PERGAMON

Journal of Steroid Biochemistry & Molecular Biology 86 (2003) 245–253

The Journal of Steroid Biochemistry & Molecular Biology

www.elsevier.com/locate/jsbmb

Breast cancer tissue estrogens and their manipulation with aromatase inhibitors and inactivators☆

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Abstract

Despite the dramatic fall in plasma estrogen levels at menopause, only minor differences in breast tissue estrogen levels have been reported comparing pre- and postmenopausal women. Thus, postmenopausal breast tissue has the ability to maintain concentrations of estrone (E_1) and estradiol (E_2) that are 2–10- and 10–20-fold higher than the corresponding plasma estrogen levels. This finding may be explained by uptake of estrogens from the circulation and/or local estrogen production. Local aromatase activity in breast tissue seems to be of crucial importance for the local estrogen production in some patients while uptake from the circulation may be more important in other patients. Beside aromatase, breast tissue expresses estrogen sulfotransferase and sulfatase as well as dehydrogenase activity, allowing estrogen storage and release in the cells as well as conversions between estrone and estradiol. The activity of the enzyme network in breast cancer tissue is modified by a variety of factors like growth factors and cytokines. Aromatase inhibitors have been used for more than two decades in the treatment of postmenopausal metastatic breast cancer and are currently investigated in the adjuvant treatment and even prevention of breast cancer. Novel aromatase inhibitors and inactivators have been shown to suppress plasma estrogen levels effectively in postmenopausal breast cancer patients. However, knowledge about the influence of these drugs on estrogen levels in breast cancer tissue is limited. Using a novel HPLC-RIA method developed for the determination of breast tissue estrogen concentrations, we measured tissue E_1, E_2 and estrone sulfate (E_1S) levels in postmenopausal breast cancer patients before and during treatment with anastrozole. Our findings revealed high breast tumor tissue estrogen concentrations that were effectively decreased by anastrozole. While E_1S was the dominating estrogen fraction in the plasma, estradiol was the estrogen fraction with the highest concentration in tumor tissue. Moreover, plasma estrogen levels did not correlate with tissue estrogen concentrations. The overall experience with aromatase inhibitors and inactivators concerning their influences on breast tissue estrogen concentrations is summarized.

Keywords: Breast cancer; Estrogens; Aromatase inhibitors; Tissue estrogens; Anastrozole; Letrozole; Exemestane

1. Introduction

Plasma estradiol (E_2) levels decrease at menopause by about 90% and one could expect that breast tissue E_2 levels would decrease simultaneously. However, several investigators have confirmed that breast tissue E_2 levels are maintained at nearly the same level as seen in premenopausal women. Thus, breast cancer tissue E_2 levels are about 10–20-fold higher and breast tissue estrone (E_1) levels 2–10-fold higher than their corresponding plasma levels [1–4]. This observation may be explained by local synthesis and/or active estrogen uptake from the circulation as well as altered estrogen metabolism. Moreover, the ratios of the major estrogen fractions seem to differ significantly between the circulation and breast tissue. While estrone sulfate (E_1S) is the dominating estrogen fraction in the plasma followed by E_1 and E_2, E_2 is the major estrogen fraction in postmenopausal breast cancer tissue followed by E_1 and E_1S.

Estrogen deprivation is an effective approach for the treatment of hormone sensitive breast cancer. Thus, plasma estrogen measurements have been performed to evaluate treatment options like castration in premenopausal women and treatment with aromatase inhibitors or inactivators in postmenopausal women. However, the major aim of estrogen depriving therapies is to decrease the estrogen concentrations in the malignant tissue and in non-malignant tissues surrounding the tumor. Moreover, plasma estrogen levels do not reflect tissue estrogen levels. Thus, there is an increasing interest in tumor tissue estrogen levels.

The main reasons for our limited knowledge about tumor tissue estrogen levels are the methodological problems connected to tissue estrogen measurements. Firstly, breast tissue is well-known to be a difficult type of tissue to work with

☆ Presented at the VI International Aromatase Conference, AROMATASE 2002, Kyoto, Japan, 26–30 October 2002.

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doi:10.1016/S0960-0760(03)00364-9

Table 1
Overview: publications on breast cancer tissue estrogen levels

Author [reference]	Tumor E_1 concentration	Tumor E_2 concentration	Tumor E_1S concentration
Blankenstein et al. [5]	n.d.	320 fmol/g (87 pg/g)	n.d.
Bonney et al. [6]	0.31 ng/g (310 pg/g)	0.48 ng/g (480 pg/g)	n.d.
Chetrite et al. [7]	320 pg/g	388 pg/g	454 pg/g
De Jong et al. [8]	951 fmol/g (257 pg/g)	840 fmol/g (229 pg/g)	n.d.
Geisler et al. [9]	173.6 fmol/g (47 pg/g)	217.9 fmol/g (59 pg/g)	80.7 fmol/g (29 pg/g)
van Landeghem et al. [4]	0.5 pmol/g (135 pg/g)	0.75 pmol/g (204 pg/g)	n.d.
Miller et al. [10]	0.81 pmol/g (219 pg/g)	0.75 pmol/g (204 pg/g)	n.d.
Pasqualini et al. [2]	1.0 pmol/g (270 pg/g)	1.4 pmol/g (381 pg/g)	3.3 pmol/g (1205 pg/g)
Recchione et al. [13]	n.d.	46 pg/g	n.d.
Reed et al. [11]	327 pg/g	n.d.	n.d.
Thijssen et al. [12]	0.29 pmol/g (78 pg/g)	0.60 pmol/g (163 pg/g)	n.d.

Abbreviations: E_1, estrone; E_2, estradiol; E_1S, estrone sulfate; n.d., not done. The values have been converted to pg/g whenever other units had been used in the original publications.

for technical reasons, as it may contain significant amounts of connective tissue, fat and sometimes necrotic tissue. Secondly, a large variety of steroid hormones that may possibly cross-react in the detection assays are suggested to be present in breast tissue simultaneously. This risk of cross-reacting metabolites is substantially increased during treatment with steroidal drugs used for the treatment of breast cancer. Despite these methodological problems, several investigators have measured normal breast and breast cancer tissue estrogen levels using a variety of methods [2,4–13]. The results of these studies are given in Table 1.

