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Research Paper

The serum estradiol concentration is the main determinant of the estradiol concentration in normal breast tissue

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ABSTRACT

Background: The estrogen concentration has been determined in breast tissue, focusing largely on samples obtained from breast cancers. In this study, estradiol concentration was determined in normal breast tissue obtained from women undergoing esthetic, and oncoplastic surgery.
Methods: Normal breast tissue was obtained during 68 operations for esthetic or reconstructive indications in women with and without a history of breast cancer. Our study included six different groups of women. The first three groups were normal cycling women, women taking oral contraceptives, and normal postmenopausal women, all undergoing a bilateral esthetic breast reduction. The second three groups were premenopausal and postmenopausal women, with a history of breast cancer and currently taking tamoxifen treatment, or postmenopausal women currently taking an aromatase inhibitor, needing contra-lateral corrective esthetic surgery.
Findings: In the group of women without history of breast cancer, normal cycling women ($n = 24$) presented a strong correlation ($r = 0.853$; $P < 0.0001$) between 17β-estradiol concentration in serum (median: 29.7 pg/mL; IQR: 10.8–82.3 pg/mL) and in breast tissue (30.6 pg/g; IQR: 18.6–183.8 pg/g). Postmenopausal women had low 17β-estradiol concentrations both in serum and breast tissue ($r = 0.813$; $P < 0.0001$, $n = 16$). Women taking oral contraceptives ($n = 12$) had low serum and breast tissue levels of estradiol ($r = 0.376$; $P =$ n.s.).

Premenopausal women ($n = 6$, mean age: 44.2 years) with a history of breast cancer and currently taking tamoxifen, had very high concentrations of 17β-estradiol both in serum (277.9 pg/mL; IQR: 96.2–544.7 pg/mL) and in epithelial cells (251.9 pg/g; IQR: 115.0–426.5 pg/g) ($r = 0.803$; $P < 0.001$). Postmenopausal women taking tamoxifen ($n = 4$, mean age: 48.3) had low concentrations of 17β-estradiol in serum (7.0 pg/mL; IQR: 5.7–16.3 pg/mL) and in epithelial cells (14.6 pg/g IQR: 13.3–16.3 pg/g) ($r = 0.10$; $P =$ n.s.). The estradiol concentration in the breast of premenopausal women taking tamoxifen was 8.2 times higher that observed in the breast of normal cycling women, and 17.3 times higher that observed in postmenopausal women taking tamoxifen. Women taking adjuvant aromatase inhibitors had extremely low concentrations of 17β-estradiol both in serum and in breast tissue.
Interpretation: This study shows that serum estradiol levels largely determine estrogen levels in normal breast tissue.

1. Introduction

Endogenous 17β-estradiol secretion by the follicular cell is stimulated by pituitary gonadotropins, mainly FSH, which are under control of the pulsatile secretion of the hypothalamic LH releasing hormone (LHRH). The latter is regulated through feed back by estradiol. When the hypothalamo-pituitary feedback is unlocked by an anti-estrogen blocking the cellular estrogen receptor, such as tamoxifen, the estradiol secretion is stimulated [1]. The latter seems to be an undesirable side effect in patients with estrogen-sensitive breast cancer. Tamoxifen has proven to be an effective adjuvant hormonal treatment in pre- and postmenopausal women with

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estrogen receptor-positive breast cancer, and has saved many lives [2]. In prospective trials tamoxifen was also effective in preventing both in situ and invasive breast cancer in pre- and postmenopausal women with increased risk of breast cancer [3–6]. It is still puzzling why tamoxifen is so effective in premenopausal women since it induces a high serum concentration of estradiol. Attempts to reduce this high 17β-estradiol serum concentration, with LHRH-agonists have failed to increase the effectivity of the tamoxifen treatment [7,8]. On the other hand, other studies clearly show a benefit of suppressing ovarian function during tamoxifen treatment, in premenopausal patients with a high risk for relapse [9,10]. Studies apparently give conflicting results. However, it could be that tamoxifen is effective, but only to a certain extent. Once estrogen levels are too high i.e. in young women [10], these levels may actually reduce the effectivity of tamoxifen. This overruling effect of high estradiol levels may be even more important in 'high risk' (node positive, high grade, large tumors) patients. There is certainly a rationale for the possible interference of high levels of estradiol since. It is known that steroid hormones diffuse freely from serum through the phospholipid membrane of the cells [11].

Several studies have measured estrogens in breast cancer tissue homogenates [12–15]. In breast cancer tissue estradiol production may be largely determined by up regulated endogenous local production. However, little is known on how this serum 17β-estradiol correlates with in situ 17β-estradiol in normal breast tissue. This may be important to understand the behavior of normal of premalignant breast epithelial cells during hormonal replacement or during chemoprevention trials using tamoxifen. In our study we quantified 17β-estradiol in normal glandular breast cells obtained from operation specimens of pre- and postmenopausal healthy women where a bilateral breast reduction was performed for esthetic reason. We also included pre- and postmenopausal women taking tamoxifen and postmenopausal women taking an aromatase inhibitor that required a unilateral breast reduction for asymmetry.

