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CHANGES IN HUMAN SERUM ALCOHOL DEHYDROGENASE ACTIVITY DURING RETINOIC ACID TREATMENT OF CANCER PATIENTS
ALI REZA WALADKHANI*, PETER KUNZ, WERNER ZIMMERMANN1 and MICHAEL ROLAND CLEMENS

Krankenanstalt Mutterhaus der Borromäerinnen, Feldstraße 16, 54219 Trier and 1Pharmazeutisches Institut der Universität Tübingen, 72076 Tübingen, Germany

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Abstract — Retinoids can inhibit cell growth and induce cell differentiation in experimental tumour models. Human alcohol dehydrogenase (ADH) exists as a group of enzymes that can be placed into five classes based upon structural and functional distinctions. Human class I ADH catalyses the oxidation of a wide variety of alcohols including ethanol and retinol, whereas human class II ADH does not catalyse the oxidation of retinol. Using specific fluorescent substrates, class I and class II ADH activity in human sera was determined. No significant changes in class I or II activity were observed after 4 weeks of treatment with c/j-retinoic acid (cRA). While total ADH activity was increased from 84 ± 78 mU/1 to 206 ± 70 mU/1 (mean ± SD, P < 0.02) after 1 week of treatment, there were no further significant changes after 4 weeks of treatment with cRA. Sex-related differences were observed on total ADH activity after 1 week of treatment with cRA. Although total ADH activity of patients with cancer of the cervix increased significantly after 1 week of treatment, there were no significant changes in total activity in head and neck cancer patients. This sex-related difference might be dependent on the stage of the menstrual cycle. The elimination of ethanol in women can be either faster or slower than in men depending on the stage of menstrual cycle. This study therefore suggests that the main ADH activity observed in serum belongs to class II, and not to class I ADH. The data from this study also suggest that retinoic acid has a positive feedback effect on total ADH activity after 1 week of treatment.

INTRODUCTION

Synthetic analogues of retinol (retinoids) are capable of inhibiting cell growth and inducing cell differentiation in experimental tumour models (Meyskens et al., 1985; Lipman et al., 1987a,b). They can suppress carcinogenesis in a variety of epithelial tissues, including skin, trachea, lungs, and the oral mucosa in animals and humans (Lippman et al., 1987a; Bertram et al., 1987; Kraemer et al., 1988). The precise mechanism of the anticarcinogenic effects of retinoids is not fully understood (Lippman et al., 1987a,b).

Studies on the enzymatic pathway controlling the synthesis of retinoic acid from its vitamin A precursor, retinol, have shown that enzyme alcohol dehydrogenase (ADH EC1.1.1.1) from humans and other mammals functions as a cytosolic retinol dehydrogenase in the conversion of retinol to retinoic acid in vitro (Mezey and Holt, 1971; Kim et al., 1992; Yang et al., 1993). ADH is a well-characterized enzyme that converts ethanol into acetaldehyde reversibly using NAD+/NADH as cofactors (Branden et al., 1975). Human ADH exists as a group of enzymes that can be placed into five classes based upon structural and functional distinctions (Strydom and Vallee, 1982; Algar et al., 1983; Yin et al., 1990; Moreno and Pares, 1991; Pares et al., 1992; Jörnvall and Höög, 1995). The classes also contain isoenzymes, particularly those of class I. Human class I ADH (ADH1, ADH2, and ADH3) catalyses the oxidation of a wide variety of alcohols including ethanol (Li, 1977) and retinol (Mezey and Holt, 1971), whereas human class II ADH does not catalyse retinol oxidation. It has been demonstrated that the ADH3 gene can be induced by retinoic acid in tissue culture cells (Shean and Duester, 1992), and that the ADH3 promoter possesses a retinoic acid response element. Further evidence of class I ADH playing a controlling role...
Table 1. Blood parameters for cancer patients

<table>
<thead>
<tr>
<th>Patients and numbers</th>
<th>Hb (g/dl)</th>
<th>GOT (U/l)</th>
<th>GPT (U/l)</th>
<th>ω-GT (U/l)</th>
<th>Albumin (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total patients (n = 14)</td>
<td>12.7 ± 1.6</td>
<td>16.4 ± 18.8</td>
<td>15.5 ± 14.3</td>
<td>79 ± 112</td>
<td>20.7 ± 19.4</td>
</tr>
<tr>
<td>Cervical cancer patients (n = 7)</td>
<td>11.7 ± 1.2</td>
<td>17.3 ± 27.2</td>
<td>11.1 ± 9.4</td>
<td>81 ± 130</td>
<td>16.6 ± 17.6</td>
</tr>
<tr>
<td>Head and neck cancer patients (n = 7)</td>
<td>13.2 ± 1.6</td>
<td>16.0 ± 12.8</td>
<td>17.6 ± 15.8</td>
<td>78 ± 100</td>
<td>19.9 ± 16.6</td>
</tr>
</tbody>
</table>

Values are means ± SD. Abbreviations used: Hb, haemoglobin; GOT, glutamate-oxaloacetate aminotransferase; GPT, glutamate-pyruvate aminotransferase; ω-GT, ω-glutamyltransferase.

in human retinoic acid synthesis was provided by the finding that retinoic acid feedback activates expression of the human class I ADH gene, ADH3 (Duester et al., 1991).

