Experimental and Molecular Pathology 91 (2011) 631–635

Contents lists available at ScienceDirect

Experimental and Molecular Pathology

journal homepage: www.elsevier.com/locate/yexmp

Alcohol dehydrogenase (ADH) isoenzymes and aldehyde dehydrogenase (ALDH) activity in the sera of patients with acute and chronic pancreatitis

Wojciech Jelski [a,*], Emilia Kutylowska [b], Magdalena Laniewska-Dunaj [a], Karolina Orywal [a], Wiktor Laszewicz [c], Maciej Szmitkowski [a]

[a] Department of Biochemical Diagnostics, Medical University, Bialystok, Poland
[b] Analytical Laboratory, General Hospital of Wysokie Mazowieckie, Wysokie Mazowieckie, Poland
[c] Department of Medicine and Gastroenterology, Regional Hospital of Bialystok, Bialystok, Poland

ARTICLE INFO

Article history:
Received 8 March 2011
and in revised form 12 June 2011
Available online 23 July 2011

Keywords:
Alcohol dehydrogenase isoenzymes
Aldehyde dehydrogenase
Acute pancreatitis
Chronic pancreatitis

ABSTRACT

Objective: Acute and chronic pancreatitis is a major complication of alcohol abuse. The pancreas can metabolize ethanol via oxidative pathway involving the enzymes — alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) as well as the nonoxidative pathway. Human pancreas tissue contains various ADH isoenzymes and possesses also ALDH activity. In this paper we have measured the activity of alcohol dehydrogenase isoenzymes, and aldehyde dehydrogenase in the sera of patients with acute and chronic pancreatitis.

Methods: Serum samples were taken for routine biochemical investigation from 46 patients suffering from acute pancreatitis and 32 patients with chronic pancreatitis. Total ADH activity was measured by photometric method with p-nitrosodimethylaniline (NDMA) as a substrate and ALDH activity by the fluorometric method with 6-methoxy-2-naphtaldehyde as a substrate. For the measurement of the activity of class I isoenzymes we employed the fluorometric methods, with class-specific fluorogenic substrates. The activity of class III alcohol dehydrogenase was measured by the photometric method with n-octanol and class IV with m-nitrobenzaldehyde as a substrate.

Results: A statistically significant increase of class III alcohol dehydrogenase isoenzymes was found in the sera of patients with acute and chronic pancreatitis. The median activity of this class isoenzyme in the patients group increased about 35% in the comparison to the control level. The total alcohol dehydrogenase activity was also significantly higher (23.5%) among patients with pancreatitis than healthy ones. The activities of other tested ADH isoenzymes and total ALDH were unchanged. The activity of the class I ADH isoenzyme was significantly higher in the sera of heavy drinkers with pancreatitis.

Conclusion: We can state that the increase of the activity of class III alcohol dehydrogenase isoenzyme in the sera of pancreatitis patients seems to be caused by the release of this isoenzyme from damaged pancreatic cells.

© 2011 Elsevier Inc. All rights reserved.

Introduction

Acute and chronic pancreatitis are diseases of heterogenous etiology. Alcohol abuse is the major cause of inflammation of the pancreas but the mechanism by which ethanol and other etiologic factors cause destructive changes in the pancreas are unknown (Pandol and Raraty, 2007). The risk of developing pancreatitis increases with increasing doses of alcohol, suggesting that alcohol exerts dose-related toxic effects on the pancreas. The pancreas can metabolize ethanol by means of oxidative and nonoxidative pathways. The major oxidative enzyme system uses alcohol dehydrogenase (ADH) (Gukovskaya et al., 2002). Human ADH exists in multiple molecular forms grouped into several classes (Riveros-Rosas et al., 1997). The comparison of the ADH isoenzyme activities in the pancreas showed that four classes of isoenzymes exist. Among the ADH isoenzymes the highest activity in the pancreas is represented by isoenzymes of class III (Chrostek et al., 2003; Haber et al., 1998). The activity of ADH I was very low and activities of class II and IV isoenzymes were barely detectable (Chrostek et al., 2003). The pancreas possesses also aldehyde dehydrogenase (ALDH) activity, which catalyzes the oxidation of acetaldehyde to acetic acid. In our previous study we have shown that the activity of class III isoenzyme was significantly higher in pancreatic cancer cells than in healthy tissue (Jelski et al., 2007). We have found also that the serum total alcohol dehydrogenase activity is changed in the course of pancreatic cancer (Jelski et al., 2008).

We hypothesized that the changed activities of ADH and ALDH in damaged pancreatic cells in the course of pancreatitis are reflected in the serum. In the present study, we have investigated the effect of

* Correspoding author at: Department of Biochemical Diagnostics, Medical University, Waszyngtona 15 A, 15-269 Bialystok, Poland. Fax: +48 85 7450 585.
E-mail address: wjelski@umwb.edu.pl (W. Jelski).

