Estrone and Dehydroepiandrosterone Sulfatase Activities and Plasma Estrone Sulfate Levels in Human Breast Carcinoma

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ABSTRACT

The activity of the two membrane-bound sulfatases, estrone and dehydroepiandrosterone sulfatases, are reported in human breast carcinoma tissues. In 21 tested tumors (12 from postmenopausal women and 9 from nonmenopausal women), the two sulfatases were consistently present. The apparent Km values for estrone and dehydroepiandrosterone sulfatases were, respectively, 6.8 and 14.9 μM.

In terms of maximal velocity, the sulfatase activities are not correlated to the estrogen or progesterone receptor status of the tumors or to the hormonal status of the donors. It may be concluded that these two activities are not hormone dependent. Estrone sulfate, the substrate of estrone sulfatase, has been measured in plasma of postmenopausal women. The mean levels (nmol/liter) of plasma estrone sulfate were compared in postmenopausal women with (n = 51) or without (n = 39) breast cancer.

For the first age group (48 to 55 years old), no statistically significant difference in these levels was observed [1.91 ± 1.06 versus 1.50 ± 1.04 (mean ± S.D.; S.E.)]. For the two other age groups (56 to 65 and 66 to 80 years of age), the differences were statistically significant [1.46 ± 0.43 versus 0.77 ± 0.21 (p < 0.02) and 1.77 ± 0.53 versus 0.81 ± 0.22 (p < 0.01)].

The usefulness of plasma estrone 3-sulfate levels as an indicator of the real estrogen status of postmenopausal women is discussed.

INTRODUCTION

High concentrations of E1-S^3 (9, 15, 21, 22) in blood provide a potentially high flux of free estrogens to mammary tumors, due to the presence of arylsulfatase C (aryl sulfate sulfohydrolase; EC 3.1.6.1) (6, 13, 31). The transformation of E1-S into 17β-estradiol in human mammary carcinoma has been reported by Wilking et al. (31), and Vignon et al. (30) concluded that estrone sulfates can act in vitro as estrogen precursors in human breast cancer cells containing sulfatases. The biological role of E1-S as an estrogen precursor has also been found in other mammalian tissues (5, 16, 24).

The first step for the activation of E1-S into the physiologically active 17β-estradiol and estrone (5, 20, 24) is its hydrolysis by sulfatases. Estrone sulfatase is a membrane-bound enzyme (20, 29) and, in rat liver, it has been reported that this enzyme is identical to arylsulfatase C (7, 12). In mammalian tissues, the question remains whether the hydrolysis of E1-S and DHEA-S is catalyzed by a single or by 2 distinct membrane-bound enzymes (10–12, 20). However, Dao et al. (6) reported that, when human breast tumors exhibited estrone sulfatase activity, this enzyme was not consistently associated with DHEA sulfatase.

In this study, human breast cancers were investigated in order to relate membrane-bound sulfatase activities to the hormonal status of the donors and to the hormone responsiveness of the tumors as determined by estrogen and progesterone cytosol receptors. The levels of plasma estrone-sulfate, the substrate of estrone sulfatase, were also determined in postmenopausal women with or without breast cancer.

MATERIALS AND METHODS

Chemicals

DHEA, estrone, cortisol, diethylstilbestrol, E1-S, and DHEA-S were purchased from Sigma Chemical Co. (St. Louis, MO.). R-5020 (17,21-dimethyl-19-nor-4,9-pregnadiene-3,20-dione) was a generous gift from Roussel Uclaf (Paris, France). [17α-methyl-3H]R-5020 (specific activity, 87 Ci/mmol; [6,7-3H]E1-S (specific activity, 53.0 Ci/mmol; [7-3H]DHEA-S (specific activity, 24.0 Ci/mmol), and [4,14C]estradiol (specific activity, 57.8 mCi/mmol) were purchased from New England Nuclear (Boston, Mass.). [4,14C]estrone (specific activity, 52.0 mCi/mmol) was purchased from the Radio Chemical Centre (Amersham, England). Before use, steroids were purified as described previously (5, 20). TED buffer contained 10 mM Tris-HCl, pH 7.40, containing 1.5 mM Na2EDTA and 0.5 mM diethiothreitol. All reagents were of analytical grade (Merck, E. Merck, Darmstadt, Chelles, France).

