Effect of Tamoxifen or Anastrozole on Steroid Sulfatase Activity and Serum Androgen Concentrations in Postmenopausal Women with Breast Cancer

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Abstract. Background: In postmenopausal women estrogens can be formed by the aromatase pathway, which gives rise to estrone, and the steroid sulfatase (STS) route which can result in the formation of estrogens and androstenediol, a steroid with potent estrogenic properties. Aromatase inhibitors, such as anastrozole, are now in clinical use whereas STS inhibitors, such as STX64, are still undergoing clinical evaluation. STX64 was recently shown to block STS activity and reduce serum androstenediol concentrations in postmenopausal women with breast cancer. In contrast, little is known about the effects of aromatase inhibitors or anti-estrogens on STS activity or serum androgen levels. Patients and Methods: Study 1: Blood was collected from ten postmenopausal women with breast cancer before and after two-week treatment with anastrozole and serum concentrations of androstenediol and other androgens and estrogens were assessed. Study 2: Blood samples were collected from 15 breast cancer patients before and after four-week treatment with anastrozole and 10 patients before and after four-week treatment with tamoxifen. Blood was used to assess STS activity in peripheral blood lymphocytes (PBLs) and serum dehydroepiandrosterone sulfate and dehydroepiandrosterone levels. Results: Neither anastrozole nor tamoxifen had any significant effect on STS activity as measured in PBLs. Anastrozole did not affect serum androstenediol concentrations. Conclusion: Anastrozole and tamoxifen did not inhibit STS activity and serum androstenediol concentrations were not reduced by aromatase inhibition. As androstenediol has estrogenic properties, it is possible that the combination of an aromatase inhibitor and STS inhibitor may give a therapeutic advantage over the use of either agent alone.

Anti-estrogens and aromatase inhibitors are now widely used for the treatment of postmenopausal women with hormone-dependent breast cancer (1, 2). While anti-estrogens, such as tamoxifen, block the interaction of estradiol (E2) with the estrogen receptor (ER), aromatase inhibitors such as anastrozole, letrozole and exemestane inhibit the conversion of androstenedione to estrone (E1), the major source of estrogen in postmenopausal women (3). Recent clinical studies have revealed that third-generation aromatase inhibitors are of greater clinical benefit compared with tamoxifen in postmenopausal women with breast cancer (4, 5).

In addition to the aromatase pathway of estrogen synthesis, the steroid sulfatase (STS) route is also thought to contribute to the in situ synthesis of estrogens in breast tumors (6). STS is responsible for the hydrolysis of estrone sulfate (E1S) and dehydroepiandrosterone sulfate (DHEAS) to E1 and dehydroepiandrosterone (DHEA) respectively. E1 and DHEA can be reduced by 17β-hydroxysteroid dehydrogenase type I (17β-HSD1) to steroids with potent estrogenic properties, i.e. E2 and androstenediol. 17β-HSD1 is expressed in many tissues in the body including breast tumors (7, 8). Several potent STS inhibitors have now been developed including STX64 (also known as 667 Coumate,
double isotopic infusion technique, STS activity can readily be measured in PBLs. In addition, serum concentrations of androstenediol were assessed by radioimmunoassay, together with the levels of androstenedione, testosterone, E₁, E₂ and E₁S, which were measured using gas chromatographic-tandem mass spectroscopy (GC-MS/MS).

Patients and Methods

**Study 1.** Blood was collected for the measurement of serum steroid concentrations from ten postmenopausal women with breast cancer before and two weeks after starting treatment with anastrozole (1 mg/d). Three of the patients in this group achieved an objective response. For these patients, serum concentrations of androstenediol, androstenedione, testosterone, E₁, E₂ and E₁S were measured.