To more precisely measure tissue estrogen levels, we have recently established a novel, highly sensitive and specific HPLC-RIA especially designed for the simultaneous detection of breast tissue levels of E_1, E_2 and E_1S [14]. Briefly, tissue homogenates are incubated with ^{3}H-labeled estrogens (E_1, E_2 and E_1S) as recovery controls and crude fractions are separated by ether extraction. The E_1S fraction is hydrolyzed with sulfatase followed by elution on a Sephadex LH-20 column. A HPLC system was used to purify the individual estrogen fractions prior to RIA analysis. E_1 and E_1S are converted into E_2, and all three estrogen fractions are finally measured by the same highly sensitive and specific RIA using estradiol-6-carboxy-methyloxime-[2-^{125}I]-iodohistamine as a ligand. Final estrogen values are corrected for the amount of tissue used in each individual sample (wet weight) as well as for the recovery of the amount of [^{3}H]-hormone added as internal standard. The detection limits for breast tissue levels of E_2, E_1 and E_1S are 4.3, 19.8, and 11.9 fmol/g tissue, respectively. We have recently measured E_1, E_2 and E_1S concentrations in the plasma and breast cancer tissue of 12 untreated postmenopausal women with advanced breast cancer (Fig. 1) and following treatment with anastrozole [9]. Thus, the described method proved to be suitable for the detection of very low estrogen levels, as expected in

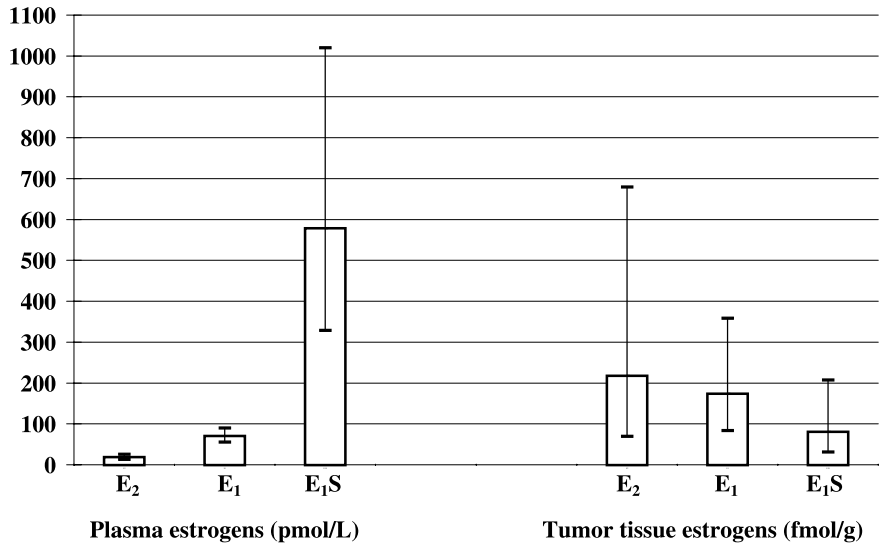

Fig. 1. Mean (geometric) plasma and tissue estrogen levels in postmenopausal breast cancer patients ($n = 12$; [9]).

samples collected during treatment with the highly potent third generation aromatase inhibitors and inactivators.

2. Determinants of breast cancer tissue estrogen levels in postmenopausal women

Several authors have discussed possible sources of high levels of estrogens found in breast tumor samples in postmenopausal women [1,15–21]. The major determinants that may certainly contribute to the high tumor estrogen levels are: uptake of estrogens (or estrogen precursors) from the circulation, local estrogen synthesis in the tumor (or in tissues nearby the tumor) and estrogen metabolism. Some of the major sources for breast (cancer) tissue estrogen levels are summarized in Fig. 2.

2.1. Uptake of estrogens from the circulation

Animal studies have provided evidence that estrogen receptor rich tissues like breast tissue more readily concentrate estradiol causing high tissue/plasma gradients [15]. In addition, breast tissue in a rat model was shown to concentrate estradiol to a greater extent at low than at high circulating E_2 levels [17]. This high affinity uptake/binding of estradiol was explained by high affinity binding components that may possibly represent the estrogen receptors itself [17]. However, the importance of these findings for the hormonal situation in postmenopausal women is unknown. Miller and Dixon have studied the influence of several aromatase inhibitors and inactivators on the ability of breast tissue to take up estrogens from the circulation. Although a marked variation between the paired non-malignant samples was observed, a

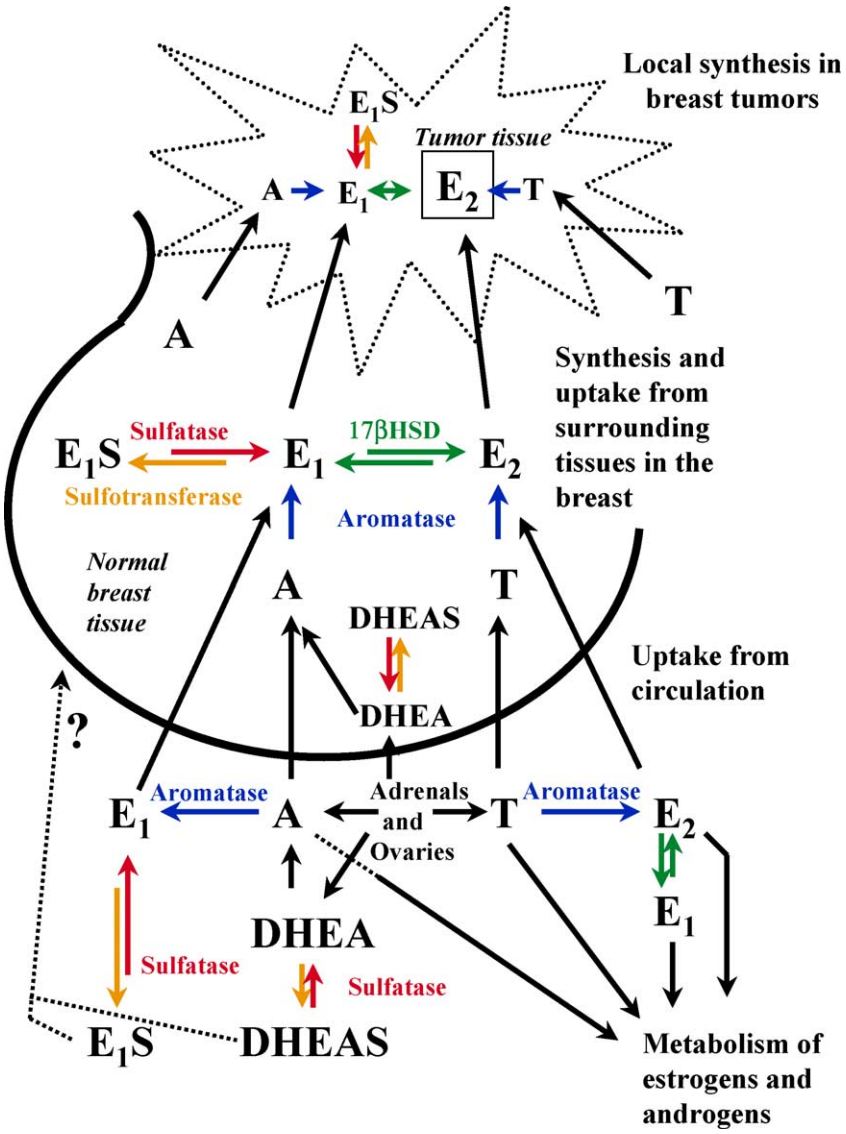

Fig. 2. Some determinants of breast tissue estrogen levels in postmenopausal women.

tendency for increased estrogen uptake was seen following treatment with aromatase inhibitors and inactivators [22].

