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Comparison of estrogen concentrations, estrone sulfatase and aromatase activities in normal, and in cancerous, human breast tissues

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Abstract

In the present study, the concentrations of estrone (E_1), estradiol (E_2) and their sulfates (E_1S and E_2S), as well as the sulfatase and aromatase activities, were evaluated in post-menopausal patients with breast cancer. Comparative studies of the evaluation of these parameters were carried out in (a) tumor tissue, (b) areas surrounding the tumor, and (c) areas distant from the tumor (glandular tissue) which were considered as normal tissue. The levels (in pm/g; mean $\pm$ SEM) were: for E_1 in the (a) area: 320 ± 95; in (b): 232 ± 86; and in (c): 203 ± 71; for E_2 in the (a) area: 388 ± 106; in (b): 224 ± 48; and in (c): 172 ± 80; for E_1S in the (a) area: 454 ± 110; in (b): 259 ± 90; and in (c): 237 ± 65; for E_2S in the (a) area:318 ± 67; in (b): 261 ± 72; and in (c): 232 ± 75, respectively. The values of E_1S and E_2 were significantly higher in the tumor tissue than in the area considered as normal. In all the tissues studied, the sulfatase activity was much higher than aromatase (130–200). In addition, the sulfatase levels were significantly higher in the peripheral and in the tumor tissue than in the area considered as normal. The levels of aromatase were significantly higher in tumoral than in normal tissue. The present data extend the "intracrine concept" for breast cancer tumors. The physiopathology and clinical significance as promoter parameters in breast cancer is to be explored.

1. Introduction

There is substantial information that the human mammary cancer tissue contains the enzyme systems (aromatase, sulfatase, 17β-hydroxysteroid dehydrogenase) necessary for the last steps in the formation of estradiol (E_2), the hormone that plays an important role in the origin and evolution of this disease [1–3]. Estrogens in this carcinoma tissue can originate through two main pathways, one from aromatase, which converts androgens to estrogens [4–6] and the other from sulfatase, which converts estrone sulfate into estrone (E_1) [7–12]. Estrone is converted to the biologically active estradiol by the action of the 17β-hydroxysteroid dehydrogenase (Type I) [13–16]. Quantitative determinations in breast cancer tissues indicate that the "estrone sulfatase pathway" is 40–500 times that of the "aromatase pathway" [17,18]. The present study compares the concentrations of E_1, E_2, and their sulfates (E_1S and E_2S), as well as the aromatase and estrone sulfatase activities in (i) breast cancer tissue, (ii) the peripheral area of the tissue, and (iii) the area of the mammary gland considered as normal, in patients with breast cancer.

2. Materials and methods

2.1. Chemicals

[6,7-^{3}H]-Estrone sulfate (SA: 49.0 Ci/mmol), [7-^{3}H]-testosterone (SA: 27.7 Ci/mmol, [6,7-^{3}H]-estrone (SA:

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41.9 Ci/mmol), [4-^{14}C]-estrone (SA: 57.6 mCi/mmol), [4-^{14}C]-estradiol (SA: 57.0 mCi/mmol), were obtained from New England Nuclear Division (Du Pont de Nemours, Les Ulis, France). Estrone sulfate ammonium salt and β-NADPH were purchased from Sigma–Aldrich Chimie (St Quentin, Fallavier, France). Estrone and estradiol were obtained from Steraloids (Touzart et Matignon, Vitry-sur-Seine, France).

2.2. Patients and biological material

This study was carried out with 14 peri- and postmenopausal patients (aged 48–75 years) with breast cancer. None of the patients had a history of endocrine, metabolic or hepatic diseases or had received treatment in the previous 2 months. Each patient received local anesthesia (lidocaine 1%) and three regions of the mammary tissue were selected for the various analyses: (A) the tumoral tissue, (B) the surrounding area of the tumor, and (C) a distant zone (glandular tissue) which was considered as normal. The different tissue specimens removed at the time of surgery were promptly trimmed of fat and connecting tissues. A sample of each tissue was used for histology studies and classified according to the epithelial density. Samples were placed in liquid nitrogen and stored at −80°C until hormone or enzyme activity analysis.

2.3. Hormonal analysis

The various estrogens: estrone, estradiol, estrone sulfate, and estradiol sulfate, were evaluated in the tissues by radioimmunoassay (RIA) as described previously by Gelly et al. [19] and Pasqualini et al. [20]. The sensitivity (smallest quantity which is significantly different from zero at $p < 0.01$) of both E_2 and E_1 standard curves was determined to be 5 pg.