**Study 2.** Blood samples were also collected from a further 15 postmenopausal women with breast cancer before and four weeks after treatment with anastrozole (1 mg/d) and ten patients before and after four-week treatment with tamoxifen (20 mg/d). In addition, blood samples were collected from these patients before and after four-week treatment for the assessment of STS activity in PBLs. For this blood was collected into CPT vacutainers (Beckton Dickinson, Franklin Lakes, NJ, USA). After centrifugation (1,500 g at 22˚C for 30 min), isolated PBLs were washed with phosphate-buffered saline (PBS, 5 ml ×2) and stored at –20˚C until assayed. The STS in PBLs was solubilised before assaying using Triton-X 100/PBS (0.2%). STS activity was assayed using [6,7-3H] E₁S (2-3 nmol/l, 46-57 Ci/mmol; Perkin-Elmer Life Sciences, Wellesley, MA, USA) over a 20 h period using [4-14C] E₁ to monitor procedural losses (11, 22).

The protocols for these studies were approved by the Hammersmith Hospital’s Ethics Committees and all patients gave written informed consent for their participation in the study.

Steroid concentrations. For serum steroid concentration measurements in samples obtained from patients in the first study, androstenediol concentrations were measured by radioimmunoassay (RIA) after organic solvent extraction and Celite column partition chromatography (23). Concentrations of androstenedione, testosterone, E₁, E₂ and E₁S were measured using a GC-MS/MS method by SBFC Taylor (Princeton, NJ, USA) (24, 25). The limits of quantitation of E₁, E₂ and E₁S in this assay were 5.8, 2.3 and 8.9 pmol/l, respectively. For samples of serum obtained from the second study, concentrations of DHEAS and DHEA were assayed using kits obtained from DSL (Webster, TX, USA) according to the manufacturer’s instructions. Intra- and inter-assay coefficients of variation for these assays were <15%.

Statistics. Mean and standard error of the mean (sem) levels of STS activity and serum steroid concentrations were calculated using the Instat 3 programme (GraphPad Software Inc., La Jolla, CA, USA). The significance of differences in mean concentrations of serum steroids and STS activity in PBLs was assessed using the paired Student’s t-test. Data are presented as mean±sem.

Results

**Study 1.** In the first study, the effect of two-week therapy with anastrozole on serum androgen and estrogen concentrations was assessed (Figure 1). Basal levels of
androstenediol, androstenedione, testosterone, $E_1$, $E_2$ and $E_3S$ were similar to those previously reported by this group in postmenopausal women with breast cancer (11). It is evident from this study that daily treatment with anastrozole had no significant effect on serum concentrations of androstenediol, androstenedione or testosterone. Pretreatment levels of $E_1$, $E_2$ and $E_3S$ in this study were 60±25, 17.7±8.3 and 980±233 pmol/l, respectively. As shown in Figure 1, $E_3S$ concentrations were significantly lower ($p<0.01$) at the end of the two-week period, but detectable in all the samples assayed at the end of this period (range, 26-210 pmol/l). The mean level of aromatase suppression calculated from the change in serum $E_3S$ concentrations was 94%. There was no significant difference in the serum $E_3S$ concentrations for three patients who subsequently showed a response to aromatase inhibitor therapy (52±9 pmol/l) and that of non-responders (72±35 pmol/l). For all patients, serum concentrations of $E_1$ after two-week treatment with anastrozole were below the limits of quantitation for this assay (5.8 pmol/l). For $E_2$, significant concentrations (3.1 and 2.9 pmol/l) were still detectable after treatment with anastrozole for two weeks in two of the patients who did not subsequently respond to aromatase inhibitor therapy.

**Study 2.** As previous reports had suggested that tamoxifen may influence STS activity and possible effects of aromatase inhibitors on STS are not yet known, PBLs were collected from a second group of patients receiving either tamoxifen or anastrozole over a four-week period. STS activity in these PBLs was similar to the range detected in basal samples collected from postmenopausal women participating in the STS inhibitor trial (11). It was clear that treatment with either tamoxifen or anastrozole for the four-week period had no significant effect ($p>0.05$) on STS activity in PBLs from these groups of patients (Figure 2). Similarly, serum concentrations of DHEAS and DHEA were not affected by treatment with either tamoxifen or anastrozole (Figure 3). There is no obvious explanation for the differences in the pre- and post-treatment levels of DHEAS and DHEA for patients receiving tamoxifen or anastrozole. Interestingly, differences in basal levels of DHEAS and DHEA were previously seen by this group in different cohorts of patients receiving either the 5 or 20 mg of STX64 (11).