2.2. Local estrogen synthesis in postmenopausal breast cancer tissue: the enzymes

Human breast tissue contains all important enzymes necessary to synthesize, convert and store estrogens. Miller and Dixon studied the sources of breast cancer tissue estrogen levels in postmenopausal women. In terms of oestrogen biosynthesis, evidence for in situ production was found in 36 of 47 (77%) tumors [22]. The major enzymes involved in estrogen synthesis and conversion in human breast tissue are aromatase, steroid sulfatase (STS) and 17β-hydroxysteroid dehydrogenase (17β-HSD). The importance of these enzymes and their possible contributions to the breast tissue estrogen content are discussed in the following paragraphs.

2.2.1. Aromatase

Aromatization of androgens derived from the adrenals and the ovaries is the only established production pathway for estrogens in postmenopausal women. First evidence for intratumoral aromatization of androgen precursors in breast cancer tissue was provided by Miller and Forrest [23]. Aromatase transcription is highly regulated by at least nine major promoter sites that respond to cyclic nucleotides, cytokines, gonadotropins, phorbol esters, glucocorticoids and growth factors. The control of aromatase expression in normal human breast tissue and breast cancer tissue by tissue-specific promoters has been discussed in several overview publications in recent years [24]. Aromatase expression in normal breast tissue is almost exclusively located in the fibroblast component of the adipose tissue employing mainly the cytokine plus glucocorticoid-induced promoter I.4, while adipose tissue surrounding breast tumors uses predominantly promoter I.3 or promoter II for aromatase expression. Several candidate factors for the regulation of aromatase expression via promoter I.3 and II, like PGE_2 [25–27], have been suggested [28,29]. Complex mechanisms involving a great variety of factors regulate aromatase activity in human breast tissue. Today, it is generally accepted that the aromatase-pathway (either in peripheral tissues or in the tumor itself) is the most important source for breast cancer tissue estrogen levels in postmenopausal women. The importance of this pathway is underlined by the dramatic fall of plasma and tissue estrogen levels in postmenopausal women treated with novel, highly selective aromatase inhibitors or inactivators alone [30–35].

2.2.2. Steroid sulfatase (STS) and estrogen sulfotransferase (EST)

Steroid sulfatase (arylsulfatase C) is the enzyme that hydrolyses both E_1S and dehydroepiandrosterone sulfate (DHEA-S) to their active, unsulfated forms [36]. Steroid sulfatase and its counterpart, a sulfotransferase, are found ubiquitously in both normal and malignant breast tissue [37–39]. Steroid sulfatase activity has been reported to be higher in malignant and benign breast tumors than in adjacent, non-involved tissues [7,40]. Moreover, patients with tumors containing high levels of steroid sulfatase mRNA were found to have a significantly shorter disease free survival as compared to those with low levels [41]. Thus, the 'sulfatase-pathway' has been suggested to be of major importance for the estrogen production in breast cancer tissue [21,40,42]. The V_{max} for estrone sulfatase activity is in general 10^5–10^6-fold higher than the aromatase levels in human breast cancer [21], however, estrone sulfatase has a much lower affinity for the substrate ($K_m = 15\ \mu mol/l$) compared to aromatase ($K_m = 0.027\ \mu mol/l$) [43]. It has been postulated by some authors that more estrogens could be formed by the sulfatase pathway than the aromatase-pathway; see [15] for references. A significant reduced activity of STS was found in MCF-7 cells following exposure to estradiol suggesting a negative feedback of high estradiol concentrations on sulfatase activity [44]. Although some authors have suggested a high tissue–plasma ratio for E_1S in breast cancer patients [40,45], we could not confirm this finding in one of our own studies [9]. Due to its hydrophilic nature, E_1S is probably not able to pass cell membranes and the free estrogen has to be cleaved from its conjugate form before uptake into tumor cells. Tracer studies performed in animal models [46] as well as in postmenopausal women with advanced breast cancer [47] support this point of view. Thus, intracellular E_1S is suggested to originate from the conversion via sulfotransferase of intracellular E_1. Nevertheless, E_1S is of biological importance for the overall production of unconjugated estrogens in peripheral tissues. One might speculate whether the comparably low levels of E_1S in breast cancer tissue samples observed in our study [9] may be explainable by an increased activity of estrone sulfatase, allowing the use of liberated E_1 for further conversion to E_2 in the malignant breast tissue.

Recently, transmembrane transporters for estrogen sulfates like human multidrug resistance protein-1 (MRP-1) (efflux) and the organic anion transporting polypeptide family (OATP-family) (uptake) have been identified in vitro [48]. While the *MRP-1* gene has been shown to be frequently expressed in human primary breast cancers [49], OATPs are expressed in different human tissues [50]. However, whether these transporters have any importance for the balance of steroids in human breast cancer tissue is currently unknown.

2.2.3. 17β-Hydroxysteroid dehydrogenase (17β-HSD)

The 17β-HSD catalyze reductions and oxidations of steroids (estrogens, androgens and progestins) in position 17 [51,52]. At least 10 distinct 17β-HSD isoenzymes have been characterized so far, differing in their co-factor requirements, reaction kinetics and subcellular distribution [51,53–55]. The isoform 17β-HSD type 1 seems to be the major isoenzyme involved in glandular estrogen production in humans but is also expressed in human breast tissue and its tumors in a variable way [56,57]. However, the expression of 17β-HSD

type 1 in human breast tumor is not necessarily higher than in normal tissue [53,56]. 17β-HSD type 1 is responsible for the interconversion of E_1 into E_2 and vice versa, but favors probably the reduction of E_1 to E_2 in vitro [58]. Some studies support the hypothesis that the conversion of E_1 to E_2 may be the preferred conversion in malignant breast tumors and the conversion of E_2 to E_1 in non-malignant tissue (53). However, others have suggested the opposite [6]. Thus, 17β-HSD type 1 may be one of the main factors contributing to the high tissue–plasma ratio of E_2 in postmenopausal breast cancer patients. Interestingly, 17β-HSD type 1 is often but not always expressed simultaneously with aromatase activity in human breast carcinomas [59]. A significant correlation was observed between the expression of aromatase and 17β-HSD type 1 in invasive lobular carcinoma but not in ductal carcinoma [59]. Activity of 17β-HSD type 1 is regulated by gonadotrophins, modulated by sex hormone levels and paracrine growth factors. Santner et al. have suggested that the enzyme activity may differ in subtypes of breast tissue [60]. The isoform 17β-HSD type 2 has been suggested to counteract the 17β-HSD type 1 in vitro [61].