The aim of this study was to determine if there were any changes in ADH activity in cancer patients undergoing retinoic acid therapy.

SUBJECTS AND METHODS

Subjects

The study was carried out on the serum of 14 cancer patients suffering from metastatic squamous cell carcinoma. The patients were divided into 'cervical cancer patients' (female, n = 7) and 'head and neck cancer patients' (male, n = 7) (Table 1). Eight healthy volunteers were also included, four females and four males. Heparinized blood samples were collected and stored until measurement at −70°C.

Chemicals

4-Methylpyrazole and 4-methoxy-1-naphthaldehyde (IA) were purchased from Sigma (Germany). The syntheses of 6-methoxy-2-naphthaldehyde (IIA), 6-methoxy-2-naphthylmethanol (IIIB), and 4-methoxy-1-naphthylmethanol (IIB) have been described (Wierzchowski et al., 1989). p-Nitrosodimethylaniline, n-butanol, and pyrazole were purchased from Aldrich (Germany). Both oxidized and reduced nicotinamide adenine dinucleotide (NAD⁺ and NADH respectively) were from Boehringer Mannheim (Germany). Horse liver ADH was purchased from Sigma (Germany).

Determination of class I and class II ADH activities

The concentrations of substrates were determined spectrophotometrically using a molar absorption of 5700 (M⁻¹ cm⁻¹) at 296 nm for IIB and 1270 (M⁻¹ cm⁻¹) at 330 nm for IIB. The fluorimetric ADH procedure was performed as described previously (Wierzchowski et al., 1992). All reaction mixtures were prepared in 4-ml fluorometric cuvettes by the addition of 150 μl of a 300 μM solution of either IA or IIA, 100 μl of 1 mM NADH, and 2690 μl of buffer. After thermal equilibration for 5 min, 60 μl of serum were added and the change in fluorescence recorded with a Perkin–Elmer LS-3B, excitation wavelength 316 nm, bandwidth 10 nm, for both substrates and emission wavelength 370 nm, bandwidth 10 nm, for IIA and 360 nm for IIA. The linear increase in fluorescence was recorded for up to 10 min. After this period, the signal was once again compensated for by zero suppression, and 60 μl of the product, final concentration 4–6 μM, were added to provide an internal standard.

Determination of total ADH activity

Minute activities of ADH in blood serum can be determined by lessening of the yellow colour of p-nitrosodimethylaniline in a recycling reaction. This aldehyde-like substance is reduced enzymatically to a colourless compound in the presence of NADH which is generated by ADH from NAD⁺ and n-butanol. p-Nitrosodimethylaniline (NDMA), a strongly coloured substance (E₄₄₀ = 35 mM⁻¹) is reduced by liver ADH by adding NADH to the colourless hydroxylamine derivate. NDMA is reduced enzymatically by NADH, whereas alcohol is oxidized enzymatically by NAD⁺, the enzyme recycling between its oxidized and reduced forms. ADH from human serum and horse liver shows a high relative activity with n-butanol as substrate in comparison with ethanol and other alcohols.

Serum ADH activity was measured using the modified incubation method of Skursky et al. (1979). The reaction mixture (600 μl) contained...
Table 2. Determination of class I and class II ADH and total ADH activity in plasma from cancer patients after 4 weeks of
13-cis retinoic acid therapy

<table>
<thead>
<tr>
<th>Subjects and numbers</th>
<th>Time (week)</th>
<th>Class I ADH (mU/l)</th>
<th>Class II ADH (mU/l)</th>
<th>Total ADH (mU/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female controls (n = 4)</td>
<td>0</td>
<td>0.47 ± 0.19</td>
<td>22.0 ± 5.6</td>
<td>—</td>
</tr>
<tr>
<td>Male controls (n = 4)</td>
<td>0</td>
<td>0.69 ± 0.28</td>
<td>11.2 ± 2.6</td>
<td>—</td>
</tr>
<tr>
<td>Total patients (n = 14)</td>
<td>0</td>
<td>1.40 ± 2.02</td>
<td>20.1 ± 6</td>
<td>127 ± 148</td>
</tr>
<tr>
<td>Total patients (n = 14)</td>
<td>4</td>
<td>2.10 ± 3.02</td>
<td>21.0 ± 11</td>
<td>228 ± 221</td>
</tr>
<tr>
<td>Cervical cancer patients (n = 7)</td>
<td>0</td>
<td>1.80 ± 2.01</td>
<td>18.7 ± 8</td>
<td>145 ± 186</td>
</tr>
<tr>
<td>Cervical cancer patients (n = 7)</td>
<td>4</td>
<td>2.90 ± 3.01</td>
<td>21.5 ± 11</td>
<td>306 ± 277</td>
</tr>
<tr>
<td>Head and neck cancer patients (n = 7)</td>
<td>0</td>
<td>1.01 ± 1.03</td>
<td>22.0 ± 3</td>
<td>103 ± 109</td>
</tr>
<tr>
<td>Head and neck cancer patients (n = 7)</td>
<td>4</td>
<td>1.10 ± 1.04</td>
<td>20.4 ± 13</td>
<td>124 ± 63</td>
</tr>
</tbody>
</table>