Tumor Tissue Preparation

Human primary tumor specimens removed at the time of surgery were promptly trimmed of fat and connective tissues and frozen in liquid nitrogen. In all cases, histological diagnosis of carcinoma was confirmed from block sections. Tissues were processed within 2 weeks of storage. The specimens, weighing 300 to 500 mg, were pulverized from the supercooled state (Thermovac, Touzart et Matignon, Vitry sur Seine, France) and homogenized with a Potter-Elvehjem instrument with 6 volumes (w/v) of TED buffer. The homogenate was centrifuged at 105,000 × g for 1 hr. The supernatant (cytosol) was used for receptor assays. The pellet was suspended in an equal volume of 50 mM Tris-HCl, pH 7.0, containing 0.25 mM saccharose and centrifuged at 100,000 × g for 10 min. This supernatant (enzyme preparation) was used for DHEA and estrone sulfatase activities.

PGR and ER Assays

A single saturation assay was used according to the method of Pichon and Milgrom (18). Each assay was done in triplicate.

PGR. Briefly, cytosol (100 μl) was added to a test tube containing 20 mM (3H)R-5020 and 10 μM of cortisol. Parallel tubes also contained 2 μM of R-5020. After incubation for 2 hr at 4°C, 100 μl of TED buffer, 40 mM Na2MoO4, glycerol 60% (v/v) were added. After 2 hr at 4°C, 200 μl of TED was added and the tubes were centrifuged for 15 min at 100,000 × g. An aliquot of the supernatant was added to a test tube containing 100 mM trichloroacetic acid (TCA). After 1 hr at 4°C, the tubes were centrifuged at 100,000 × g, and the supernatant was discarded. The pellets were boiled for 1 hr with scintillation solution and counted in a Packard liquid scintillation counter.

ER. The assay was performed as described by Pichon and Milgrom (18), with the following modifications. The incubation period was 16 hr at 4°C. After incubation and centrifugation, the pellets were dissolved in 0.1 N NaOH, and the radioactivity was measured by liquid scintillation spectrometry. The results were expressed as fmoles of bound hormone or as percent of total hormone bound.
buffer, 20 mM Na₂MoO₄ glycerol 30% (v/v), 0.5% Norit A, and 0.05% dextran T-70 (Pharmacia, Bois d’Arcy, France) were added. After 30 min at 4°, the tubes were centrifuged, and an aliquot was removed for counting.

ER. The same protocol was used as for PGR with the following differences. The cytosol (100 μl) was incubated with 10 nm [³H]17β-estradiol with or without 100-fold excess of unlabeled diethylstilbestrol. After 18 hr at 4°, 100 μl of TED buffer and 200 μl of TED buffer, 0.5% Norit A, and 0.05% dextran were added and, after 3 hr at 4°, the tubes were centrifuged, and an aliquot was removed for counting.

Specific binding was considered to be the difference between total and nonsaturable binding (statistical analysis of differences was calculated using Student’s t test). Tumors with receptor values below 10 fmol/mg cytosol protein were considered as clinically receptor poor (PGR- and ER-).

**Estrone and DHEA Sulfatase Assays**

The method has been described previously (20). Briefly, assays were carried out at 5 substrate concentrations of 2, 3, 5, 10, and 20 μM in 0.05 M Tris-HCl buffer, pH 7.3, for DHEA sulfatase or 1, 2, 3, 5, and 10 μM in 0.05 M Tris-HCl buffer, pH 7.0, for estrone sulfatase in a final volume, including enzyme preparation, of 0.5 ml. The reaction was started by adding the enzyme preparation and stopped after 1 hr at 37° by adding 1 ml of 0.1 M Na₂CO₃. Liberated DHEA or estrone was extracted by 4 ml of petroleum benzine or diethyl ether, respectively, and the methodological losses were corrected by [¹⁴C]DHEA or [¹⁴C]estrone. The method was optimized in terms of incubation time, quantity of enzyme preparation, and concentrations in substrates for breast cancers. Lineweaver-Burk reciprocal plots were drawn (lines were calculated by the method of least mean squares) to determine Km and Vₘₐₓ.

**Hormone Assays**

Blood samples were collected on EDTA by venipuncture between 8 and 10 a.m. from subjects who had fasted for 12 hr. Plasma was stored at −20° in several assay-tubes until processing. Commercial RIA kits purchased from BioMérieux (Lyon, France) were used for the determination of plasma 17β-estradiol, progesterone, and FSH according to the manufacturer’s procedure as reported previously (20, 21). E₁-S in plasma was measured by a specific method developed in our laboratory (21). Briefly, free steroids were extracted from plasma with diethyl ether, and E₁-S was isolated with use of Vitors’ reagent (methylene blue in diluted H₂SO₄/Na₂SO₄ solution). After enzyme hydrolysis, free estrone was isolated by Celite chromatography and measured by RIA (Estrone RIA kit; BioMérieux). The precision was 10%.