Finally, while all the enzymes mentioned above are certainly of importance for the estrogen concentrations in human breast cancer tissue, no consistent relationship between enzyme activities and tissue estrogen concentrations could be established for any of these enzymes [3,62].

2.3. Metabolism of estrogens

Estrogens are eliminated from the human body by a variety of metabolic conversions to hormonally inactive or less active metabolites. These conversions take place mainly by oxidative (largely hydroxylations) and conjugative metabolism (glucuronidation, sulfonation and *O*-methylation) [63]. Two of the major metabolic pathways for estrogens in humans are the 2- and 16α-hydroxylation of estrone [64]. While 2-hydroxylated estrogens cause very weak estrogenic effects, 16α-hydroxylated estrogens are suggested to have strong carcinogenicity in vivo [65]. The highest capacity for these hydroxylations is probably present in the human liver tissue. However, both pathways have also been shown to be present in human breast tissue [66]. 4-Hydroxylation is a less important pathway in the liver but has been suggested to be one of the major pathway of estrogens in peripheral tissues like the breast; for references see [63]. 4-Hydroxyestradiol has been suggested to be a strong carcinogen in vivo and comparably high activity of 4-hydroxylase has been measured in human breast cancer tissues [67]. In conclusion, some of the estradiol effects in human breast tissue may not be caused by estradiol per se, but may result from intra-tissue formation of estradiol or estrone metabolites which function as local mediators either by activating estrogen receptors or by receptor independent mechanisms. These metabolites are in general not recognized with RIA methods used to measure tissue estrogen levels in human breast cancer samples.

3. Influences of aromatase inhibitors and inactivators on breast cancer tissue estrogen levels

While plasma estrogen levels have been studied extensively during treatment with aromatase inhibitors and inactivators, only a limited number of studies have been published on tissue estrogen levels during treatment with these compounds. One explanation for this is that methodological problems connected to the detection of tissue estrogens in general and to the low estrogens levels during treatment with aromatase inhibitors in particular render tissue measurements difficult. The limited data concerning suppression of tissue estrogens with various aromatase inhibitors and inactivators is summarized in the following paragraphs.

3.1. Formestane (4-hydroxyandrostenedion)

Reed et al. were among the first investigators looking at the influences of an aromatase inactivator on breast tissue estrogen levels. This pioneer work evaluated the influence of the second generation, steroidal aromatase inactivator 4-hydroxyandrostenedion (4-OHA; formestane; Lentaron®) on tumor tissue levels of E_1 [11] in four postmenopausal women with advanced breast cancer. Tissue E_1 levels were detected by radioimmunoassay after extraction with diethyl ether and preparation on Sephadex LH-20 columns [6]. In breast tumor tissues, the mean concentration of E_1 was 327 pg/g tissue at baseline and 112 pg/g tissue following 2 weeks on treatment with 4-OHA (mean decrease 62%). Individual E_1 tumor tissue levels before and after treatment are given in Fig. 3. In five additional samples of normal breast tissue the E_1 levels were 140 pg/g before treatment and 51 pg/g following treatment with 4-OHA (mean suppression of E_1: 62%).

3.2. Vorozole

The influence of vorozole, an aromatase inhibitor belonging to the triazole family, on tumor tissue estrogen levels

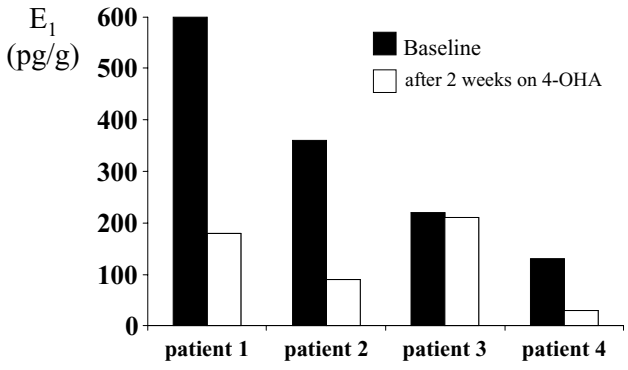

Fig. 3. Influence of treatment with 4-hydroxyandrostenedione (4-OHA) on tumor tissue E_1 (estrone) levels in four postmenopausal women with advanced breast cancer as published by Reed et al. [11].

has been estimated by De Jong et al. [8]. Tumor tissue levels of E_1 and E_2 obtained after 7 days of treatment with vorozole (2.5 mg daily) preceding surgery were compared to historical data for untreated patients (due to the lack of pre-treatment tissue estrogen levels). Tissue estrogen levels were measured using a method described previously [68]. During treatment with vorozole, tumor tissue levels of E_1 and E_2 were 64 and 80% lower ($n = 5$), respectively, when compared to historical untreated controls ($n = 9$).

3.3. Letrozole

Miller et al. determined breast tumor E_1 and E_2 levels in patients before and following treatment with letrozole (Femara®) [34]. While the exact percentage of tissue estrogen suppression could not be calculated due to the concomitant infusion of tracer steroids used to determine estrogen production rates, endogenous levels of estrogens (E_1 and E_2) fell during treatment with letrozole in all 10 tumor pairs examined.

