Activity of Alcohol Dehydrogenase (ADH) Isoenzymes and Aldehyde Dehydrogenase (ALDH) in the Sera of Patients With Breast Cancer

Wojciech Jelski,^{1*} Lech Chrostek,¹ Włodzimierz Markiewicz,² and Maciej Szmítkowski¹

¹Department of Biochemical Diagnostics, Medical University of Białystok, Białystok, Poland

²Department of Surgery, Regional Center of Oncology, Białystok, Poland

Numerous experiments have shown that alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) play a significant role in the metabolism of many biological substances. Some metabolic disorders that can lead to breast carcinogenesis may be the cause of changes in ADH and ALDH activity. In previous experiments we found a changed level of class I ADH activity in breast cancer tissues. The changed level of this enzyme in cancer cells may be reflected by the enzyme's activity in the serum. Therefore, in this study we measured the activity of ADH isoenzymes and ALDH in the sera of

patients with breast cancer. Serum samples were taken for routine biochemical investigation from 45 women with breast cancer before treatment. Among all tested classes of ADH isoenzymes, only class I had higher activity in the serum of patients with breast cancer in stage IV. The total ADH and ALDH activities were not significantly higher in patients with breast cancer than in healthy controls. The changes in activity, especially in class I ADH, appear to be caused by isoenzymes being released from the organ damaged by metastatic disease. *J. Clin. Lab. Anal.* 20:105–108, 2006. © 2006 Wiley-Liss, Inc.

Key words: alcohol dehydrogenase isoenzymes; aldehyde dehydrogenase; breast cancer

INTRODUCTION

Human alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH), the principal enzymes responsible for ethanol metabolism, exist in multiple molecular forms that have been grouped into several classes (1). Isoenzymes of ADH and ALDH are present in large quantities in the liver. ADH also occurs in the gastrointestinal tract, lungs, and kidneys (2). Isoenzymes of ADH are also observed in breast parenchyma (3). Many studies have demonstrated that ADH and ALDH play a significant role in breast carcinogenesis because they catalyze the oxidation or reduction of many biological substances (4). The best-characterized function of ADH is protection against excess endogenous alcohols, products of lipid peroxidation, and some exogenous xenobiotics (5). Isoenzymes of ADH participate in bioamine, prostaglandin, and retinoid acid metabolism, and catalyze the oxidation of S-hydroxymethylglutathione (6). The second enzyme tested in this study, ALDH, is responsible for the oxidation of aldehydes and participates in polyamine and histamine

catabolism and retinoic acid synthesis (oxidation of retinal) (7). In a previous study we showed that ADH and ALDH activities are present in breast cancer cells. In addition, the activities of class I ADH isozymes were significantly lower in cancer cells than in healthy mucosa (unpublished results).

Breast cancer is one of the most frequently occurring aggressive neoplasms. In this study we investigated the activity of ADH and its isoenzymes and the total activity of ALDH in the sera of patients with breast cancer. We hypothesized that the changed activities of these enzymes in cancer cells would be reflected in the serum and could thus be helpful for diagnosing breast cancer.

*Correspondence to: W. Jelski, M.D., Department of Biochemical Diagnostics, Medical University, Waszyngtóna 15A, PL-15-269 Białystok, Poland. E-mail: wjelski@amb.edu.pl

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MATERIALS AND METHODS

Serum samples were taken before treatment from 45 women (49–71 years old) with breast cancer (carcinoma ductale invasivum) and 45 healthy women (51–67 years old). None of the patients had received chemo- or radiotherapy before the blood samples were collected.

Pretreatment staging procedures included physical and blood examinations, mammography, mammary ultrasound scanning, breast core biopsies, chest X-ray, and computed tomography (CT). Postoperative pathological staging was performed according to surgical and histological findings. Tumors were classified and staged in accordance with the International Union Against Cancer tumor-node metastasis (UICC-TNM) classification. The patients were divided into four groups: stage I ($n = 12$), stage II ($n = 10$), stage III ($n = 12$), and stage IV ($n = 11$). Of the patients in stage IV, six had bone metastasis, three had liver metastasis, and two had lung metastasis. All of the patients drank alcohol occasionally.