2. Materials and methods

2.1. Patients

Women ($n = 68$) undergoing breast surgery for esthetic or reconstructive reasons were recruited for this study. Most women ($n = 52$) underwent breast reducing surgery for esthetic reasons. Sixteen women taking tamoxifen (20 mg/d) (6 premenopausal/4 postmenopausal) or an aromatase inhibitor (1.0 mg anastrozol or 2.5 mg letrozol) (6 postmenopausal), underwent a contra lateral correction because of asymmetry. This asymmetry resulted from breast conservative surgery followed by radiotherapy, which diminished the volume of the treated breast. In some patients a skin sparing mastectomy with immediate reconstruction (DIEP flap) [16] also resulted in a smaller breast. In the course of their follow up, while taking adjuvant tamoxifen or aromatase inhibitors, these 16 women requested a contra lateral breast reduction to correct this breast asymmetry. The histology of the removed tissue was carefully examined. No malignant of premalignant abnormalities were detected. Women taking tamoxifen were asked to stop medication 10 days prior to surgery to avoid thrombotic events.

The study was approved by the Ghent University Hospital ethical review board (EC UZG 2005/022).

2.2. Tissue and serum 17β-estradiol analysis

During surgery, blood and breast biopsies were collected. Serum was obtained by centrifugation (10 min at $600 \times g$, room temperature) after coagulation and aliquots were stored at −20 °C until analysis. The investigators were blinded to the treatments when working with the samples. The tissue samples were immediately frozen in liquid nitrogen and stored at −80 °C until analysis. The biopsies were first dissected into fractions containing almost exclusively glandular tissue, based on macroscopic inspection. Areas of adipose tissue intimately intermixed with fibro glandular tissue were avoided and connective tissue was removed. Next, estrogens were extracted as described by Chetrite et al. [17,18]: approximately 200 mg adipose or glandular breast tissue were homogenized in 5 mL ethanol/water (70/30, v/v) using a T10 ULTRA-TURRAX® (Ika, Werk Staufen, Germany), and, after precipitation (2×24 h at −20 °C), extracted with 5 mL ethyl acetate/hexane (60/40, v/v). The organic phase was evaporated to dryness at 37 °C under a gentle stream of N_2 and reconstituted in 500 μL steroid-free serum (Std0-DRG, DRG Instruments GmbH, Marburg, Germany). Finally, samples were analyzed for 17β-estradiol using a quantitative immunoassay (EIA-4499, DRG Instruments GmbH, Marburg, Germany). According to the manufacturer, this kit has a sensitivity of <5.13 pmol/L serum, an intra- and inter-assay CV of 6.4% and 7.6%, respectively, and a cross-reactivity of 0.2% with estrone, 0.05% with estriol, and <0.05% with 17α-estradiol.

2.3. Statistics

Statistical analyses were performed using the MedCalc® software (MedCalc Ltd, Ostend, Belgium) and included the Kolmogorov–Smirnov test, the calculation of medians and 95% confidence intervals (CI), and the Spearman's correlations.

3. Results

3.1. Correlation between estradiol concentration in serum and in breast tissue in women without oncological history

Overall, a strong positive correlation ($r = 0.865$, $P < 0.0001$, $n = 68$) (Table 1) was found between circulating and tissue 17β-estradiol concentrations. In normal cycling women ($n = 24$) the median 17β-estradiol concentration was 29.7 pg/mL (IQR: 10.8–82.3 pg/mL) in the serum and 30.6 pg/g (IQR: 18.6–183.8 pg/g) in breast epithelial cells ($r = 0.853$, $P < 0.0001$). The estradiol concentration was in concordance with the recorded day of their menstrual cycle. In postmenopausal controls, a positive correlation was found ($r = 0.813$, $P < 0.0001$, $n = 16$) between 17β-estradiol concentrations in serum (median: 10.0 pg/mL, IQR: 5.4–15.6 pg/mL) and in tissues (median: 18.8 pg/g, IQR: 8.9–46.3 pg/g). In women taking oral contraceptives 17β-estradiol concentrations both serum and breast tissue were low (8.2 pg/mL IQR: 5.0–20.3 and 14.3 IQR: 10.8–18.2 pg/g, respectively, $n = 12$) ($r = 0.376$; $P =$ n.s.).

3.2. Correlation between serum estradiol and breast tissue estradiol in women with an oncological history of receptor positive breast cancer

Premenopausal women ($n = 6$, mean age: 44 years, mean BMI = 24.6) with a history of breast cancer and currently taking tamoxifen, had a very high concentration of 17β-estradiol both in serum (277.9 pg/mL; IQR 96.2–544.7 pg/mL) and epithelial cells (251.9 pg/g; IQR: 115.0–426.5 pg/g) and these were positively correlated ($r = 0.803$; $P < 0.001$). Postmenopausal women ($n = 4$, mean age: 55, mean BMI = 23.0) had a low concentration of 17β-estradiol in serum (7.0 pg/mL) and in epithelial cells (14.6 pg/g) ($r = 0.10$; $P =$ n.s.). Women taking aromatase inhibitors ($n = 6$), had extremely low levels of 17β-estradiol, both in serum (5.0 pg/mL IQR: 5.0–5.0) and in epithelial cells (5.1 pg/g, IQR: 4.3–6.2 pg/g).