3.4. Anastrozole

Anastrozole (Arimidex®) is a selective aromatase inhibitor widely used for the treatment of postmenopausal breast cancer. The drug has been shown to inhibit in vivo aromatization by 96–97% in postmenopausal women with advanced breast cancer [30]. Anastrozole has been shown to block estrogen synthesis both peripherally and within the breast in postmenopausal women with large operable breast cancers [10]. In accordance with these findings, we have recently reported on the effects of anastrozole on both plasma and intra-tumoral estrogen levels in a group of 12 postmenopausal women with locally advanced breast cancer [9]. Briefly, tumor tissue was obtained before treatment and following 15 weeks on treatment with anastrozole. Intra-tumoral estrogen levels were determined using a novel highly sensitive RIA following a multistep purification procedure involving high-pressure liquid chromatography [14]. Treatment with anastrozole suppressed tumor tissue E_2, E_1 and E_1S levels by 89.0, 83.4 and 72.9%, respectively (Fig. 4A–C). The finding of better suppression of tissue E_1 and E_2 compared to E_1S levels may be partly explained by the fact that some patients had E_1S tissue levels below the detection limit even before initiation of treatment. In addition, 7 out of 12 patients had tissue E_1S levels below the detection limit while on treatment with anastrozole. In general, it was noticed that patients with high tissue estrogen levels experienced better estrogen suppression compared to patients with low tissue estrogen levels at baseline. All in all, our values of breast cancer tissue E_1 and E_2 concentrations were in accordance to the findings made by other groups. However, in marked contrast to a previously study published by Pasqualini et al. [2] which reported breast tissue levels of E_1S to be 10 times higher than in plasma, we found tissue levels of E_1S to be only about 20% of

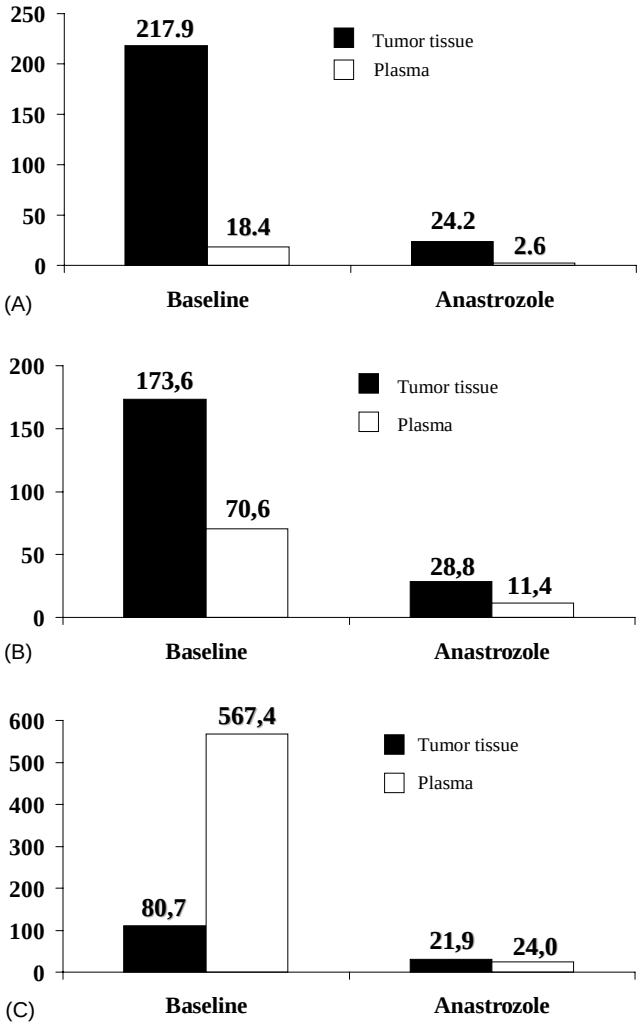

Fig. 4. (A) Plasma (pmol/l) and tumor tissue (fmol/g) estradiol levels at baseline and on treatment with anastrozole [9]. (B) Plasma (pmol/l) and tumor tissue (fmol/g) estrone levels at baseline and on treatment with anastrozole [9]. (C) Plasma (pmol/l) and tumor tissue (fmol/g) estrone sulfate levels at baseline and on treatment with anastrozole [9].

the plasma levels. One possible explanation may be that intra-tumoral E_1S becomes deconjugated and used to synthesize E_2. This steroid is the dominant estrogen fraction in breast cancer tissue in our patient group.

3.5. Exemestane

To my knowledge, no data are available about the influence of the steroidal aromatase inactivator exemestane (Aromasin®) on breast tissue estrogen levels. This may be partly explainable by cross-reacting metabolites of exemestane in many of the currently available assays [22,69]. However, when testing our novel HPLC-RIA method for the estrogen measurements in plasma samples obtained in postmenopausal women treated with exemestane with doses up to 200 mg daily, we did not find any sign for cross-reactions (unpublished observations). Thus, we suggest that our

method will probably be suitable to measure tissue estrogens in patients treated with exemestane.

4. Summary and conclusions

Human breast cancer tissue is able to concentrate estrogens through several mechanisms including local estrogen production and uptake of estrogens (or estrogen precursors) from the circulation. Tracer studies published by Miller et al. [34] and James et al. [70] evaluated the origin of tumor tissue estrogens. They demonstrated substantial inter-individual variation regarding the sources of tissue estrogens. They suggested that the bulk of estrogens originate from uptake from the circulation in some patients but in local production of estrogens in malignant breast tissue in others. Several major pathways have been discussed in the literature to be of major importance for the synthesis of high estrogen levels in breast cancer tissue-like the aromatase- and the sulphatase-pathway. However, the effects of monotherapy with the novel, highly selective aromatase inhibitors and inactivators on plasma and tissue estrogen levels indicate that the aromatase-pathway certainly is the most important source of tissue estrogen levels in breast cancer tissue. Otherwise, it would be difficult to explain that the selective inhibition of the aromatase enzyme is able to suppress plasma and tissue estrogen levels in postmenopausal women by 80–95%. There is obviously no other source of estrogens in vivo that can effectively compensate for the fall in plasma and tissue estrogen levels caused by aromatase inhibition. However, breast cancer tissue may adapt to estrogen deprivation, as resistance to treatment with aromatase inhibitors occurs frequently. Yue et al. [15] have suggested that human breast tumors may compensate for low peripheral estrogen levels by enhanced uptake of estrogens from the circulation and/or by increased local production of estrogens (e.g. by increased aromatase activity). Other possible mechanisms involved in the adaptation process to low estrogen levels have recently been reviewed [71].

Acknowledgements

This work was supported by the Norwegian Cancer Society. I am grateful to Mrs. Hildegunn Helle and Mr. Dagfinn Ekse (Breast Cancer Research Group, Haukeland University Hospital, Bergen, Norway) for their skilful technical assistance during the plasma and tissue estrogen measurements performed at our hospital. The discussion of the manuscript with Prof. P.E. Lønning is highly appreciated.

References

[1] J. Fishman, J.S. Nisselbaum, C.J. Menendez-botet, M.K. Schwartz, Estrone and estradiol content in human breast tumors: relationship to estradiol receptors, J. Steroid Biochem. 8 (1997) 893–896.

