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The Activity of Class I, II, III, and IV Alcohol Dehyrogenase Isoenzymes and Aldehyde Dehydrogenase in Endometrial Cancer

Karolina Orywal,[1]* Wojciech Jelski,[1] Michał Zdrodowski,[2] and Maciej Szmitkowski[1]
[1]*Department of Biochemical Diagnostics, Medical University, Bialystok, Poland*
[2]*Department of Gynecology, Medical University, Bialystok, Poland*

Objective: The metabolism of cancerous cells is in many ways different than in healthy cells. In endometrial cancer, cells exhibit activity of alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH), which participate in the metabolism of many biological substances. The aim of this study was to compare the metabolism of endometrial cancer cells and normal endometrial cells by measurement of ADH isoenzymes and ALDH activities in these tissues. *Methods*: The study material consists of cancerous endometrial tissues obtained from 34 patients. Total ADH activity was measured using the photometric method and ALDH activity using the fluorometric method. For the measurement of class I and II ADH isoenzyme activity, we employed the fluorometric method, with class-specific fluorogenic substrates. The activity of class III and IV ADH was measured using the photometric method. *Results*: The activity of the class I ADH isoenzyme was significantly higher in the endometrial cancer tissues when compared with normal endometrial tissues. The other classes of ADH tested did not show significant differences between activity of cancerous cells and healthy endometrium. The activity of total ADH was also significantly higher in endometrial cancer. *Conclusion*: The increased activity of total ADH in endometrial cancer, especially the class I isoenzyme and normal activity of ALDH, may be the cause of disorders in metabolic pathways that use these isoenzymes and could increase the concentration of acetaldehyde, which is cancerogenic substance. J. Clin. Lab. Anal. 24:334–339, 2010.
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Key words: alcohol dehydrogenase isoenzymes; aldehyde dehydrogenase; endometrial cancer

INTRODUCTION

Endometrial cancer has the fifth highest incidence in the classification of female cancers (1). Risk factors can be associated with almost every aspect of women's lives. Family history, life style, diet, smoking, and alcohol, all increase the likelihood of endometrial cancer developing. The growth of endometrial cancer has also been related to exposure to endogenous or exogenous estrogens (2).

Recent evidence suggests that alcohol intake positively influences endogenous estrogens, which could be associated with endometrial cancer incidence (3). The mechanism by which alcohol increases the risk of endometrial cancer is still unclear. 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). Moreover, ethanol metabolism is often considered to be a predominant factor of tissue damage, most notably through the formation of acetaldehyde. Acetaldehyde is a carcinogen because it interferes with DNA synthesis and repair, causing point mutations and chromosomal aberrations. Acetaldehyde also binds to DNA, forming stable DNA adducts, which have been found in alcohol drinkers (5).

Acetaldehyde is the primary product of ethanol oxidation, mediated by alcohol dehydrogenase (ADH), and is further metabolized by aldehyde dehydrogenase (ALDH). ADH and ALDH exist in multiple molecular forms that have been grouped into several classes (6).

*Correspondence to: Karolina Orywal, Department of Biochemical, Diagnostics, Medical University, Waszyngtona 15 A, PL 15–276, Bialystok, Poland. E-mail: orywalk@umwb.edu.pl

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Recent data show that the uterus contains ADH class I, III, and IV isoenzymes (7). These enzymes also play a significant role in the metabolism of many biological substances. ADH and ALDH possess a high affinity for ethanol and catalyze the oxidation or reduction of a wide spectrum of substrates (6). It was also found that class I ADH isoenzymes participate in the metabolism of bioamines and prostaglandins, and together with the class IV ADH isoenzymes in retinoic acid metabolism (8). Moreover, class III of ADH catalyzes the oxidation of S-hydroxymethylglutathione much more effectively than ethanol (9). The main physiological role of ALDH is the oxidation of acetaldehyde to acetic acid. ALDH is responsible also for the oxidation of other aldehydes and participates in polyamine and histamine catabolism and retinoic acid synthesis (oxidation of retinal) (10).

The aim of this study was to determine the differences in the activity of ADH isoenzymes and ALDH in cancerous and normal endometrial tissues in pre- and postmenopausal women.

