	0.20	0.50	−0.43	0.14	−0.14	0.65	0.24	0.43

* linear dependence

The activities of class I alcohol dehydrogenase isoenzymes correlated with those of alanine and aspartate aminotransferases in the first period of the disease (sample I, II and III), but did not correlate with markers of cholestasis such as γ-glutamyltransferase and alkaline phosphatase (tab. 2).

The activities of class II alcohol dehydrogenase isoenzymes were also significantly increased during the course of viral hepatitis (fig. 2). The highest level was observed at the beginning of the study (sample I) and was evaluated at 46.85 ± 36.73 mU/l, about 4 times above the mean control value. In the second period (sample II), activity of class II isoenzymes decreased to 38.73 ± 29.29 mU/l and was almost at the same level in sample IV (34.16 ± 29.39 mU/l). The multiples of mean control value in these three samples were about 2−3 times. At the end of the investigation (sample V), the activity of class II alcohol dehydrogenase isoenzymes slightly decreased, reaching a mean value of 22.05 ± 16.85 mU/l, which was only 83% higher than the control value.

In comparison to other enzyme activities, it was found that the multiples of class II alcohol dehydrogenase isoenzyme activity were similar to those of alkaline phosphatase (2.5 times), lower than the multiple of γ-glutamyltransferase and aminotransferases but higher than those of lactate dehydrogenase (1.5 times) (tab. 1).

The correlation study (tab. 2) showed a good correlation coefficient between the activities of class II alcohol de-

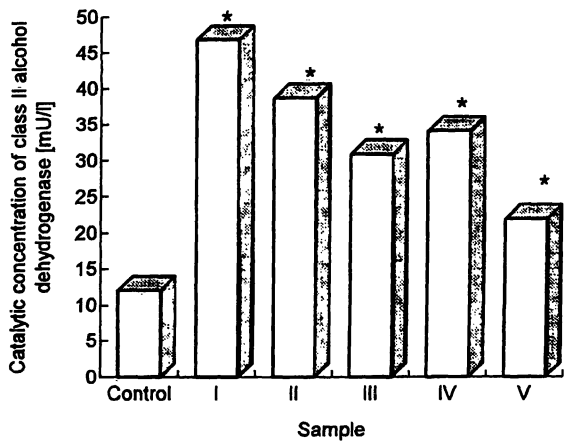

Fig. 2 Catalytic concentration of class II alcohol dehydrogenase in the sera of patients with viral hepatitis.
I−V = samples in the course of viral hepatitis
* $p < 0.05$, compared with control

hydrogenase isoenzymes and alanine aminotransferase, aspartate aminotransferase and lactate dehydrogenase in the first two samples, and in the last period of the study.

The total alcohol dehydrogenase activity measured at pH 9.2 was higher than that of the controls (fig. 3). The greatest increase of activity was noted in the second sample (60% in comparison to mean control value), and subsequently the activity gradually decreased but did not reach the level of the control group. Total alcohol dehydrogenase activity measured by a spectrophotometric method correlated only with the activity of class II isoenzymes in the first week of hospitalisation.

Discussion

Viral hepatitis accompanied by cellular injury and necrosis leads to the release of cytoplasmic enzymes such as aminotransferases. The activities of these enzymes correlate well with the severity of the condition and their regular monitoring is a fundamental diagnostic marker of liver cell damage during this disease (15).

In our study we report that the serum alcohol dehydrogenase isoenzyme activity during viral hepatitis was similar to the activity of classical aminotransferases. Thus, the greatest increase of both alcohol dehydrogenase isoenzymes and aminotransferases activities was found at the onset of disease. Aminotransferase activities were greatly elevated and exceeded the mean value of the control group by about 40 fold. The next position was reserved for isoenzymes of class I of alcohol dehydrogenase activity, the mean value at the onset of disease was more than 30 times higher than the control value. Thereafter, we found the multiples of γ-glutamyltransferase activity (8.5), class II alcohol dehydrogenase (3.9), alkaline phosphatase (2.5), total alcohol dehydrogenase activity (1.4) and lactate dehydrogenase (1.5).