[2] J.R. Pasqualini, G. Chetrite, C. Blacker, M.C. Feinstein, L. Delalonde, M. Talbi, C. Maloche, Concentrations of estrone, estradiol, and estrone sulfate and evaluation of sulfatase and aromatase activities in pre- and postmenopausal breast cancer patients, J. Clin. Endocrinol. Metab. 81 (1996) 1460–1464.

[3] A. Vermeulen, J.P. Deslypere, R. Paridaens, G. Leclercq, F. Roy, J.C. Heuson, Aromatase, 17beta-hydroxysteroid dehydrogenase and intratissular sex hormone concentrations in cancerous and normal glandular breast tissue in postmenopausal women, Eur. J. Cancer Clin. Oncol. 22 (1986) 515–525.

[4] A.A. van Landeghem, J. Poortman, M. Nabuurs, J.H. Thijssen, Endogenous concentration and subcellular distribution of estrogens in normal and malignant human breast tissue, Cancer Res. 45 (1985) 2900–2906.

[5] M.A. Blankenstein, I. Maitimu-Smeele, G.H. Donker, J. Daroszewski, A. Milewicz, J.H. Thijssen, On the significance of in situ production of oestrogens in human breast cancer tissue, J. Steroid Biochem. Mol. Biol. 41 (1992) 891–896.

[6] R.C. Bonney, M.J. Reed, K. Davidson, P.A. Beranek, V.H. James, The relationship between 17beta-hydroxysteroid dehydrogenase activity and oestrogen concentrations in human breast tumours and in normal breast tissue, Clin. Endocrinol. (Oxf.) 19 (1983) 727–739.

[7] G.S. Chetrite, J. Cortes-Prieto, J.C. Philippe, F. Wright, J.R. Pasqualini, Comparison of estrogen concentrations, estrone sulfatase and aromatase activities in normal, and in cancerous, human breast tissues, J. Steroid Biochem. Mol. Biol. 72 (2000) 23–27.

[8] P.C. De Jong, J. van de Ven, H.W. Nortier, I. Maitimu-Smeele, T.H. Donker, J.H. Thijssen, P.H. Slee, R.A. Blankenstein, Inhibition of breast cancer tissue aromatase activity and estrogen concentrations by the third-generation aromatase inhibitor vorozole, Cancer Res. 57 (1997) 2109–2111.

[9] J. Geisler, S. Detre, H. Berntsen, L. Ottestad, B. Lindtjorn, M. Dowsett, P. Eystein Lønning, Influence of neoadjuvant anastrozole (Arimidex) on intratumoral estrogen levels and proliferation markers in patients with locally advanced breast cancer, Clin. Cancer Res. 7 (2001) 1230–1236.

[10] W.R. Miller, J.M. Dixon, M. Stuart, T. Sahmoud, Anastrozole (‘Arimidex’) blocks estrogen synthesis both peripherally and within the breast in postmenopausal women with large operable breast cancer, in: Proceedings of the American Association for Cancer Research, 2000.

[11] M.J. Reed, G.W. Aherne, M.W. Ghilchik, S. Patel, J. Chakraborty, Concentrations of oestrone and 4-hydroxyandrostenedione in malignant and normal breast tissues, Int. J. Cancer 49 (1991) 562–565.

[12] J.H. Thijssen, M.A. Blankenstein, G.H. Donker, J. Daroszewski, Endogenous steroid hormones and local aromatase activity in the breast, J. Steroid Biochem. Mol. Biol. 39 (1991) 799–804.

[13] C. Recchione, E. Venturelli, A. Manzari, A. Cavalleri, A. Martinetti, G. Secreto, Testosterone, dihydrotestosterone and oestradiol levels in postmenopausal breast cancer tissues, J. Steroid Biochem. Mol. Biol. 52 (1995) 541–546.

[14] J. Geisler, H. Berntsen, P.E. Lonning, A novel HPLC-RIA method for the simultaneous detection of estrone, estradiol and estrone sulphate levels in breast cancer tissue, J. Steroid Biochem. Mol. Biol. 72 (2000) 259–264.

[15] W. Yue, S.J. Santner, S. Masamura, J.P. Wang, L.M. Demers, C. Hamilton, R.J. Santen, Determinants of tissue estradiol levels and biologic responsiveness in breast tumors, Breast Cancer Res. Treat. 49 (Suppl. 1) (1998) S1–S7, discussion S33–S37.

[16] R.C. Bonney, M.J. Scanlon, M.J. Reed, D.L. Jones, P.A. Beranek, V.H. James, Adrenal androgen concentrations in breast tumours and in normal breast tissue. The relationship to oestradiol metabolism, J. Steroid Biochem. 20 (1984) 501–504.

[17] S. Masamura, S.J. Santner, P. Gimotty, J. George, R.J. Santen, Mechanism for maintenance of high breast tumor estradiol concentrations in the absence of ovarian function: role of very high affinity tissue uptake, Breast Cancer Res. Treat. 42 (1997) 215–226.