MATERIAL AND METHODS

Material

Biopsy specimens of cancerous endometrial tissues (80–100 mg) were obtained during surgical resection of endometrial carcinoma from 34 women (mean age 59 years, range 34–82 years). The patients were divided into two groups: 18 postmenopausal and 16 premenopausal women. None of the women had received chemotherapy or radiotherapy before tissue collection, and were diagnosed as either stage II or III. Control group were healthy endometrium obtained from 34 women (mean age 56 years, range 35–76 years) during resection of myoma. All the patients 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-002/179/2009). All the patients gave their informed consent for the examination.

Methods

Preparation of tissue extract

All the specimens were frozen at −80°C until analysis, which occurred within 2 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 using the photometric method using *p*-nitrosodimethylaniline as a substrate (11,12). 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

ALDH activity was measured using the fluorogenic method based on the oxidation of 6-methoxy-2-naphtaldehyde to fluorescent 6-methoxy-2-naphtoate (13,14). 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. (15). 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) using the conditions previously described (16,17). 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 the determination of class III ADH activity 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 mol NaOH–pyrophosphate buffer pH 8.0 (9). 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 the determination of class IV 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 (18). 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 using the Lowry method (19) using bovine serum albumin as the standard (Sigma Diagnostics, St. Louis).

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 were 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 endometrial cancer tissues are presented in Table 1. We have shown that ADH and ALDH activities are present in cancer cells and normal endometrium tissue, although the activity of ADH is much higher than ALDH activity in all tested groups. The comparison of ADH isoenzyme activities shows that the highest activity was exhibited by class III ADH. The median activity of this class in the total cancer group was 0.44 nmol/min/mg of protein and 0.42 nmol/min/mg of protein in healthy tissues. The activity of class I ADH was about 2.2 times lower and the activity of class IV about 9.7 times lower than that of class III ADH in cancerous cells. The activity of class II ADH isoenzyme was barely detectable. Such results were observed in all examined groups, regardless of pre- and postmenopusal age.

We have found that the activity of class I ADH ($P < 0.05$) was significantly higher in cancerous than in healthy tissues. The other classes of tested ADH isoenzymes showed higher activity in cancer cells too, but the differences were not statistically significant ($P > 0.05$). Therefore, total ADH activity was significantly

TABLE 1. Activity of ADH Isoenzymes and ALDH in Endometrial Cancer and Healthy Tissues

Tested group	ADH I Median Range Mean	ADH II Median Range Mean	ADH III Median Range Mean	ADH IV Median Range Mean	ADH Total Median Range Mean	ALDH Total Median Range Mean
Total						
Cancerous tissues ($n = 34$)	0.199	0.0026	0.44	0.046	0.99	0.078
	0.171–0.223	0.0023–0.0026	0.26–0.61	0.036–0.054	0.72–1.25	0.064–0.089
	0.196	0.0025	0.43	0.045	0.98	0.076
Health tissues ($n = 34$)	0.153	0.0025	0.42	0.045	0.89	0.074
	0.134–0.165	0.0018–0.0031	0.31–0.51	0.034–0.053	0.64–1.11	0.050–0.093
	0.145	0.0024	0.41	0.043	0.87	0.071
	$p < 0.001$	$p = 0.348$	$p = 0.186$	$p = 0.874$	$p < 0.001$	$p = 0.348$
Postmenopausal women ($n = 18$)						
Cancerous tissues	0.193	0.0026	0.45	0.043	0.91	0.076
	0.163–0.219	0.0023–0.0025	0.27–0.55	0.034–0.051	0.65–1.12	0.064–0.093
	0.191	0.0024	0.41	0.042	0.88	0.075
Healthy tissues	0.143	0.0025	0.41	0.043	0.88	0.070
	0.102–0.181	0.0021–0.0027	0.26–0.55	0.035–0.051	0.63–1.09	0.054–0.085
	0.141	0.0024	0.40	0.044	0.86	0.069
	$p < 0.001$	$p = 0.417$	$p = 0.107$	$p = 0.942$	$p < 0.001$	$p = 0.424$
Premenopausal women ($n = 16$)						
Cancerous tissues	0.206	0.0027	0.43	0.044	1.03	0.078
	0.183–0.224	0.0022–0.0029	0.35–0.53	0.035–0.054	0.71–1.28	0.053–0.101
	0.202	0.0025	0.44	0.045	0.99	0.077
Healthy tissues	0.151	0.0027	0.43	0.045	0.91	0.073
	0.125–0.172	0.0021–0.0031	0.32–0.54	0.034–0.053	0.69–1.10	0.048–0.097
	0.148	0.0026	0.43	0.044	0.90	0.072
	$p < 0.001$	$p = 0.391$	$p = 0.139$	$p = 0.876$	$p < 0.001$	$p = 0.394$

