The activity of class I, II, III and IV alcohol dehydrogenase isoenzymes and aldehyde dehydrogenase in ovarian cancer and ovarian cysts

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ABSTRACT

Purpose: The metabolism of cancerous cells is in many ways different than in healthy cells. In ovarian cancer, cells exhibit activity of alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH), which participate in metabolism of many biological substances. The aim of this study was to compare the metabolism of ovarian cancer cells, ovarian cysts and normal ovarian cells by measurement of ADH isoenzymes and ALDH activities.

Material and Methods: The study material consisted of 36 cancerous ovarian tissues. Class III, IV of ADH and total ADH activity was measured by the photometric method and class I, II ADH and ALDH activity by the fluorometric method with class-specific fluorogenic substrates.

Results: The activity of the class I ADH isoenzyme and the total ADH was significantly higher in ovarian cancer as compared to ovarian cysts and healthy tissues but there are no significant differences between ovarian cysts and healthy cells. The other classes of ADH tested, did not show significant differences between activity of cancerous cells and healthy ovary.

Conclusion: The increased activity of total ADH in ovarian cancer, especially the class I isoenzyme and normal activity of ALDH, may be the factor for the disturbances in important biological substances metabolism and could increase the concentration of highly carcinogenic acetaldehyde.

Key words: Alcohol metabolism, carcinogenesis, enzymes, acetaldehyde, gynecological cancer

INTRODUCTION

In spite of improvements in cancer treatment, ovarian cancer is the leading cause of gynecologic cancer death among women in developed countries [1]. The risk factors which can increase the likelihood of ovarian cancer developing are age, family history, smoking, dietary factors, fertility drug use and postmenopausal hormonal therapy [2]. Also excessive androgen levels have been proposed as a causal mechanism in the pathogenesis of this type of cancer [3].

Recent evidence suggest that alcohol could increase plasma estrogen levels either by promoting the induction of aromatases, which can convert androgens to estrogens, or by impairing the metabolism of estrogens in liver [4]. Ethanol is considered by the International Agency for Research on Cancer as carcinogen, most notably through the formation of acetaldehyde. Studies in vitro have shown that acetaldehyde causes many cytogenetic abnormalities in eukaryotic cells, including point mutations and gross chromosomal aberrations. Acetaldehyde also binds to proteins, resulting in structural and functional alterations and by binding to DNA, it forms stable DNA adducts [5].

Acetaldehyde is the first metabolite of alcohol oxidation, mediated by alcohol dehydrogenase (ADH). Recent data shows that healthy and also cancerous cells of endometrium and cervix contain ADH class I, III and IV isoenzymes [6,7].
Despite of contribution to ethanol oxidation, these enzymes play a significant role in the metabolism of many biological substances. Class I of ADH participate in the metabolism of bioamines and prostaglandins, and together with class IV ADH isoenzymes in retinoic acid metabolism, whereas the main role of class III of alcohol dehydrogenase is the oxidation of S-hydroxymethylglutathione [8]. The next step in ethanol metabolism is oxidation of acetaldehyde to acetic acid by aldehyde dehydrogenase (ALDH). ALDH participates also in polyamine and histamine catabolism and retinoic acid synthesis [9].

The aim of this study was to determine the differences in the activity of alcohol dehydrogenase isoenzymes and aldehyde dehydrogenase in healthy, benign changed and cancerous ovarian tissues.

MATERIAL AND METHODS

Biopsy specimens of cancerous ovarian tissues (80 - 100 mg) were obtained during surgical resection of ovarian carcinoma from 36 women (mean age 58 years, range 41 - 75 years). None of the women had received chemotherapy or radiotherapy before tissue collection. The study material were also tissues of ovarian cysts obtained from 40 women (mean age 45, range 27 - 72 years). The control group contains histologically unchanged ovarian tissues obtained during prophylactic resection of ovary from 34 women (mean age 48 years, range 36 - 61 years) with BRCA1 mutations. All of the patients had a history of occasional alcohol consumption.

All patients gave their informed written consent, and the study protocol was approved by the Ethics Committee of the Medical University of Bialystok, Poland (approval No R-I-002/179/2009).

Preparation of tissue extract

All specimens were frozen at −80°C until analysis, which occurred within two weeks. The examined tissues (1.4 w/v) were homogenized in a potassium phosphate buffer (0.1 M; pH 7.4) for 20 sec using an ultrasonificator (Sonoplus HD 70, Bandelin, Germany) and then were centrifuged at 14,000 rpm for 30 min. with cooling to 4°C. Supernatant was used to determine the ADH and ALDH activities.

Determination of total ADH activity

Total ADH activity was estimated by the photometric method using p-nitrosodimethylaniline (NDMA) as a substrate [10]. The reaction mixture (2 mL) contained supernatant (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).

Determination of total ALDH activity

Aldehyde dehydrogenase activity was measured using the fluorogenic method based on the oxidation of 6-methoxy-2-naphthaldehyde to fluorescent 6-methoxy-2-naphtoate [11]. The reaction mixture contained 60 µL of supernatant, 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).