[18] W.R. Miller, Pathways of hormone metabolism in normal and nonneoplastic breast tissue, Ann. N.Y. Acad. Sci. 586 (1990) 53–59.
[19] W.R. Miller, R.A. Hawkins, A.P. Forrest, Significance of aromatase activity in human breast cancer, Cancer Res. 42 (1982) 3365s–3368s.
[20] R.J. Santen, D. Leszczynski, N. Tilson-Mallet, P.D. Feil, C. Wright, A. Manni, S.J. Santner, Enzymatic control of estrogen production in human breast cancer: relative significance of aromatase versus sulfatase pathways, Ann. N.Y. Acad. Sci. 464 (1986) 126–137.
[21] S.J. Santner, P.D. Feil, R.J. Santen, In situ estrogen production via the estrone sulfatase pathway in breast tumors: relative importance versus the aromatase-pathway, J. Clin. Endocrinol. Metab. 59 (1984) 29–33.
[22] W.R. Miller, J.M. Dixon, Local endocrine effects of aromatase inhibitors within the breast, J. Steroid Biochem. Mol. Biol. 79 (2001) 93–102.
[23] W.R. Miller, A.P. Forrest, Oestradiol synthesis by a human breast carcinoma, Lancet 2 (1974) 866–868.
[24] S. Chen, D. Zhou, C. Yang, T. Okubo, Y. Kinoshita, B. Yu, Y.C. Kao, T. Itoh, Modulation of aromatase expression in human breast tissue, J. Steroid Biochem. Mol. Biol. 79 (2001) 35–40.
[25] Y. Zhao, V.R. Agarwal, C.R. Mendelson, E.R. Simpson, Estrogen biosynthesis proximal to a breast tumor is stimulated by PGE2 via cyclic AMP, leading to activation of promoter II of the CYP19 (aromatase) gene, Endocrinology 137 (1996) 5739–5742.
[26] E.R. Simpson, Y. Zhao, V.R. Agarwal, M.D. Michael, S.E. Bulun, M.M. Hinshelwood, S. Graham-Lorence, T. Sun, C.R. Fisher, K. Qin, C.R. Mendelson, Aromatase expression in health and disease, Rec. Prog. Horm. Res. 52 (1997) 185–213 (discussion 184–213).
[27] E.R. Simpson, M.D. Michael, V.R. Agarwal, M.M. Hinshelwood, S.E. Bulun, Y. Zhao, Cytochromes P450 11: expression of the CYP19 (aromatase) gene: an unusual case of alternative promoter usage, FASEB J. 11 (1997) 29–36.
[28] V.R. Agarwal, S.E. Bulun, M. Leitch, R. Rohrich, E.R. Simpson, Use of alternative promoters to express the aromatase cytochrome P450 (CYP19) gene in breast adipose tissues of cancer-free and breast cancer patients, J. Clin. Endocrinol. Metab. 81 (1996) 3843–3849.
[29] N. Harada, T. Utsumi, Y. Takagi, Molecular and epidemiological analyses of abnormal expression of aromatase in breast cancer, Pharmacogenetics, 5 (1995) (Spec. No. S59–S64).
[30] J. Geisler, N. King, M. Dowsett, L. Ottestad, S. Lundgren, P. Walton, P.O. Kormeset, P.E. Lonning, Influence of anastrozole (Arimidex): a selective, non-steroidal aromatase inhibitor, on in vivo aromatization and plasma oestrogen levels in postmenopausal women with breast cancer, Br. J. Cancer 74 (1996) 1286–1291.
[31] M. Dowsett, A. Jones, S.R. Johnston, S. Jacobs, P. Trunet, I.E. Smith, In vivo measurement of aromatase inhibition by letrozole (CGS 20267) in postmenopausal patients with breast cancer, Clin. Cancer Res. 1 (1995) 1511–1515.
[32] J. Geisler, B. Haynes, G. Anker, M. Dowsett, P.E. Lonning, Influence of letrozole and anastrozole on total body aromatization and plasma estrogen levels in postmenopausal breast cancer patients evaluated in a randomized, cross-over study, J. Clin. Oncol. 20 (2002) 751–757.
[33] J. Geisler, N. King, G. Anker, G. Ornati, E. Di Salle, P.E. Lonning, M. Dowsett, In vivo inhibition of aromatization by exemestane a novel irreversible aromatase inhibitor, in postmenopausal breast cancer patients, Clin. Cancer Res. 4 (1998) 2089–2093.
[34] W.R. Miller, J. Telford, C.D. Love, R. Leonard, S. Hillier, H. Grundacker, H. Smith, J.M. Dixon, Effects of letrozole as primary medical therapy on in situ oestrogen synthesis and endogenous oestrogen levels within the breast, The Breast 7 (1998) 273–276.
[35] W.R. Miller, M. Stuart, T. Sahmoud, M. Dixon, Anastrozole ('Arimidex') blocks estrogen synthesis both peripherally and within the breast in postmenopausal women with large operable breast cancers, in: Proceedings of the American Association for Cancer Research, 2000, pp. 4707.
[36] J.H. MacIndoe, The hydrolysis of estrone sulfate and dehydroepiandrosterone sulfate by MCF-7 human breast cancer cells, Endocrinology 123 (1988) 1281–1287.
[37] R.A. Hawkins, M.L. Thomson, E. Killen, Oestrone sulphate, adipose tissue, and breast cancer, Breast Cancer Res. Treat. 6 (1985) 75–87.
[38] J.B. Adams, T. Pewnim, D.P. Chandra, L. Archibald, M.S. Foo, A correlation between estrogen sulfotransferase levels and estrogen receptor status in human primary breast carcinoma, Cancer Res. 39 (1979) 5124–5126.
[39] T.L. Dao, C. Hayes, P.R. Libby, Steroid sulfatase activities in human breast tumors, Proc. Soc. Exp. Biol. Med. 146 (1974) 381–384.
[40] J.R. Pasqualini, J. Cortes-Prieto, G. Chetrite, M. Talbi, A. Ruiz, Concentrations of estrone, estradiol and their sulfates, and evaluation of sulfatase and aromatase activities in patients with breast fibroadenoma, Int. J. Cancer 70 (1997) 639–643.
[41] T. Utsumi, N. Yoshimura, S. Takeuchi, J. Ando, M. Maruta, K. Maeda, N. Harada, Steroid sulfatase expression is an independent predictor of recurrence in human breast cancer, Cancer Res. 59 (1999) 377–381.
[42] J.R. Pasqualini, C. Gelly, B.L. Nguyen, C. Vella, Importance of estrogen sulfates in breast cancer, J. Steroid Biochem. 34 (1989) 155–163.
[43] R.J. Santen, J. Martel, M. Hoagland, F. Naftolin, L. Roa, N. Harada, L. Hafer, R. Zaino, S.J. Santner, Stromal spindle cells contain aromatase in human breast tumors, J. Clin. Endocrinol. Metab. 79 (1994) 627–632.
[44] J.K. Tobacman, M. Hinkhouse, Z. Khalkhali-Ellis, Steroid sulfatase activity and expression in mammary myoepithelial cells, J. Steroid Biochem. Mol. Biol. 81 (2002) 65–68.
[45] J.R. Pasqualini, G. Chetrite, B.L. Nguyen, C. Maloche, L. Delalonde, M. Talbi, M.C. Feinstein, C. Blacker, J. Botella, J. Paris, Estrone sulfate-sulfatase and 17beta-hydroxysteroid dehydrogenase activities: a hypothesis for their role in the evolution of human breast cancer from hormone-dependence to hormone-independence, J. Steroid Biochem. Mol. Biol. 53 (1995) 407–412.
[46] S. Masamura, S.J. Santner, R.J. Santen, Evidence of in situ estrogen synthesis in nitrosomethylurea-induced rat mammary tumors via the enzyme estrone sulfatase, J. Steroid Biochem. Mol. Biol. 58 (1996) 425–429.
[47] A. Purohit, A.A. Riaz, M.W. Ghilchik, M.J. Reed, The origin of oestrone sulphate in normal and malignant breast tissues in postmenopausal women, Horm. Metab. Res. 24 (1992) 532–536.
[48] S.P. Cole, G. Bhardwaj, J.H. Gerlach, J.E. Mackie, C.E. Grant, K.C. Almquist, A.J. Stewart, E.U. Kurz, A.M. Duncan, R.G. Deeley, Overexpression of a transporter gene in a multidrug-resistant human lung cancer cell line, Science 258 (1992) 1650–1654.
[49] D.W. Dexter, R.K. Reddy, K.G. Geles, S. Bansal, M.A. Myint, A. Rogakto, J.C. Leighton, L.J. Goldstein, Quantitative reverse transcriptase-polymerase chain reaction measured expression of MDR1 and MRP in primary breast carcinoma, Clin. Cancer Res. 4 (1998) 1533–1542.
[50] I. Tamai, J. Nezu, H. Uchino, Y. Sai, A. Oku, M. Shimane, A. Tsuji, Molecular identification and characterization of novel members of the human organic anion transporter (OATP) family, Biochem. Biophys. Res. Commun. 273 (2000) 251–260.
[51] A. Krazeisen, R. Breitling, G. Moller, J. Adamski, Phytoestrogens inhibit human 17beta-hydroxysteroid dehydrogenase type 5, Mol. Cell. Endocrinol. 171 (2001) 151–162.
[52] I. Dufort, P. Rheault, X.F. Huang, P. Soucy, V. Luu-The, Characteristics of a highly labile human type 5 17beta-hydroxysteroid dehydrogenase, Endocrinology 140 (1999) 568–574.
[53] K. Pollow, E. Boquoi, J. Baumann, M. Schmidt-Gollwitzer, B. Pollow, Comparison of the in vitro conversion of estradiol-17beta to estrone of normal and neoplastic human breast tissue, Mol. Cell. Endocrinol. 6 (1977) 333–348.
[54] M. Miettinen, M. Mustonen, M. Poutanen, V. Isomaa, M. Wickman, G. Soderqvist, R. Vihko, P. Vihko, 17Beta-hydroxysteroid dehydrogenases in normal human mammary epithelial cells and breast tissue, Breast Cancer Res. Treat. 57 (1999) 175–182.
[55] J. Adamski, F.J. Jakob, A guide to 17beta-hydroxysteroid dehydrogenases, Mol. Cell. Endocrinol. 171 (2001) 1–4.