Data are expressed as nmol/min/mg of protein. *p*, cancerous tissues vs healthy tissues; ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase.

TABLE 2. The Comparison of ADH Isoenzymes and ALDH Activities in Endometrial Cancer and Healthy Tissues in Pre- and Postmenopausal Women

Tested group	ADH I Median Range Mean	ADH II Median Range Mean	ADH III Median Range Mean	ADH IV Median Range Mean	ADH Total Median Range Mean	ALDH Total Median Range Mean
Endometrial cancer						
Postmenopausal	0.193	0.0026	0.45	0.043	0.91	0.076
women ($n = 18$)	0.163–0.219	0.0023–0.0025	0.27–0.55	0.034–0.051	0.65–1.12	0.064–0.093
	0.191	0.0024	0.41	0.042	0.88	0.075
Premenopausal	0.206	0.0027	0.43	0.044	1.03	0.078
women ($n = 16$)	0.183–0.224	0.0022–0.0029	0.35–0.53	0.035–0.054	0.71–1.28	0.053–0.101
	0.202	0.0025	0.44	0.045	0.99	0.077
	$p < 0.001$	$p = 0.348$	$p = 0.264$	$p = 0.645$	$p < 0.001$	$p = 0.433$
Healthy tissues						
Postmenopausal	0.143	0.0025	0.41	0.043	0.88	0.070
women ($n = 18$)	0.102–0.181	0.0021–0.0027	0.26–0.55	0.035–0.051	0.63–1.09	0.054–0.085
	0.141	0.0024	0.40	0.044	0.86	0.069
Premenopausal	0.151	0.0027	0.43	0.045	0.91	0.073
women ($n = 16$)	0.125–0.172	0.0021–0.0031	0.32–0.51	0.034–0.053	0.69–1.10	0.048–0.097
	0.148	0.0026	0.43	0.044	0.90	0.072
	$p < 0.001$	$p = 0.349$	$p = 0.156$	$p = 0.567$	$p = 0.329$	$p = 0.518$

Data are expressed as nmol/min/mg of protein. *p*, premenopausal women vs postmenopausal women; ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase.

higher in cancer tissue than in helathy ($P < 0.05$). The activity of ALDH was also higher in cancerous cells as compared with healthy tissue, however, the difference was not statistically significant.

The comparison of ADH isoenzyme and ALDH activity in cancerous tissues of pre- and postmenopausal women is demonstrated in Table 2. We have shown that the activity of class I ADH was significantly higher in premenopausal women in comparison with postmenopausal women, both in cancerous and healthy endometrial tissue. The other isoenzymes did not exhibit any characteristic change of activity correlating with age. The activity of total ADH was found to be higher in premenopausal women, but there was a statistically significant difference only in cancerous tissue. Additionally, the activity of ALDH was higher in premenopausal women, however, the difference was not statistically significant.

DISCUSSION

Endometrial carcinoma is the most common cancer of the lower female genital tract in Europe and the United States. The role of alcohol consumption in the etiology of endometrial cancer has not yet been clarified, as the results from numerous studies have been inconsistent. Although some data presented an increased risk of endometrial cancer in relation to alcohol consumption (20,21), other studies have found no such association (22,23). Hosono et al. have found that a small amount of alcohol consumption was protective against endometrial cancer among Japanese women (24). However, none of these studies examined the differential effect of alcohol within various age groups.