**RESULTS**

**Hormonal Status.** The hormonal status of postmenopausal women (FSH: 48.8 ± 6.1 IU/liter; mean ± t₀.⁹⁵ ± s/n with (n = 51) or without (n = 39) breast cancer, was compared (Table 1). Blood was collected in breast cancer patients just before mastectomy. Postmenopausal women without breast cancer were apparently healthy. Patients were grouped in 3 age groups. These groups were chosen so that the mean ages for each of them are not significantly different for women with and without breast cancer. No significant difference was noted for plasma 17β-estradiol or progesterone levels between the different groups. E₁-S was higher in postmenopausal women with breast cancer than in normal postmenopausal women. The differences were statistically significant only for the groups 56 to 65 and 66 to 80 years of age. Previously, some of us did not notice such an increase of E₁-S in postmenopausal women with breast cancer. However, the results had not been compared by age group. Although statistically nonsignificant, the higher levels of E₁-S found in women without breast cancer who were 48 to 55 years old can explain this difference (21). Factors such as the subject’s weight or degree of obesity influence the plasma E₁-S concentrations in postmenopausal women (15). However, in the present study, no statistical difference was observed in weight (p > 0.10) between postmenopausal women with or without breast cancer.

**Steroid Sulfatase Activities.** Estrone and DHEA sulfatase activities were measured in breast carcinoma tissues. Tumors were obtained from 12 postmenopausal women (age, 63.2 ± 9.0 years; plasma FSH, 35.13 ± 12.47 IU/liter; mean ± t₀.⁹⁵ ± s/n) and from 9 nonmenopausal women (age, 41.9 ± 4.8; plasma FSH, 5.49 ± 2.66). E₁ and DHEA sulfatase activities were consistently present in all tested tumors. A typical Lineweaver-Burk reciprocal plot is presented (Chart 1). This plot was chosen in order to give an example of steroid sulfatase inhibition by high substrate concentration. Such an inhibition is usual for steroid sulfatase activities (see review in Ref. 20). The Km and Vₘₐₓ values are reported in Table 2 according to the hormonal status of the donors. No statistically significant difference was observed between the 2 groups, i.e., menopausal and nonmenopausal women. It appears that the kinetic parameters of estrone and DHEA sulfatases are not influenced by the hormonal status of the donors. The mean value of Km is lower for estrone sulfatase (6.75 μM) than for DHEA sulfatase (14.89 U.M;n = 21). The activity of estrone sulfatase was consistently higher than that of DHEA sulfatase (14.89 μM;n = 21). The activity of estrone sulfatase was consistently higher than that of DHEA sulfatase, and the 2 activities were correlated in all tested tumors (r = 0.72; p = 0.001). For postmenopausal women, no statistically significant correlation was found between age and estrone or DHEA sulfatase activities (p = 0.93 and p = 0.55). Estrone and DHEA sulfatase activities were correlated with the presence of ER or PGR receptors (Chart 2). The receptor distributions, in percentage of the total tested tumors

<table>
<thead>
<tr>
<th>Classes (yr)</th>
<th>Status (n)</th>
<th>Age (yr)</th>
<th>Estrone-sulfate (pmol/liter)</th>
<th>p</th>
<th>Estradiol (pmol/liter)</th>
<th>p</th>
<th>Progesterone (pmol/liter)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>48–55</td>
<td>Without breast cancer (6)</td>
<td>51.3 ± 2.9a</td>
<td>1.50 ± 1.04b</td>
<td>&gt;0.10c</td>
<td>90 ± 46d</td>
<td>NSd</td>
<td>1.24 ± 0.82d</td>
<td>NS</td>
</tr>
<tr>
<td>56–65</td>
<td>With breast cancer (9)</td>
<td>51.4 ± 1.8</td>
<td>1.91 ± 1.06</td>
<td>&lt;0.02</td>
<td>88 ± 25</td>
<td>NS</td>
<td>1.78 ± 0.70</td>
<td>NS</td>
</tr>
<tr>
<td>66–80</td>
<td>With breast cancer (13)</td>
<td>59.5 ± 1.8</td>
<td>0.77 ± 0.21</td>
<td>&lt;0.01</td>
<td>73 ± 16</td>
<td>NS</td>
<td>1.79 ± 0.48</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>With breast cancer (17)</td>
<td>60.2 ± 1.1</td>
<td>1.48 ± 0.43</td>
<td>&lt;0.02</td>
<td>90 ± 23</td>
<td>NS</td>
<td>1.63 ± 0.41</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>With breast cancer (20)</td>
<td>73.4 ± 2.0</td>
<td>0.41 ± 0.22</td>
<td>&lt;0.01</td>
<td>82 ± 23</td>
<td>NS</td>
<td>2.18 ± 0.75</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>With breast cancer (22)</td>
<td>72.6 ± 1.8</td>
<td>1.77 ± 0.53</td>
<td>&lt;0.01</td>
<td>82 ± 23</td>
<td>NS</td>
<td>1.63 ± 0.41</td>
<td>NS</td>
</tr>
</tbody>
</table>

