Experimental and Molecular Pathology 100 (2016) 416–420

Contents lists available at ScienceDirect

Experimental and Molecular Pathology

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

The diagnostic significance of serum alcohol dehydrogenase isoenzymes and aldehyde dehydrogenase activity in renal cell cancer patients

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ARTICLE INFO

Article history:
Received 15 January 2016
and in revised form 4 April 2016
Accepted 11 April 2016
Available online 14 April 2016

Keywords:
Alcohol dehydrogenase isoenzymes
Aldehyde dehydrogenase
Renal cell carcinoma
Cancer markers
Diagnostic utility

ABSTRACT

Objectives: In previous experiments, we have found an increased level of class I ADH and total ADH activity in RCC tissues. Changes in cancer cells may be reflected by ADH activity in the serum and could thus be helpful for diagnostics of renal cancer. The aim of this study was to investigate a potential role of ADH and ALDH as tumor markers for RCC.
Material and methods: Serum samples were taken from 59 patients with RCC and 52 healthy subjects. Class III and IV of ADH and total ADH activity was measured by the photometric method. For measurement of class I and II ADH and ALDH activity, we employed the fluorometric method.
Results: The total activity of ADH and ADH I was significantly higher in the serum of patients with every stage of RCC compared to healthy subjects. The diagnostics criteria was higher for ADH I than for total ADH activity. The diagnostic sensitivity for ADH I was 73.36%, specificity 85.61%, predictive values of positive and negative results were 79.12 and 75.03% respectively. Area under ROC curve for ADH I was 0.748 and for total ADH 0.689.
Conclusion: The results suggest a potential role of ADH I as a marker for RCC.

1. Introduction

Malignant tumors of the kidney account for about 2% of all new cancer cases diagnosed each year and renal cell carcinoma (RCC) constitutes approximately 90% of all renal malignances (Gupta et al., 2008). However, no tumor markers are specific for RCC, although several biochemical indicators are thought to be useful for cancer diagnostics. Usefulness of the acute phase reactants such as erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), ferritin, basic fetoprotein (BFP) and immunosuppressive acidic protein (IAP) is well supported in the literature as markers of renal cancer staging and predicting survival (Miyata et al., 2001). Because of influence of the inflammatory processes and chronic disorders on these markers concentrations, they do not have high sensitivity or specificity.

Many studies show that changes in enzyme activity in the cancer cells in the course of different malignant diseases are reflected by the change of its activity in the serum. In our previous investigations we have shown that alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) activities are present in cancer cells of the kidney (Orywal et al., 2013). In addition, the activity of class I ADH isoenzyme and total ADH were significantly higher in cancer cells than in healthy tissue. ADH and ALDH are the main enzymes responsible for ethanol metabolism. Disproportionately high ADH and normal ALDH activity lead to increase production of acetaldehyde, the first metabolite of ethanol, what can be the factor involved in carcinogenesis (Jelski et al., 2010). It is commonly known that acetaldehyde interferes at many sites with DNA synthesis and repair what can result in tumor development. Acetaldehyde also binds to proteins, resulting in structural and functional alterations and by binding to DNA, it forms stable DNA adducts (Wang et al., 2013). It has been shown that pathomechanism of many cancers, eg upper digestive tract, liver, pancreas, colorectum and breast, is associated with disorders in ADH and ALDH activities (Jelski et al., 2010). Moreover, the changes of ADH and ALDH activity are reflected in serum cancer patients because of releasing isoenzymes from cancer cells and could thus be helpful for cancer diagnostics. While various markers have been studied in renal cell carcinoma, the diagnostic significance of ADH isoenzymes and ALDH activities have not been reported.

In the current study, which is a continuation of our previous investigations, we determined the diagnostic utility of ADH isoenzymes in renal cell carcinoma. We defined diagnostic sensitivity, specificity, predictive value for positive and negative results, and receiver-operating characteristics (ROC) curve of tested enzymes.

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http://dx.doi.org/10.1016/j.yexmp.2016.04.001
0014-4800/

2. Material and methods

2.1. Material

Serum samples were taken before treatment from 59 patients (35 men and 24 women; mean age 59 years, range 34–83 years) with clear cell renal cell carcinoma. The mean BMIs (body mass index) of patients were 24.7 kg/m^2. 21/59 patients were current smokers, 7/59 were ex-smokers and 28/59 were lifelong non-smokers. None of the patients had received chemotherapy or radiotherapy before sample collection, and were diagnosed as stage II (19 patients), III (24 patients) or IV (16 patients) of renal cell cancer. All of the patients had a history of occasional alcohol consumption. Control group were serum samples taken from 52 healthy subjects (30 men and 22 women; mean age 58 years, range 51–67 years old).