In the absence of added estrogen, the dilution of antiserum bound 52% [^{3}H]-E_2 and 48% [^{3}H]-E_1. The antiserum of E_2 was found to have no detectable cross-reaction with E_1, estriol (E_3), dehydroepiandrosterone, pregnenolone or cortisol and similarly for the antiserum of E_1 which does not cross-react with E_2, E_3, dehydroepiandrosterone or cortisol.

150–250 mg of the different tissues were homogenized in 70% ethanol using an ultraturrax apparatus (Ika-Werk, Janke & Kunkel, Staufen, Germany) and allowed to precipitate for at least 24 h at −20°C. [^{14}C]-E_2 (2000 dpm) and [^{3}H]-E_1S (10,000 dpm) were added to 5 ml of tissue homogenate in order to determine the losses of unconjugated or estrogen sulfates. After centrifugation at $900 \times g$ for 10 min, the supernatant was decanted and evaporated to dryness, then dissolved in 0.3 ml of distilled water plus 6 ml of ethanol, and precipitated again for 24 h at −20°C prior to centrifugation at $900 \times g$ for 10 min. The supernatant was evaporated to dryness, dissolved in 0.5 ml water, and the unconjugated estrogens extracted with 10 volumes of a mixture of ethyl acetate:hexane (3:2, v/v). The organic phase was evaporated and dissolved in 0.5 ml of the buffer solution (0.1 mol/l KH_2PO_4, 0.1 mol/l Na_2HPO_4, 0.15 mol/l NaCl, 0.1% gelatine, 0.1% NaN_3, pH 7.4), then processed for quantitative RIA determination of E_1 and E_2. Antisera for E_1 evaluation were purchased from Immunocorp (Montreal, Canada) and antisera for the evaluation of E_2 were a gift from the Foundation for Hormone Research (Fresnes, France). The aqueous phase was subjected to solvolysis for determination of E_1S or E_2S. Briefly, 2 ml ethanol were added to this aqueous phase and deproteinized at −20°C for 24 h, the protein pellet was removed by centrifugation and the ethanol fraction evaporated. The dry residue was dissolved in 0.9 ml of 0.9% NaCl + 0.1 ml H_2SO_4 (2 N) solutions extracted twice with 3 volumes of ethyl acetate and incubated overnight at 37°C. After neutralization with a concentrated Na_2CO_3 solution, the dry residue was dissolved in 0.5 ml of water, extracted with 10 volumes ethylacetate : hexane (3 : 2, vol/vol) and the freed estrone or estradiol were then analyzed and quantified as indicated above. These conditions of solvolysis resulted in 95–98% cleavage of authentic [^{3}H]-E_1S. The percentage recovery of E_1 was 73 ± 6 and that of E_2 68 ± 9. The blank values for E_1 and E_2 from charcoal-treated tissue extracts were negligible.

2.4. Enzyme assays

2.4.1. Estrone sulfate-sulfatase:

100–150 mg of the various areas of the breast were homogenized in 20 mmol/l Tris–HCl buffer solution (pH 7.4) for 15 s using an ultraturrax apparatus. Estrone sulfate-sulfatase activity was evaluated according to MacIndoe [21] Briefly, 100 ml of homogenate preparation (0.10–0.12 mg protein, evaluated according to Bearden [22]) and 200 ml of 20 mmol/l Tris-HCl buffer containing [^{3}H]-E_1S at 10^{-8}mol/l were incubated for 30 min at 37°C. The reaction was stopped by addition of 0.3 ml cold 0.1 mol/l Na_2CO_3 containing 5000 dpm [^{14}C]-E_1 to determine the recovery of the extraction process. The unconjugated steroids were extracted by adding 2 ml of toluene twice. Following freezing of the aqueous phase, the organic phase was transferred to a liquid scintillation vial. After evaporation of the solvent, 3 ml of Opti-fluor (Packard, Rungis, France) were added and the vials analyzed for [^{3}H] and [^{14}C] content. All determinations were performed in duplicate. E_1S-sulfatase activity is expressed in pmol of E_1 formed per mg of protein/h. The reaction rates were linear within the incubation times and the protein range employed.

2.4.2. Aromatase

Aromatase activity in homogenates of the different areas of the breast tissues was determined by isolation of [^{3}H]-E_1 and [^{3}H]-E_2 after incubation with [^{3}H]-testosterone according to Miller et al. [23]. Briefly, tissue homogenates (0.10–0.12 mg protein) were incubated for 2 h at 37°C in 20 mmol/l Tris–HCl buffer solution (pH: 7.4), [^{3}H]-testosterone at 10^{-8} mol/l and 1 mmol/l NADPH. The reaction was stopped by addition of ethanol containing [^{14}C]-E_1 and [^{14}C]-E_2 (5000 dpm of each) to monitor procedural losses. The amount of estrogens (E_1 and E_2) obtained was determined after separation by thin layer chromatography in ethyl acetate:cyclohexane (1:1, v/v). All determinations were performed in duplicate. Results are expressed in pmol of estrogens formed per mg protein/h. Formation of estrogens from testosterone was linear for the duration of the experiments (3 h) and the amount of product formed was a linear function of the amount of enzyme (preparation) incubated.