There is a strong connection between cancer and alcohol consumption, although alcohol is not a carcinogen. Chronic alcohol consumption is a risk factor for many cancers, e.g. upper digestive tract, liver, pancreas, colorectum, and breast. A main role in carcinogenesis can be attributed to acetaldehyde (the first metabolite of ethanol), because of its potential carcinogenicity and toxicity. Formation and degradation of acetaldehyde in the body is regulated by the activity of ADH and ALDH. In our study, we found that the activity of human ADH was significantly higher in cancerous tissues than in healthy tissues, however, the activity of ALDH was not different between both tissues types. The increased activity of class I ADH and decreased ALDH activity in the endometrial cancer tissue suggests that cancerous cells have a greater capability for ethanol oxidation and less ability to remove acetaldehyde when compared with healthy tissue. This may lead to the accumulation of acetaldehyde in the endometrium, contributing to the development of endometrial cancer. The activity of ADH was significantly higher in cancerous cells of premenopausal women in comparison with postmenopausal women. The difference of ADH and ALDH activity between cancerous and healthy tissue may be a factor intensifying carcinogenesis.

Our study demonstrates that the highest activity among all tested ADH isoenzymes was exhibited by

the class III ADH isoenzyme. This isoenzyme participates in the metabolism of endogenous long-chain alcohols and aldehydes and in the oxidation of *S*-hydroxymethylglutathione. The kinetic properties of ADH III indicates that this class cannot be saturated by ethanol even at high concentrations (9). Although isoenzyme class III had the highest activity among all tested classes of ADH, we did not find significant differences between cancerous and healthy tissues.

Ohno et al. have revealed the expression of ADH class I gene in rat endometrial stromal cells (7). Several reports have shown that the expression of the ADH class I gene is regulated by sex hormones. It was reported that estrogen treatment induced the expression of ADH class I mRNA in rat kidney and liver (25,26). We found that the activity of class I ADH was statistically higher in endometrial cancer when compared with healthy tissue, and also that class I ADH activity was statistically higher in premenopausal women than in postmenopausal, in cancerous and healthy tissue. Among all classes of ADH isoenzymes, class I is the main ethanol-metabolizing isoenzyme in human body. It was also found that this class participates in bioamine and prostaglandin metabolism, steroid dehydrogenation, and ω-oxidation of fatty acids. The increase in ADH I activity could be a cause of many metabolic disorders. The study of ADH activity in women with breast cancer has shown an inverse association. Jelski et al. demonstrated that the activity of class I ADH in invasive breast cancer was about two times lower than that in normal mammary tissues (27). A decrease of class I ADH activity in breast cancer cells might be a factor causing some disorders in the metabolism of biologically important substances (retinol), which are indispensable to correct differentiation and maturation of the breast parenchyma.

Epidemiological data show that hormonal factors are involved in the etiology of endometrial cancer. Alcohol use is correlated with endogenous hormones levels. Female alcoholics have increased levels of serum estradiol and increased plasma levels of estrone sulfate (28). In premenopausal women, alcohol consumption has been consistently, positively correlated with both total estrogen level and bioavailable estrogens (29). Alcohol could increase plasma estrogen level either by promoting the induction of aromatases, which can convert androgens to estrogens, or by impairing the metabolism of estrogen in liver, resulting in an accumulation of circulating estrogen. In postmenopausal women, data show that increased alcohol consumption is associated with decreased serum estradiol and estrone levels, but in another study it was positively associated with increased level of estrone sulfate (28,30). However, there is no simple relationship between ADH activity and the stage of malignancy but in our studies all the patients were in stage II or III. Association of ADH activity depending on tumor stage will be the subject of future studies.

In conclusion, we can state that endometrial cancer exhibited activity of ADH and ALDH, although the activity of ADH seems to be disproportionately high compared with the activity of ALDH. This suggests an increased ability of cancer cells to metabolize ethanol and form of acetaldehyde, which may intensify carcinogenesis. Among all the ADH isoenzymes studied, the activity of class I exhibited a statistically significant difference between cancerous and healthy tissues, which could be a factor for metabolic changes and disturbances in immature cancer cells.

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