**Discussion**

The main findings from the two studies presented here are that neither tamoxifen nor anastrozole has any effect on STS activity, assayed in PBLs and that serum concentrations of androgens, including androstenediol, are not altered by anastrozole therapy. This is in contrast to the results obtained with the specific STS inhibitor, STX64, where STS activity was almost completely inhibited in PBLs and a significant reduction in serum androstenediol concentrations was detected. Although it was not anticipated that anastrozole would modulate STS activity, a number of previous reports had suggested that tamoxifen may affect STS activity.
Santner and Santen examined the effect of tamoxifen on STS activity in preparations of rat mammary tumors and human breast tumors and concluded that it was a potent STS inhibitor (19). Similarly, Gelly and Pasqualini found that using R-27 cells, a tamoxifen-resistant cell line derived from MCF-7 cells, tamoxifen was able to inhibit STS activity (20).

In contrast, in other studies employing MCF-7 cells, breast tumor preparations or in vivo studies in rodents, tamoxifen was found to have either no effect or a small, but significant, stimulatory effect on STS activity (21, 26, 27). To the best of the Authors’ knowledge, this is the first study to investigate the possible effects of tamoxifen on STS activity in humans and clearly demonstrated that tamoxifen is devoid of any inhibitory effects on STS activity. The lack of an effect of aromatase inhibition on serum concentrations of DHEAS, DHEA and androstenedione is concordant with the results obtained in a previous study (14). This contrasts with the effects of STS inhibition where serum concentrations of DHEA were reduced (11).

With regard to androstenediol, as discussed previously, this steroid has potent estrogenic properties as shown by its ability to stimulate breast cancer cell growth in vitro and induced mammary tumors in vivo (15-17). The finding that serum androstenediol concentrations are not affected by treatment by anastrozole may be one possible reason why patients on this form of therapy do eventually progress. However, three of the patients in the first cohort studied did subsequently respond to aromatase inhibitor therapy, demonstrating that responses can occur in the presence of relatively high levels of androstenediol. Further studies, involving the combination of an aromatase inhibitor with an STS inhibitor will be required to examine whether reduction of serum androstenediol levels improves the response or extends the duration of endocrine therapy for breast cancer.

In the present study serum concentrations of E₁, E₂ and E₁S were measured using a GC-MS/MS method. There has been considerable debate recently about the merits of RIA when trying to measure estrogen levels in postmenopausal women and, in particular, in serum from women receiving aromatase inhibitor therapy. It is well documented that the use of RIA to measure low levels of estrogen in postmenopausal women is markedly difficult, mainly due to cross-reacting materials in the serum. Such problems led Santen to conclude that measurements of E₂ by RIA may not
be sufficiently sensitive or precise to use for serum samples from postmenopausal women (28). The measurements using GC-MS/MS in this study showed a good correlation (r=0.84) with those using a recombinant ultrasensitive bioassay (24).

In the present study, the level of aromatase inhibition calculated from the suppression of serum E1S concentrations was 94%, which is in good agreement with the level of suppression derived from measurements of E1S using an RIA in association with an extensive purification procedure (29, 30). While the levels of E1S were, therefore, effectively suppressed by anastrozole, levels above the limit of quantitation for this steroid conjugate were detected in all the samples analysed. Again this would suggest that, although these levels of E1S do not preclude an initial response to aromatase inhibitor treatment, they may contribute to disease progression in the longer term.

In summary, the results from the present investigations revealed that neither tamoxifen nor anastrozole has any effect on STS activity in PBLs. Anastrozole was also found not to affect serum concentrations of androstenediol, a steroid with potent estrogenic properties. Serum concentrations of E1, E2 and E1S were effectively suppressed by anastrozole treatment for two weeks but significant concentrations of E1S were still detectable. Given the findings that reduced concentrations of E1S are still detectable after aromatase inhibitor treatment and that serum concentrations of androstenediol are not affected by this form of therapy, it will be important to test whether combination of an aromatase inhibitor with a sulfatase inhibitor may give a therapeutic advantage over the use of either agent alone.

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References