Total ADH activity was measured by the photometric method with p-nitrosodimethylaniline (NDMA) as a substrate, and ALDH activity was measured by the fluorometric method with 6-methoxy-2-naphthaldehyde (oxidation). To measure the activity of class I and II isoenzymes we employed fluorometric methods with class-specific fluorogenic substrates: 4-methoxy-1-naphthaldehyde for class I, and 6-methoxy-2-naphthaldehyde for class II (reduction) (8). The activity of classes III and IV of ADH was measured by the photometric method with n-octanol and m-nitrobenzaldehyde as a substrates,

respectively. The assays were performed as previously described (8,9). Statistical analysis was performed using Wilcoxon's test and Spearman's correlation coefficients. Differences were considered significant at $P < 0.05$.

The protocol was approved by the Human Care Committee of the Medical University of Bialystok (approval Nr R-I-003/85/2004). All of the patients gave informed consent for the examination.

RESULTS

The activities of total ADH, ALDH, and ADH isoenzymes in the sera are demonstrated in Table 1. The total ADH activity was not significantly higher (about 6.3%) in patients with breast cancer than in healthy controls. The analysis of ALDH activity did not indicate a significant difference between the patient group and healthy controls.

The comparison of ADH isoenzymes activities showed that the highest difference was exhibited by class I ADH. The mean activity of this class of isoenzymes in the total cancer group increased about 7.1% (1.286 ± 0.48 mU/L) in comparison to the control level (1.195 ± 0.56 mU/L). This increase was not statistically significant. Other tested ADH isoenzymes also did not exhibit marked differences in activities between the patient and control groups.

When we analyzed the activity of particular ADH isoenzymes depending on the progression stage of the carcinoma, we observed a tendency toward increased ADH I activity in accordance with the advance of

TABLE 1. ADH and ALDH Activity in the Sera of Patients with Breast Cancer

	ADH I (mU/L)	ADH II (mU/L)	ADH III (mU/L)	ADH IV (mU/L)	ADH total (U/L)	ALDH total (mU/L)
($n = 45$)						
Breast cancer	1.286 ± 0.48	9.58 ± 1.23	10.96 ± 1.24	4.75 ± 0.94	0.582 ± 0.053	2.456 ± 0.66
Control	1.195 ± 0.56	9.26 ± 0.98	11.32 ± 1.82	4.58 ± 0.82	0.545 ± 0.046	2.416 ± 0.54
	$P = 0.062$	$P = 0.562$	$P = 0.745$	$P = 0.342$	$P = 0.546$	$P = 0.658$
I ($n = 12$)						
Breast cancer	1.243 ± 0.35	9.56 ± 1.55	10.58 ± 1.62	4.66 ± 0.74	0.538 ± 0.068	2.475 ± 0.46
Control	1.195 ± 0.56	9.26 ± 0.98	11.32 ± 1.82	4.58 ± 0.82	0.545 ± 0.046	2.416 ± 0.54
	$P = 0.082$	$P = 0.584$	$P = 0.776$	$P = 0.404$	$P = 0.832$	$P = 0.595$
II ($n = 10$)						
Breast cancer	1.257 ± 0.55	9.42 ± 1.27	11.05 ± 1.84	4.53 ± 0.63	0.557 ± 0.032	2.402 ± 0.49
Control	1.195 ± 0.56	9.26 ± 0.98	11.32 ± 1.82	4.58 ± 0.82	0.545 ± 0.046	2.416 ± 0.54
	$P = 0.071$	$P = 0.644$	$P = 0.718$	$P = 0.965$	$P = 0.668$	$P = 0.836$
III ($n = 12$)						
Breast cancer	1.294 ± 0.68	9.77 ± 1.18	10.83 ± 1.053	4.89 ± 0.65	0.590 ± 0.065	2.461 ± 0.68
Control	1.195 ± 0.56	9.26 ± 0.98	11.32 ± 1.82	4.58 ± 0.82	0.545 ± 0.046	2.416 ± 0.54
	$P = 0.066$	$P = 0.473$	$P = 0.744$	$P = 0.132$	$P = 0.354$	$P = 0.745$
IV ($n = 11$)						
Breast cancer	1.325 ± 0.64	9.62 ± 1.35	10.96 ± 1.08	4.82 ± 0.51	0.612 ± 0.055	2.473 ± 0.63
Control	1.195 ± 0.56	9.26 ± 0.98	11.32 ± 1.82	4.58 ± 0.82	0.545 ± 0.046	2.416 ± 0.54
	$P < 0.001$	$P = 0.577$	$P = 0.755$	$P = 0.368$	$P = 0.182$	$P = 0.520$