*a Mean ± t₀.⁹⁵ ± s/n
b Mean of duplicate determinations.
c Values were compared statistically by Mann-Whitney U test.
d NS, not significant.
were ER+PGR+, 55.0%; ER+PGR-, 20.0%; ER-PGR+, 10.0%; and ER-PGR-, 15.0%. (The small number of cases has prevented us from making a distinction between menopausal and nonmenopausal women.) No significant difference between the presence and the absence of receptors was found in the activities of estrone sulfatase or DHEA sulfatase.

DISCUSSION

The isotope dilution method used to study estrone and DHEA sulfatase activities was the preferred method so as to be able to: (a) evaluate 2 kinetic parameters, \( V_{\text{max}} \) and \( K_m \), and (b) obtain the highest possible sensitivity to detect low amount of enzyme. Published procedures (6, 31) determine sulfatase activities at one level of substrate. The measured velocities may be decreased by the presence and concentration of endogenous steroids used as substrates or endogenous inhibitors. Using 5 different concentrations of substrate, we could express enzyme activities in terms of \( V_{\text{max}} \). This kinetic parameter is independent of substrate concentration and remains unchanged in the presence of endogenous competitive inhibitors. These reasons explain why there were consistently estrone and DHEA sulfatase activities in breast carcinoma tissues. Dao et al. (6) tested 85 tumor preparations; 31 tumors exhibited estrone sulfatase activity, and 19 tumors also exhibited sulfatase activity toward DHEA-sulfate. Using a histochemical method, Koudstaal (13) showed arylsulfatase activity in only 14 of the 83 breast carcinomas studied. The activities, reported in terms of pmol product/mg protein/min in the relevant literature, were lower than those reported here [(mean, 0.414 ± 0.071; \( n = 27 \)) for estrone sulfatase activity by Wilking et al. (31) and 0.297 ± 0.095 \( (n = 16) \) and 0.015 ± 0.05 \( (n = 9) \) for estrone and DHEA sulfatase activities by Dao et al. (6)]. In agreement with the latter authors, the levels of DHEA sulfatase activity have been consistently found to be lower than those of estrone sulfatase. The \( K_m \) values reported here are widely dispersed. An endogenous factor may be responsible for the variation of the \( K_m \) from tumor to tumor. In sheep brain, such an endogenous factor decreasing arylsulfatase C \( K_m \) has been reported (14). However, the mean \( K_m \) values found for estrone and DHEA sulfatases are in the same order of magnitude (10^{-6} M) as those reported in different human tissues (11, 20, 27).

Estrone and DHEA sulfatase activities are present in all tested human breast tumors regardless of their ER or PGR status. Moreover, these 2 activities are not correlated to the hormonal status of the donors. Adams et al. (3), Pewnim et al. (17), and Wilking et al. (31) reported a correlation between steroid receptor status and estrogen sulfotransferase or 17β-hydroxysteroid de...
hydrogenase activities in human mammary carcinoma. This difference may be explained only if we suppose that, in breast cancers as in normal endometrium (28, 29), these 2 enzymes are induced by progestogen, whereas estrone and DHEA sulfatases are not hormone dependent. DHEA-S as well as Estr-S may be sources of estrogen, since free DHEA liberated from DHEA-S may be converted into estrogens in many human breast tumors (1, 4).

5-Androstene-3ß,17ß-diol, another product of DHEA, has been implicated as having a possible, influential estrogen action in hormone-responsive breast cancer (2). The intracellular levels of estrone and DHEA liberated from their sulfon conjugated precursors depend on the estrone and DHEA sulfatase activities and the circulating hormone concentrations. A significant increase of plasma Estr-S has been found in postmenopausal women with breast cancer. Studies in the plasma levels of unconjugated estrogens in breast cancer patients have been largely inconclusive (see review in Refs. 19 and 23). Samojlik et al. (25) have recently supported the usefulness of plasma Estr-S determinations to measure the real estrogen status of postmenopausal women, since Estr-S levels are approximately 10-fold higher than unconjugated estrogens (21, 26). In postmenopausal women, an emerging concept of stimulation of estrogen-responsive breast cancers can be formulated. Estrone sulfatase regulated the transport of Estr-S from the plasma to the cell tumors as unconjugated estrone. The biological activity of estrogen in hormone-responsive tissues has been reported by Vignon et al. (30) in human breast cancer cells and by Eckert and Katzenellenbogen (8) in human endometrial cells.

REFERENCES


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