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. [12]. The assays were performed in a reaction mixture containing a supernatant (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) under the conditions previously described [13]. 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.

Determination of class III ADH isoenzyme

The assay mixture for class III alcohol dehydrogenase contained a supernatant (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 M NaOH-pyrophosphate buffer pH 8.0 [13]. 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.

Determination of class IV ADH isoenzyme

The assay mixture for class IV of ADH activity contained a supernatant (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 [14]. The oxidation of NADH was monitored at 340 nm and 25°C on a Shimadzu UV/VIS 1202 spectrophotometer.

Protein assays

Protein concentration was measured by the Lowry method using bovine serum albumin as the standard (Sigma Diagnostics, St. Louis, USA) [15].

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 test was used for statistical analysis. Data was presented using
median, range and mean values. Statistically significant differences were defined as comparisons resulting in p < 0.05.

**RESULTS**

The activities of total ADH, ALDH and ADH isoenzymes in the ovarian cancer and ovarian cysts tissues are presented in Tab. 1. We have shown that ADH and ALDH activities are present in cancer and cysts cells and in healthy ovarian tissue, although the activity of ADH is much higher than ALDH activity in all tested groups.

The activity of class I ADH was significantly higher (p<0.05) in cancerous than in healthy tissues and in comparison to ovarian cysts. The median activity of this class in cancer group was 0.147 nmol/min/mg of protein. The median activity of ADH I in cysts cells was 0.115 nmol/min/mg of protein and in healthy tissue 0.109 nmol/min/mg of protein. The difference between ovarian cysts group and healthy ovary was not significant (p>0.05). The analysis of the other classes of ADH isoenzyme activity did not indicate any significant differences. The comparison of ADH isoenzyme activities shows that the highest activity was exhibited by class III ADH in all tested group. The activity of class I ADH was about 1.9 times lower and the activity of class IV about 7.5 times lower than that of class III ADH in cancerous cells. The activity of class II ADH isoenzyme was barely detectable in all tested groups.

The total activity of ADH was significantly higher (p<0.05) in cancerous cells in comparison with both - healthy and cysts tissues. The median ADH activity in cancer group was 0.703 nmol/min/mg of protein. The median activity of total ADH in cysts cells was slightly increased (0.681 nmol/min/mg of protein) compared to healthy tissue (0.674 nmol/min/mg of protein) but the difference were not significant (p>0.05). Analysis of ALDH activity did not show statistically significant differences between cancer, cysts and unchanged ovarian cells.

**DISCUSSION**

Ovarian cancer is the sixth most commonly diagnosed cancer among women in the world and the leading cause of death from gynecologic malignancies [16]. Despite the high incidence and mortality rates, the etiology of this disease is still poorly understood. The role of alcohol consumption in etiology of ovarian cancer has not yet been clarified, as the results from numerous studies have been inconsistent. Neither studies of population-based cohorts, studies of alcoholic cohorts nor case control studies have found a clear association between alcohol consumption and ovarian cancer risk, although a few recent studies have reported a positive or negative correlation [2].

Many data shows that hormonal factors are involved in the etiology of ovarian cancer. Normal ovarian surface epithelial cells proliferate if stimulated by estrogen and 60% of ovarian tumors are estrogen-receptor positive [17]. Therefore using estrogen-only hormone replacement therapy is associated with a significant higher ovarian cancer risk

Table 1. The activity of ADH isoenzymes and ALDH in ovarian cancer, ovarian cyst and healthy tissues.

<table>
<thead>
<tr>
<th>TESTED GROUP</th>
<th>ADH I Median</th>
<th>ADH II Median</th>
<th>ADH III Median</th>
<th>ADH IV Median</th>
<th>ADH Total Median</th>
<th>ALDH Total Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovarian cancer (n=36)</td>
<td>0.147</td>
<td>0.0016</td>
<td>0.263</td>
<td>0.035</td>
<td>0.703</td>
<td>0.047</td>
</tr>
<tr>
<td></td>
<td>0.093–0.219</td>
<td>0.00024</td>
<td>0.175–0.388</td>
<td>0.023–0.051</td>
<td>0.561–0.910</td>
<td>0.042–0.063</td>
</tr>
<tr>
<td></td>
<td>0.156</td>
<td>0.0018</td>
<td>0.281</td>
<td>0.037</td>
<td>0.735</td>
<td>0.052</td>
</tr>
<tr>
<td>Ovarian cyst (n=40)</td>
<td>0.115</td>
<td>0.0013</td>
<td>0.255</td>
<td>0.034</td>
<td>0.681</td>
<td>0.045</td>
</tr>
<tr>
<td></td>
<td>0.080–0.176</td>
<td>0.00022</td>
<td>0.166–0.371</td>
<td>0.022–0.048</td>
<td>0.523–0.921</td>
<td>0.039–0.060</td>
</tr>
<tr>
<td></td>
<td>0.128</td>
<td>0.0016</td>
<td>0.268</td>
<td>0.036</td>
<td>0.722</td>
<td>0.049</td>
</tr>
<tr>
<td>Healthy tissues (n=34)</td>
<td>0.109</td>
<td>0.0014</td>
<td>0.251</td>
<td>0.034</td>
<td>0.674</td>
<td>0.044</td>
</tr>
<tr>
<td></td>
<td>0.075–0.169</td>
<td>0.00022</td>
<td>0.168–0.357</td>
<td>0.024–0.047</td>
<td>0.527–0.914</td>
<td>0.038–0.058</td>
</tr>
<tr>
<td></td>
<td>0.122</td>
<td>0.0017</td>
<td>0.263</td>
<td>0.035</td>
<td>0.721</td>
<td>0.048</td>
</tr>
</tbody>
</table>