[56] M. Poutanen, V. Isomaa, V.P. Lehto, R. Vihko, Immunological analysis of 17beta-hydroxysteroid dehydrogenase in benign and malignant human breast tissue, Int. J. Cancer 50 (1992) 386–390.
[57] V.V. Isomaa, S.A. Ghersevich, O.K. Maentausta, E.H. Peltoketo, M.H. Poutanen, R.K. Vihko, Steroid biosynthetic enzymes: 17beta-hydroxysteroid dehydrogenase, Ann. Med. 25 (1993) 91–97.
[58] M. Poutanen, V. Isomaa, H. Peltoketo, R. Vihko, Role of 17beta-hydroxysteroid dehydrogenase type 1 in endocrine and intracrine estradiol biosynthesis, J. Steroid Biochem. Mol. Biol. 55 (1995) 525–532.
[59] H. Sasano, A.R. Frost, R. Saitoh, N. Harada, M. Poutanen, R. Vihko, S.E. Bulun, S.G. Silverberg, H. Nagura, Aromatase and 17beta-hydroxysteroid dehydrogenase type 1 in human breast carcinoma, J. Clin. Endocrinol. Metab. 81 (1996) 4042–4046.
[60] S.J. Santner, D. Leszczynski, C. Wright, A. Manni, P.D. Feil, R.J. Santen, Estrone sulfate: a potential source of estradiol in human breast cancer tissues, Breast Cancer Res. Treat. 7 (1986) 35–44.
[61] M.M. Miettinen, M.V. Mustonen, M.H. Poutanen, V.V. Isomaa, R.K. Vihko, Human 17beta-hydroxysteroid dehydrogenase type 1 and type 2 isoenzymes have opposite activities in cultured cells and characteristic cell- and tissue-specific expression, Biochem. J. 314 (Part 3) (1996) 839–845.
[62] A.A. van Landeghem, J. Poortman, M. Nabuurs, J.H. Thijssen, Endogenous concentration and subcellular distribution of androgens in normal and malignant human breast tissue, Cancer Res. 45 (1985) 2907–2912.
[63] B.T. Zhu, A.H. Conney, Functional role of estrogen metabolism in target cells: review and perspectives, Carcinogenesis 19 (1998) 1–27.
[64] C.R. Jefcoate, J.G. Liehr, R.J. Santen, T.R. Sutter, J.D. Yager, W. Yue, S.J. Santner, R. Tekmal, L. Demers, R. Pauley, F. Naftolin, G. Mor, L. Berstein, Tissue-specific synthesis and oxidative metabolism of estrogens, J. Natl. Cancer Inst. Monogr. (2000) 95–112.
[65] H.L. Bradlow, R. Hershcopf, C. Martucci, J. Fishman, 16 alpha-hydroxylation of estradiol: a possible risk marker for breast cancer, Ann. N.Y. Acad. Sci. 464 (1986) 138–151.
[66] N.T. Telang, D.M. Axelrod, G.Y. Wong, H.L. Bradlow, M.P. Osborne, Biotransformation of estradiol by explant culture of human mammary tissue, Steroids 56 (1991) 37–43.
[67] J.G. Liehr, M.J. Ricci, 4-Hydroxylation of estrogens as marker of human mammary tumors, Proc. Natl. Acad. Sci. U.S.A. 93 (1996) 3294–3296.
[68] A.A. van Landeghem, J. Poortman, A. Helmond-Agema, J.H. Thijssen, Measurement of endogenous subcellular concentration of steroids in tissue, J. Steroid Biochem. 20 (1984) 639–644.
[69] D.C. Johannessen, T. Engan, E. Di Salle, M.G. Zurlo, J. Paolini, G. Ornati, G. Piscitelli, S. Kvinnsland, P.E. Lonning, Endocrine and clinical effects of exemestane (PNU 155971), a novel steroidal aromatase inhibitor, in postmenopausal breast cancer patients: a phase I study, Clin. Cancer Res. 3 (1997) 1101–1108.
[70] V.H. James, M.J. Reed, L.C. Lai, M.W. Ghilchik, G.H. Tait, C.J. Newton, N.G. Coldham, Regulation of estrogen concentrations in human breast tissues, Ann. N.Y. Acad. Sci. 595 (1990) 227–235.
[71] J. Geisler, P.E. Lonning, Resistance to endocrine therapy of breast cancer: recent advances and tomorrow's challenges, Clin. Breast Cancer 1 (2001) 297–308 (discussion 309).