disease. Significantly higher activity of class I ADH was found in stage IV cancer patients compared to the control group ($P < 0.05$). The mean activity of ADH I was 1.328 ± 0.4 mU/L in the sera of three patients with liver metastasis, 1.318 ± 0.66 mU/L in two patients with lung metastasis, and 1.318 ± 0.66 mU/l in six patients with bone metastasis. The other isoenzymes did not exhibit any characteristic changes of activity in patients in various advancing stages of tumor.

The activity of total ADH was not statistically different, although there was a tendency toward increased activity in the more advanced stages of tumor.

DISCUSSION

Breast cancer is one of the most frequently occurring aggressive neoplasms. Early detection of cancer in this organ is very important. Therefore, it is important to find markers that may be helpful for diagnosing breast cancer. Some authors have demonstrated ADH and ALDH activities in the cells of healthy parenchyma (10). In a previous study (unpublished results) we found that the activity of total ADH was lower (insignificantly) in breast cancer cells than in healthy tissues, but the activity of class I ADH was significantly lower. Therefore, the activity of this isoenzyme in the case of breast cancer is contrary to that of other cancers. For example, the activities of total ADH and class I of ADH are significantly higher in colorectal cancer than in healthy colon mucosa (11). Therefore, the activity of class I ADH isoenzymes is significantly elevated in the sera of patients with colorectal cancer (unpublished results). The present study demonstrates that the activity of class I ADH was insignificantly higher in the sera of patients with breast cancer than in healthy controls. However, the majority of the patients with colorectal cancer were drinkers, and the patients with breast cancer drank alcohol occasionally. The serum activity of class I ADH isoenzymes appeared to have a tendency to increase in accordance with the advance of disease. The activity of ADH I was significantly higher in the sera of patients with stage IV breast cancer compared to healthy controls. Tumors were classified and staged in accordance with the UICC-TNM classification system. Of the patients in stage IV, six had bone metastasis, three had liver metastasis, and two had lung metastasis.

The pretreatment staging procedures included chest X-ray and CT. The activities of enzymes (ALT and AST) and bilirubin concentration, tested as markers of liver lesions were significantly elevated. Class I ADH isoenzymes are expressed mainly in the liver, but they are also present in lungs (12). The elevated activity of class I isoenzymes in our study apparently was caused by the enzyme released from metastatic cells or organ

damaged by metastatic disease. Metastatic cancers are infiltrating tumors that act aggressively on healthy tissues and lead to the release of the enzyme from normal cells (13). These suppositions are in accordance with those of Chrostek and Szmikowski (14), who showed increased ADH I activity in the sera of patients with metastatic liver tumors. The other isoenzymes did not exhibit any characteristic changes of activity in patients in various advancing stages of tumor. The activity of total ADH was not statistically different, although there was a tendency toward increased activity in the more advanced stages of tumor. The increase of serum total ADH in the course of breast cancer is probably due to an elevation of class I ADH isoenzymes. In a previous study we showed that the differences of other tested classes of ADH isoenzymes activities (except ADH I) between cancer cells and healthy parenchyma were not statistically significant. The activity of ALDH also did not differ between the two types of tissues (unpublished results). The activity of ALDH in serum did not show significant one-way changes in the more advanced cancers, and ALDH activity in breast cancer was very low, especially when compared with ADH activity.

In conclusion, we can state that the activity of class I ADH isoenzymes was elevated in the sera of patients with stage IV breast cancer. We believe the observed changes resulted from isoenzymes being released from the organ damaged by metastatic disease, rather than from secondary tumors.

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