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Original research article

The alterations in alcohol dehydrogenase and aldehyde dehydrogenase activities in the sera of patients with renal cell carcinoma

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ABSTRACT

Purpose: In a previous study we showed that the total activity of alcohol dehydrogenase (ADH) and its isoenzyme class I was significantly higher in renal cancer (RCC) cells compared to normal kidney. The aim of this study was to compare the activities of ADH isoenzymes and aldehyde dehydrogenase (ALDH) in the sera of patients with different stages of RCC and healthy subjects.
Materials and methods: Serum samples were taken from 54 patients with clear cell RCC (17 patients in stage II, 22 in stage III and 15 in stage IV) and 52 healthy patients. Class III, IV of ADH and the total ADH activity was measured by the photometric method. For the measurement of ADH class I, II and the total ALDH activity we employed the fluorometric method.
Results: The total activity of ADH and its isoenzyme class I were significantly higher in the sera of patients with every stage of RCC compared to healthy subjects. The analysis of ALDH activity did not indicate significant differences between tested groups.
Conclusions: The increased activity of total ADH and its isoenzyme class I in the sera of patients with RCC, seems to be caused by isoenzymes being released from cancerous cells and may be useful for diagnostics of renal cancer.

1. Introduction

Renal Cell Carcinoma (RCC) is one of the most common malignancies of the genitourinary tract, accounting for about 3% of all adult cancers [1]. Despite advances in diagnosis, about 20–30% of all patients are diagnosed already with metastatic disease and extremely poor prognosis. Moreover one-third of RCC patients develops distant metastases after resection of the primary tumor [2]. Tumor status has been associated with a number of metabolic and biochemical alterations. The subject of a new studies are certain molecules which could have a meaning in carcinogenesis and tumor progression. Several data have shown a role of tetraspanins, CD 133, calbindin, glutathione S-transferase P, retinal dehydrogenase 1 and aldehyde dehydrogenase in pathogenesis of renal cell carcinoma [3–6].

Studies of *Jelski and Szmitkowski* show that differences of alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) activity between cells of many cancers and healthy tissue may be one of the factors intensifying carcinogenesis [7]. Moreover, changes of ADH and ALDH activity in cancer cells are reflected in serum of patients because of releasing these enzymes from malignant tissue [7]. Human alcohol dehydrogenase and aldehyde dehydrogenase exist in multiple molecular forms that have been grouped into several classes. The best characterized function of these enzymes is a metabolism of ethanol and the other alcohols, also role in protection against products of lipid peroxidation and some exogenous xenobiotics. It was also found that ADH and ALDH take part in biosynthesis of retinoic acid, an important factor for cell differentiation and regeneration [8,9].

In our previous study we showed that normal and cancerous cells of kidney exhibit the activity of alcohol dehydrogenase and aldehyde dehydrogenase. Furthermore, the activity of class I ADH isoenzymes and total ADH were significantly higher in cancer tissue than in unchanged renal cells. Moreover, the activity of ADH seems to be disproportionately high compared to the activity of ALDH, what suggests an increased ability of cancer cells to form the highly toxic and mutagenic acetaldehyde and initiating disorders in metabolism of many biologically important substances [10].

While various markers have been studied in RCC, the clinical significance of alcohol dehydrogenase and aldehyde dehydrogenase has not been elucidated. In this study we investigated the

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activity of ADH, its isoenzymes and the total activity of ALDH in the sera of patients with renal cell carcinoma.

2. Material and methods

2.1. Material

Serum samples were taken before surgery from 54 patients (31 men and 23 women; mean age 58 years, range 34–83 years) with clear cell renal cell carcinoma. The mean BMIs (body mass index) of patients were 24.5 kg/m^2. 18/54 patients were current smokers, 6/54 were ex-smokers and 30/54 were lifelong non-smokers. None of the patients had received chemotherapy or radiotherapy before sample collection, and were diagnosed as stage II (17 patients), III (22 patients) or IV (15 patients) of renal cell cancer. Control group were serum samples taken from 52 healthy subjects (30 men and 22 women; mean age 58 years, range 51–67 years old) with mean BMI 23.6 kg/m^2. 19/52 were current smokers and 33/52 non-smokers. All of the patients from tested and control group had a history of occasional alcohol consumption.

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 from tested and control group 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 [11]. 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 [12]. 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. [13]. 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 [14]. 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 [15]. 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 [16]. The oxidation of NADH was monitored at 340 nm and 25° C on a Shimadzu UV/VIS 1202 spectrophotometer.

2.2.6. 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. All data was expressed in mIU/l and 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 total activity of alcohol dehydrogenase was significantly higher (about 26%) in the serum of patients with renal cancer than in healthy subjects. The median total activity of ADH was 1213.0 mIU/l in the RCC group and 895.0 mIU/l in the control group. The analysis of ALDH activity did not indicate significant differences between total tested group and healthy persons.

The comparison of ADH isoenzymes activities showed that the highest difference was exhibited by class I ADH. The median activity of this class of isoenzymes in the cancer group increased by about 24% (1.785 mIU/l) in comparison to the control level (1.338 mIU/l). The increase of ADH I activity was statistically significant. The other tested classes of ADH isoenzymes had higher activities in the serum of patients with cancer but the differences were not statistically significant ($p > 0.05$).

The analysis of particular ADH isoenzymes activities depending on the progression stage of carcinoma, showed the tendency of ADH I activity to increase in accordance with the advance of disease (Fig. 1). Significantly higher ADH class I activity was found in every stage (from II to IV) of cancer compared to the control group. In the stage II of cancer advancement we observed about 20% increase of ADH I activity compared to the control group. In the stage III the increase in class I activity was above 23% and in the IV stage – 26% in comparison to healthy subjects. The activity of total ADH was found to be also significantly higher in patients with renal cancer without dependence on tumor stage. The other isoenzymes did not exhibit any characteristic changes of activity correlating with stage of disease. The total activity of ALDH also did not indicate significant differences between advancement stages.

4. Discussion

The malignant transformation is based on the loss of control mechanisms, disorders in cell differentiation and uninhibited growth. The metabolism of cancer cells is in many ways different then in healthy tissue.

Renal cell carcinoma is characterized by an abnormally high glycogen deposition caused by altered enzyme activities [17]. Many studies reported decreased expression of glucose 6-phosphatase, aminoacylase-I and aldehyde dehydrogenase-1 in RCC [18,19]. ALDH catalyses an oxidation of aldehydes to carboxylic compounds and this reaction is considered as a general detoxification process. *Sreerama at al* revealed that ALDH-1 take part in the