2.5. Statistical analysis

Results were expressed as the mean ± SEM. Statistical comparisons were made using the *t*-test. $P \leq 0.01$ was considered significant. For the different parameters studied, the intra-assay coefficient of variation was less than 9%, and the inter-assay coefficient of variation was less than 11%.

3. Results

3.1. Estrogen concentrations in the different areas of the breast

Mammary tissues of post-menopausal patients with breast tumors were divided into three areas: tumoral, peripheral and normal tissue. Fig. 1 shows the levels of the most important free and conjugated estrogens: estrone (E_1), estradiol (E_2), estrone sulfate (E_1S) and estradiol sulfate (E_2S) in each of these three areas. It was observed that: (1) the concentrations of the four estrogens were higher in the tumoral tissue than in the peripheral tissue or the areas of the breast considered as normal; (2) the conjugated estrogens and particularly E_1S were the most elevated in the three areas; (3) the level of E_1S was significantly higher in the tumoral tissue than in the peripheral tissue or the areas of the breast considered as normal ($p \leq 0.025$); the concentration of E_2 was significantly higher in the tumoral tissue than in the areas of the breast considered as normal ($p \leq 0.05$).

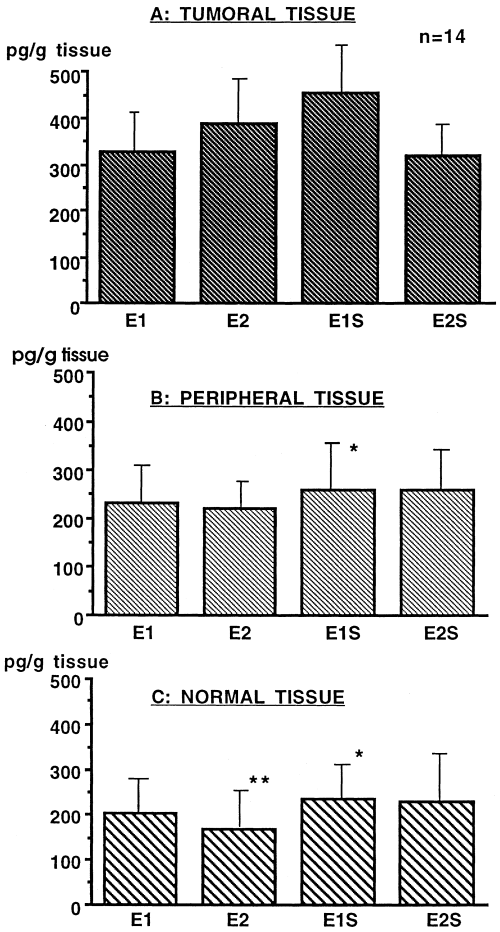

Fig. 1. Concentrations of estrone (E_1), Estradiol (E_2), Estrone-sulfate (E_1S) and estradiol sulfate (E_2S) in different tissular areas (tumoral, peripheral and normal) of patients with breast cancer. The different estrogens were evaluated as indicated in Section 2. Values (in pg/g tissue) are expressed as the mean ± SEM ($n = 14$). $*p \leq 0.025$ vs. E_1S in the tumoral tissue. $**p \leq 0.05$ vs. E_2 in the tumoral tissue.

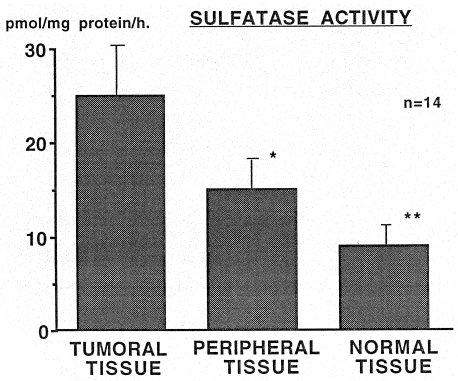

Fig. 2. Estrone sulfatase activity in different areas of breast cancer. Estrone sulfatase activity in the tissues of breast cancer patients ($n = 14$) was evaluated as indicated in Section 2. Values (in pmol/mg protein/h) are expressed as the mean ± SEM. $*p \leq 0.05$ vs. estrone sulfatase value in the tumoral tissue. $**p < 0.005$ vs. estrone sulfatase value in the tumoral tissue.