\[ p_a < 0.001 \quad p_a = 0.639 \quad p_a = 0.483 \quad p_a = 0.404 \quad p_a < 0.001 \quad p_a = 0.423 \]
\[ p_b < 0.001 \quad p_b = 0.583 \quad p_b = 0.387 \quad p_b = 0.389 \quad p_b < 0.001 \quad p_b = 0.583 \]
\[ p_c = 0.473 \quad p_c = 0.376 \quad p_c = 0.648 \quad p_c = 0.486 \quad p_c = 0.235 \quad p_c = 0.365 \]

Data are expressed as nmol/min/mg of protein.

\[ p_a - \text{ovarian cancer vs. control group} \]
\[ p_b - \text{ovarian cancer vs. ovarian cyst} \]
\[ p_c - \text{ovarian cyst vs control group} \]
compared with users of combined preparations [18]. Alcohol consumption is correlated with endogenous hormones levels. In pre-menopausal women, alcohol consumption has been consistently, positively correlated with both total estrogen level and bioavailable estrogens but post-menopausal alcoholics have elevated levels of serum estradiol and plasma levels of estrone sulphate [19,20].

Chronic alcohol consumption is related with pathomechanism for many cancers, e.g. upper digestive tract, liver, pancreas, colorectal, breast and uterus. The main factor responsible for alcohol-related carcinogenesis is acetaldehyde, which formation and degradation depends mostly on the activity of alcohol dehydrogenase and aldehyde dehydrogenase. In our study, we found that the activity of ADH (especially class I) was significantly higher in cancerous tissues than in ovarian cysts and healthy tissues but the activity of ALDH, responsible for degradation of toxic acetaldehyde, was not changed. These findings suggest increased ability to produce acetaldehyde by cancerous ovarian cells compared to benign changes and normal ovary. Acetaldehyde interferes with DNA synthesis and repair causing inhibition in the O6-methylguanine-DNA methyltransferase (MGMT) activity [21]. However ovarian cancer is the second highest tumor MGMT activity, following the colorectal malignancies [22]. The study of ADH activity in women with uterus cancer are consistent with ovarian cancer results. In endometrial cancer and cervical cancer cells was found higher activity of ADH I and the total ADH without any changes in ALDH activity [2,7]. The study in women with breast cancer has shown an inverse association. Jelski et al. demonstrated that the activity of class I ADH in breast cancer was lower than in normal mammary tissues and was decreasing together with the progression of the disease [23]. ADH I perform a protective function by reducing aldehyde products generated from lipid peroxidation to their less toxic alcohols. Many studies suggests a role of aldehyde products of lipid peroxidation in carcinogenesis of the breast gland, what can be associated with decreased of ADH I activity in cancer tissue [24].

Retinoids have been shown to be important in oncogenesis for many tissues and alterations in vitamin A homeostasis are found in many tumors, including breast, oral, prostate and leukemia. Many studies indicate that the retinoic acid pathway plays a role also in ovarian carcinogenesis. ADH and ALDH catalyze the oxidation or reduction of a wide spectrum of substrates. Besides of the ethanol oxidation, I ADH isoenzymes participate in the metabolism of many biological substances: bioamines, prostaglandins and retinoic acid synthesis (RA) [25]. ADH I catalyzes oxidation of retinol, which is a principal mediator of retinoid actions required for maintaining epithelia in a differentiated states. New-created retinal is in next step catalyzed by ALDH to retinoic acid. Disturbances between ADH and ALDH activities in cancer cells and not-cancerous ovarian tissues can be a factor entangled in ovary carcinogenesis. Moreover, alcohol exposure may lower RA levels either by blocking alcohol dehydrogenase-activated RA biosynthesis due to the competition, as dehydrogenase substrates, between alcohol and vitamin A [26].

CONCLUSIONS

In conclusion, we can state that healthy ovarian tissue, ovarian cyst and ovarian cancer cells exhibited activity of ADH and ALDH. However, the activity of ADH in cancer cells is disproportionately high compared to the activity of ALDH, what can lead to induce acetaldehyde production and support carcinogenesis in ovary. Among all of the ADH isoenzymes studied, the activity of class I exhibited a statistically significant difference between cancerous and healthy or benign changed tissues, what could be a factor for the disturbances in the metabolism many important biological substances, including retinoic acid.

ACKNOWLEDGEMENTS

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REFERENCES