The research protocol was approved by the Medical University of Białystok's Human Care Committee located in Bialystok, Poland (Approval Nr R-I-002/436/2013). All patients gave their informed consent for the examination.

2.2. Methods

2.2.1. Determination of total ADH activity

Total ADH activity was estimated by the photometric method using p-nitrosodimethylaniline (NDMA) as a substrate (Skursky et al., 1979). The reaction mixture (2 mL) contained serum (0.1 mL), 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 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).

2.2.2. Determination of total ALDH activity

Aldehyde dehydrogenase activity was measured using the fluorogenic method based on the oxidation of 6-methoxy-2-naphtaldehyde to fluorescent 6-methoxy-2-naphtoate (Wierzchowski et al., 1997). 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 also contained 50 μL 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 and an emission wavelength of 360 nm on a Shimadzu RF–5301 spectrofluorophotometer (Shimadzu Europa GmbH, Duisburg, Germany).

2.2.3. Determination of class I and II ADH isoenzymes

Class I and II ADH 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 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) using the conditions previously described (Jelski & Szmitkowski, 2008). The measurements were performed on a Shimadzu RF–5301 spectrofluorophotometer 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.

2.2.4. Determination of class III ADH isoenzyme

The assay mixture for class III alcohol dehydrogenase contained a serum (100 μL), formaldehyde as a substrate (100 μL of 1 mM), glutathione (100 μL of 1 mM) and NAD (240 μL of 1.2 mM) in 0.1 mol NaOH-pyrophosphate buffer pH 8.0 (Koivusalo et al., 1989). The final volume was 2 mL. The reduction of NAD was monitored at 340 nm and 25 °C on a Shimadzu UV/VIS 1202 spectrophotometer.

2.2.5. Determination of class IV ADH isoenzyme

The assay mixture for class IV of ADH activity contained serum (50 μL), m-nitrobenzaldehyde as a substrate (132 μL of 80 μM) and 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.

2.2.6. Diagnostic values calculation

The diagnostic criteria, such as the diagnostic sensitivity, diagnostic specificity, predictive value of positive (PPV) and negative (NPV) results and the ROC curve, were determined using GraphRoc Program for Windows (University of Turku, Turku, Finland) (Kairisto et al., 1993). For the diagnostic accuracy calculations and the ROC curve we used the total RCC patients group vs. control group.

2.3. Statistical analysis

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. To test the hypothesis about the differences between different grades of bladder cancer, ANOVA rank Kruskal–Wallis test was performed. Data was presented using median, range and mean values. Statistically significant differences were defined as comparisons resulting in $p < 0.05$.

3. Results

The activities of total ADH, ALDH and ADH isoenzymes in the sera of patients with renal cell carcinoma are presented in Table 1. The comparison of ADH isoenzymes activities showed that only the activity of ADH I was significantly higher in the serum of cancer patients in contrast to healthy subjects. The median activity of this class ADH was 1.788 mIU/l in the renal cancer patients and 1.284 mIU/l in the control group. The other tested classes of ADH isoenzymes had higher activities in the serum of patients with cancer but the differences were not statistically significant ($p > 0.05$). Moreover we have found that the total alcohol dehydrogenase activity was significantly higher in patients with renal cell carcinoma. The median total activity of ADH was 1.216 IU/l in the sera of cancer patients and 0.895 IU/l in the healthy subjects. We have also observed that ALDH activity did not indicate significant differences between tested groups.

The analysis of the alcohol dehydrogenase isoenzymes activities in the different advancement stages of renal cancer showed the tendency of ADH I and the total ADH activities to increase in accordance with the disease progression. Significantly higher ADH class I activity was observed in the cancer patients regardless of the cancer stage in comparison to the control group. The activity of ADH I in stage II of RCC was 1.636 mIU/l, 1.765 mIU/l in stage III and was highly elevated in stage in stage IV (1.838 mIU/l). The serum level of total ADH activity was found to be also significantly higher in every stage of disease compared to the healthy subjects. The other isoenzymes and the total ALDH did not exhibit any characteristic changes of activity correlating with stage of renal cell carcinoma. The analysis of ADH and ALDH activities did not show any significant differences between different stages of renal cancer ($p = 0.617$ for ADH I; $p = 0.501$ for ADH II; $p = 0.241$ for ADH III; $p = 0.439$ for ADH IV, $p = 0.846$ for ADH total; $p = 0.106$ for ALDH).

Table 2 shows the diagnostic criteria for ADH total and ADH I. The sensitivity (73%) and specificity (85%) of ADH I were higher than values for ADH total (67% and 72% respectively). Both the predictive value of positive and negative results were also the highest for ADH I.

The relationship between diagnostic sensitivity and specificity was illustrated by a ROC curve (Fig. 1). It shows that area under the ROC curve for ADH I (0.748) was higher than the ROC area of ADH total (0.689).