0014-4800/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.yexmp.2011.06.008

pancreas cell inflammation in acute and chronic pancreatitis on the serum activity of alcohol dehydrogenase isoenzymes and aldehyde dehydrogenase.

Materials and methods

Patients

The protocol was approved by the Human Care Committee of the Medical University in Bialystok, Poland (Approval Nr R-I-002/24/2010). All patients gave informed consent for the examination.

Serum samples were taken for routine biochemical investigations from 46 patients suffering from acute pancreatitis (33 males and 13 females, mean age 44 years, range 36–65 years) and 32 patients with chronic pancreatitis (22 males and 10 females, mean age 50 years, range 41–68 years) hospitalized in the Department of Medicine and Gastroenterology, Regional Hospital of Bialystok. The group with acute pancreatitis included 32 patients with mean alcohol intake greater than 20 g/day (26 men) and 10 g/day (6 women). This group included also 14 patients (7 men and 7 women) who drank alcohol moderately (1 drink/day 5–6 days/week). The group of patients with chronic pancreatitis included 20 heavy drinkers (16 men, 4 women) and 12 moderate drinkers (6 men and 6 women). Serum samples were also obtained from 80 healthy subjects (control group, 55 males, 25 females, aged 35–65 years). The control group included 45 heavy drinkers (35 men, 10 women) and 35 moderate drinkers (20 men and 15 women). Control groups were selected from healthy community residents who attend the hospitals for routine physical check-ups at the Department of Preventive Medicine and heavy drinkers were recruited from patients admitted to the hospital for alcohol detoxification. One and all were volunteers and were defined as those with normal results of all physical, blood examinations and computerized tomography (CT) of the abdomen. Before the examinations control group had not consumed alcohol for almost 1 month and ethanol did not exist in serum samples of any subject when it was collected.

Biochemical assays

Determination of total ADH activity

Total ADH activity was estimated by the photometric method with p-nitrosodimethylaniline (NDMA) as a substrate (Skursky et al., 1979). The reaction mixture (2 ml) contained 1.9 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 (Jelski et al., 2004). The reaction mixture contained 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

Fluorometric assays of alcohol dehydrogenase isoenzyme activities (class I and II) are more sensitive and specific than the previously used classical method. 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 reduction reaction according to Wierzchowski et al., 1989. 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 of sodium phosphate buffer, pH 7.6 (2.69 ml) in conditions previously described (Jelski et al., 2002). The measurements were performed on a Shimadzu RF–5301 spectrofluorophotometer (Shimadzu Europa GmbH, Duisburg, Germany) at excitation wavelenght 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 (Koivusalo et al., 1989). 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 of (132 μl of 80 μM), NADH (172 μl of 86 μM) in 0.1 M sodium phosphate buffer pH 7.5 (Dohmen et al., 1996). 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's test was used for statistical analysis. Data were 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 reported in Table 1. The total activity of alcohol dehydrogenase was significantly higher in patients with acute pancreatitis and chronic pancreatitis than in healthy subjects (25 and 22% respectively). The median total activity of ADH was 695 mU/l in patients with acute pancreatitis, 662 mU/l in chronic disease and 522 mU/l in control group. The analysis of ALDH activity did not indicate significant difference between tested groups and healthy controls. The comparison of ADH isoenzymes activities showed that the high difference was exhibited by class III ADH. The median activity of this class isoenzyme in the acute pancreatitis and chronic pancreatitis group increased respectively about 38% (14.77 mU/l) and 32% (14.04 mU/l) in the comparison to the control level (11.65 mU/l). This increase was statistically significant ($p<0.001$). The other tested classes of ADH isoenzymes had higher activities in the serum of patients with pancreatitis, but the differences were not statistically significant in all patient groups ($p>0.05$).

The analysis of ADH, ALDH and ADH isoenzymes activities in the serum did not indicate significant differences between patients with acute pancreatitis and chronic pancreatitis.

The total activity of alcohol dehydrogenase significantly differs when comparing heavy drinking or moderately drinking patients with control group (Table 2). The activities of total ADH differed significantly (by 22%) for heavy drinkers with pancreatitis (acute and chronic) in comparison to moderate drinkers group. In contrast, the analysis of ALDH activity did not show a significant difference between heavy drinkers and moderately drinking patients. The activity of class III alcohol dehydrogenase isoenzyme significantly differs when comparing heavy drinkers or moderately drinking patients with suitable control group.

Download English Version:

https://daneshyari.com/en/article/2775264

Download Persian Version:

https://daneshyari.com/article/2775264

Daneshyari.com