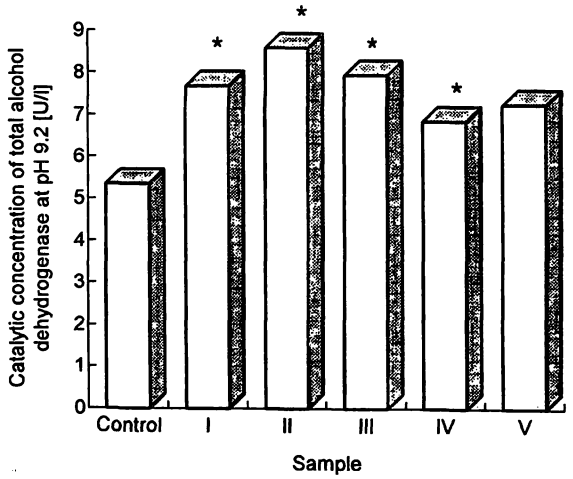

Fig. 3 Catalytic concentration of total alcohol dehydrogenase measured by spectrophotometric method in the sera of patients with viral hepatitis.
I–V = samples in the course of viral hepatitis
* $p < 0.05$, compared with control

Schmidt et al. showed that β-glutamyltransferase appears to be an extremely sensitive marker (sensitivity 95%) of liver dysfunction (16). According to these authors, other enzymes are less sensitive but are often more specific for a particular hepatic syndrome. It is known that class I and especially class II alcohol dehydrogenase isoenzymes are the most specific of all tested enzmyes because serum activity of class II isoenzyme is derived only from liver cells (3), and hepatic class I isoenzymes represent 80–95% of total activity of this class in humans (2).

This study showed that the activity of class I alcohol dehydrogenase isoenzymes in viral hepatitis increased in parallel with the development of the disease, as was the case for aminotransferases but not γ-glutamyltransferase, which was slightly altered during the illness. In the course of the disease the activity of class I alcohol dehydrogenase isoenzymes quickly decreased as did aminotransferase activities. In contrast the activity of class II alcohol dehydrogenase isoenzymes slowly decreased as did that of other enzymes, especially γ-glutamyltransferase and alkaline phosphatase, although this was not confirmed in the correlation study. These lower multiples of increase for class II alcohol dehydrogenase than class I may be explained by the fact that this class is present as ππ homodimers while class I isoenzymes are heterodimers consisting of α, β and γ subunits.

Mezey et al. reported that elevation of serum alcohol dehydrogenase activity occurred in acute parenchymal liver cell damage caused by viruses (17), and the level of enzyme activity early in the course of hepatitis generally paralleled that of serum aspartate aminotransferase. In our study we noted linear dependence of both classes of alcohol dehydrogenase with alanine and aspartate aminotransferases in the early phase of the disesae. This linearity disappeared after two or three weeks for class I and II, respectively, and was noted once again between class I alcohol dehydrogenase and alanine aminotransferase after five weeks of hospitalisation.

Because aminotransferases are present in all tissues, *Mezey* et al. (17) suggested that alcohol dehydrogenase is similar to enzymes such as ornithine carbamyl transferase and sorbitol dehydrogenase, which are liver specific and their elevated serum levels differentiate acute liver injury from acute damage to other organs.

From the data presented in the tables, it is clear that alcohol dehydrogenase activity detected by a spectrophotometric method generally did not correlate with the activity of class I and II isoenymes, which in turn correlated with the activity of aminotransferases but not with

that of γ-glutamyltransferase. These results indicate that the activities of γ-glutamyltransferase and aminotransferases did not correlate in this study. This was confirmed by us (in unreported data).

In conclusion, the activity of class I and II alcohol dehydrogenase isoenzymes measured by a fluorimetric method with a high specific substrate is a specific biochemical marker of liver cell injury during viral hepatitis, comparable to the activities of alanine and asparatate aminotransferases and much better than those of enzymes such as γ-glutamyltransferase, alkaline phosphatase and lactate dehydrogenase. Class II alcohol dehydrogenase isoenzymes, although only hepatic, yielded a much lower multiple of increase in comparison with class I but was similar to other tested enzymes with the exception of aminotransferases.

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