Values are means ± SD.
Significance: Class II ADH between healthy male volunteers and head and neck cancer patients before treatment
P < 0.05.

25 mM NDMA, 0.25 mM NAD⁺, and 12.5 mM n-butanol in a 0.1 M Na-phosphate buffer, pH 8.5. The reference solution had the same composition, but also contained 10 mM pyrazole. A 150 μl portion of serum was added to both solutions and the mixtures were incubated for 20 min at 25°C. The difference in A440 between the sample with pyrazole and the sample without pyrazole was read after the reaction had been stopped by the addition of 15 ml of 0.5 M pyrazole to the sample in which the inhibitor was absent. ADH activity was read from a calibration graph.

RESULTS

The activity of class II ADH was significantly (P < 0.05) higher in head and neck cancer patients than in healthy male volunteers before treatment with cRA (Table 2). After 1 week of treatment with cRA total ADH activity increased significantly from 84 ± 78 mU/l to 206 ± 70 mU/l (mean ± SD, P < 0.02). We therefore studied sex-related differences after 1 week of treatment with cRA on total ADH activity. Whereas the total ADH activity of cervical cancer patients increased significantly (P < 0.05) after 1 week of treatment, we found no significant differences in total ADH activity of head and neck cancer patients before or after treatment (Fig. 1).

DISCUSSION

The first study involving determination of class I and class II ADH activity in human sera was performed by Wierzchowski et al. (1992) using specific fluorescent substrates. The results of the present study and the study of Wierzchowski et al. (1992) provide evidence that class II ADH is probably the principal form of human ADH in both normal and pathological sera.

ADH is an enzyme with multiple functions, being involved in the transformation of many alcohols and aldehydes of physiological interest (Boleda et al., 1993). The relative contribution of ADH to the detoxification of cytotoxic aldehydes depends on the amount and class of ADH present in the tissue, and on the presence of other pathways involved in this function. Class I ADH has the widest substrate specificity and, importantly, it is the most abundant in terms of amounts of protein. About 95% of class 1 ADH activity is concentrated in the liver (Boleda et al., 1989); this supports detoxification as being its main role. Class I ADH could represent a defence system to remove aldehydes resulting from lipids in the liver, and in the intestine, kidney, and testes. Class II ADH could also be involved in this function in human liver (Sellin et al., 1991; Boleda et al., 1993).

Total ADH activity increased significantly in patients with cancer of the cervix after 1 week of treatment with 13-cRA. This phenomenon was not observed in head and neck cancer patients. The positive effect of 13-cRA on ADH activity in patients with cancer of the cervix could result from induction mediated by a higher level of 13-cRA in cervical cancer patients than in head and neck cancer patients as previously reported.
It has been demonstrated that the ADH3 gene can be induced in tissue culture cells by retinoic acid (Shean and Duester, 1992) and that the ADH3 promoter possesses a retinoic acid response element. Furthermore, evidence for class I ADH playing a controlling role in human retinoic acid synthesis was provided by the finding that retinoic acid feedback activates the expression of the human class I ADH gene ADH3 (Duester et al., 1991). In the present study, we found no indication of any positive feedback mechanisms of retinoic acid on class I ADH activity. There was, however, a positive feedback mechanism on total ADH activity.

Sex-related differences in blood-alcohol concentration after an equivalent oral dose of alcohol have been widely reported (Batt, 1989; Mishra et al., 1989). The difference is said to be due to the smaller distribution volume of ethanol because of a lower tissue-water content resulting from the larger lipid content, hormone differences, and lower ADH activity in females. Indeed, lower gastric ADH activity in women has been shown to increase the bioavailability of ethanol (Frezza et al., 1990). Depending on the stage of menstrual cycle, the elimination of ethanol in women can be either faster or slower than in men (Sutker et al., 1987).

The data from this study provide evidence that retinoic acid exerts a positive feedback mechanism either on class I ADH or on class II ADH activity. Furthermore, the data also indicate a positive feedback mechanism of retinoic acid on total ADH activity after treatment for 1 week.

REFERENCES