3.2. Estrone sulfatase and aromatase activities

3.2.1. Estrone sulfatase activity

As the sulfatase pathway, which converts estrogen sulfates to estrogens, and the aromatase pathway, which converts androgens into estrogens, are the two most important routes for the intratissular biosynthesis of E_2, it was of interest to evaluate these two activities in the mammary tissue of post-menopausal patients with breast tumors. Fig. 2 indicates the level of estrone sulfatase activity in the three different areas of the breast. The values show that the sulfatase activity is significantly higher in the tumoral tissue (25.2 ± 5.1 pmol/mg protein/h) than in the peripheral tissue (14.4 ± 3.7; $p \leq 0.05$) or the areas of the breast considered as normal (9.2 ± 2.8; $p < 0.005$).

3.2.2. Aromatase activity

Fig. 3 gives the values of the aromatase activity measured in the three areas of the breast. These levels show that: (1) the aromatase activity is 130–200 times weaker than the sulfatase activity in the three different areas of the breast; (2) the aromatase activity is significantly higher in the tumoral tissue (0.131 ± 0.028 pmol/mg protein/h) than in the areas of the breast considered as normal (0.06 ± 0.017; $p \leq 0.005$).

4. Discussion

One of the important findings of the present data is the high concentration of various estrogens in the mammary gland of peri- and post-menopausal patients with breast cancer. This is of particular interest as during this period of life the ovary has ceased to produce these estrogens, suggesting that the hormone can be produced in the same organ where it exerts its biological response, which is also in agreement with the information that breast cancer tissue possesses all the enzymes necessary for the bioformation of estradiol [4–16].

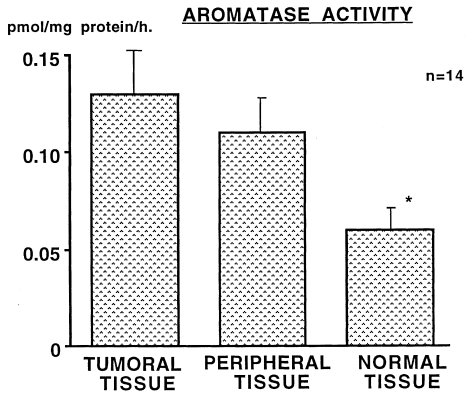

Fig. 3. Aromatase activities in different areas of breast cancer. Aromatase activity in the tissues of breast cancer patients ($n = 14$ were evaluated as indicated in Section 2. Values (in pmol/mg protein/h) are expressed as the mean ± SEM. $*p \leq 0.05$ vs. aromatase value in the tumoral tissue.

The most attractive information is the gradient in estrone sulfate (E_1S) and estradiol (E_2) when the values are between the cancer tissue and the area considered normal. In previous studies with breast cancer patients we found that the tissular concentration of estrogens, E_1S and E_2, are significantly higher than the circulating plasma levels [18].

Another aspect of these studies concerns the enzyme activities of E_1S and aromatase: the data show that sulfatase in all the tissues studied was significantly higher (130–200 times) than aromatase. With regard to the sulfatase activity the values were particularly intense in the area of the tumoral tissue. The aromatase activity was higher in the tumoral area than in the tissue considered as normal.

The present observations confirm previous studies showing that the formation of E_2 from E_1S is far more important than intratumoral aromatization [17]. The important gradient of sulfatase and also aromatase found between tumoral and normal tissue can explain the gradient in the levels of E_2 observed in these two areas. These data are in agreement with Naitoh et al. [24] and Söderqvist et al. [25] who report a lower rate of E_2 biosynthesis from E_1S in normal breast tissue than in breast cancer.

In this connection, it is interesting to mention that recently it was demonstrated that breast cancer patients with a high expression of sulfatase mRNA have significantly shorter disease-free survival compared with those whose levels are low [26].

We have also investigated the influence of the estrogen receptor (ER) status on the tissular concentration of estrogen and on the sulfatase and aromatase activities. The same 14 peri- and post-menopausal patients have been classified according to their ER (+) or ER (−) status. The results (data not shown) indicate no correlation between ER levels and those of tissular estrogens or of the two enzyme activities, indicating that ER is not significantly implicated in the local production of E_2 in breast tissue of post-menopausal patients.

5. Conclusion

The breast tumoral process is characterized by a modification of the estrogen metabolism, which corresponds to the establishment of two gradients: E_1S and E_2, and sulfatase and aromatase activities, when the tumoral and normal breast tissue are compared. These results indicate that therapeutical use of Selective Estrogen Enzyme Modulators (SEEM) can be an import-

ant additional treatment for breast cancer by reducing the rate of E_2 in the tumor.

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