Influence of anastrozole (Arimidex), a selective, non-steroidal aromatase inhibitor, on in vivo aromatisation and plasma oestrogen levels in post-menopausal women with breast cancer*

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Summary The effect of anastrozole (‘Arimidex’, ZD1033), a new, selective, non-steroidal aromatase inhibitor on in vivo aromatisation and plasma oestrogen levels was evaluated in post-menopausal women with breast cancer. Twelve patients progressing after treatment with tamoxifen were randomised to receive anastrozole 1 mg or 10 mg once daily for a 28 day period in a double-blinded crossover design. In vivo aromatisation and plasma oestrogen levels were determined before commencing treatment and at the end of each 4-week period. Treatment with anastrozole 1 and 10 mg reduced the percentage aromatisation from 2.25% to 0.074% and 0.043% (mean suppression of 96.7% and 98.1% from baseline) and suppressed plasma levels of oestrone, oestradiol and oestrone sulphate by >86.5%, >83.5% and >93.5% respectively, irrespective of dose. Notably, several patients had their oestrone and oestradiol values suppressed beneath the sensitivity limit of the assays. In conclusion, anastrozole was found to be highly effective in inhibiting in vivo aromatisation with no difference in efficacy between the two drug doses. Contrary to previous studies on other aromatase inhibitors, this study revealed an internal consistency between the percentage aromatase inhibition and suppression of plasma oestrone sulphate.

Keywords: anastrozole; aromatase inhibitor; breast cancer; hormone therapy

Breast cancer is the most common malignancy among women in the western hemisphere. Many of these patients develop metastatic disease, for which no cure is currently available. Because endocrine treatment causes fewer side-effects than chemotherapy, such therapy is first-line treatment in patients with metastatic disease and hormone receptor-positive tumours. While the anti-oestrogen tamoxifen is first choice of therapy in post-menopausal patients with metastatic breast cancer, increasing use of tamoxifen as adjuvant therapy focuses on the need for alternative endocrine treatment options on relapse in breast cancer patients.

While ovarian oestrogen synthesis ceases at the menopause, oestrogens are synthesised in peripheral tissue from circulating androgens by the process called aromatisation (Grodin et al., 1973). The main pathway is conversion of androstenedione (A) into oestrone (E1), with a minor contribution from conversion of testosterone into oestradiol (E2) (Lønning et al., 1990). Aromatase inhibitors are drugs that inhibit the peripheral conversion of androgens to oestrogens (Santen et al., 1982a), thereby suppressing plasma oestrogen levels in post-menopausal women. The first-generation aromatase inhibitor, aminogluthethimide, was implemented in breast cancer treatment more than 20 years ago (Cash et al., 1967). While the drug is effective in hormone-sensitive breast cancer, lack of specificity (inhibition of adrenal steroid-synthesising enzymes) and side-effects (such as skin rash and lethargy) provoked the development of new aromatase inhibitors (Coombes et al., 1984; Evans et al., 1992; Johnston et al., 1994; Lipton et al., 1995; Santen et al., 1989). Anastrozole (Arimidex; 2,2'-[1H-1,2,4-triazol-1-ylmethyl]-1,3-phenylene)bis(2-methylpropiononitrile, Figure 1) is a new, potent and selective aromatase inhibitor belonging to the triazole class. Pilot studies in post-menopausal women have shown the drug to suppress plasma E2 by >80% (Plourde et al., 1994), and preclinical studies as well as observations in women suggest the drug to be highly specific with no influence on adrenal steroid synthesis (Plourde et al., 1995).

A major problem in evaluating the biochemical efficacy of aromatase inhibitors has been the lack of internal consistency between the percentage aromatase inhibition and degree of plasma oestrogen suppression. While aminogluthethimide (MacNeill et al., 1992) as well as the second-generation aromatase inhibitor formestane (Jones et al., 1992) and the third-generation inhibitor fadrozole (Lønning et al., 1991) have all been found to inhibit the conversion of A to E1 in vivo by 85–92%, the same drugs have been reported to suppress plasma oestrogen levels by only 50–70% (Vermeulen et al., 1983; Dowsett et al., 1989, 1990). Accordingly, a major long-standing controversy has been whether this discrepancy could be caused by alternative oestrogen sources or could simply reflect methodological problems.

The primary aim of this study was to evaluate the effects of two different doses of anastrozole (1 and 10 mg) on in vivo aromatase inhibition and plasma oestrogen suppression in post-menopausal breast cancer patients. A secondary aim was to compare the degree of aromatase inhibition with the degree of plasma oestrogen suppression by applying recently developed, highly sensitive methodology for plasma oestrone sulphate E1S measurement in particular (Lønning and Ekse, 1995).

Patients and methods

Patients

Twelve post-menopausal women with a diagnosis of advanced or recurrent breast cancer progressing after

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previous tamoxifen treatment were enrolled in the study. The protocol was approved by the regional ethics committee at the University of Bergen. All patients gave their written informed consent. The mean age of the participating patients was 65 years and the mean weight was 67 kg.

Post-menopausal status was defined as age ≥ 50 years and no menstruation during the past 5 years or amenorrhoea for less than 5 years with follicle-stimulating hormone (FSH) levels in the post-menopausal range. Women with a drug-induced menopausal status (e.g., LHRH treatment) and those who had received treatment with an aromatase inhibitor within the previous 3 months were not eligible for the study.

All patients included had a WHO performance score of 0–2 at enrolment. Patients presenting with life-threatening visceral disease, an estimated survival of less than 3 months or a history of a systemic malignancy other than breast cancer were not eligible.

Of 12 patients that were entered into the study, two (nos. 1 and 2) were protocol deviators and lost for evaluation of in vivo aromatase activity because of administration of an incorrect isotope. One of these patients (no. 1) withdrew from the study having completed the first period of the crossover phase owing to disease progression and thus was lost for evaluation of the alterations in plasma sex hormones.

Treatment
Each patient was randomised to receive either 1 mg or 10 mg aromatase inhibitor p.o. once daily for 28 days (period I) and then crossed over to receive the alternative dose for another 28 days (period II). The study medication was administered between 08.00 and 10.00 hours. The clinical investigators as well as the biochemists were blinded to the treatment code. After the crossover period all patients received anastrozole 10 mg o.d. until evidence of disease progression.

Reagents
[6,7-3H]A (34 Ci mmol−1) and E2-6-carboxymethylloxime-2-[13C]ido-histamine (2000 Ci mmol−1) were obtained from Amersham, while [4-14C]E1 (50–60 mCi mmol−1), [6,7-3H]-E1S (60 Ci mmol−1) and [2,4,6,7-3H]E2 (85–105 Ci mmol−1) were obtained from DuPont NEN. Solvents were obtained from BDH Dagenham and were of analytical or HPLC grade. DEAE-Sephadex was obtained from Pharmacia, Amberlite and glucononidase (C 8885) from Sigma.

Measurement of whole body aromatisation
Aromatisation of A to E1 in vivo can be measured by administration of a steady-state infusion or a bolus injection of A and E1 labelled with different isotopes followed by determination of the isotope ratio in plasma or urinary oestrogens respectively. We have developed a high-performance liquid chromatography (HPLC) technique to improve the specificity and sensitivity of measuring the isotope ratio in urinary oestrogen metabolites (Jacobs et al., 1991). A recent formal assessment of sensitivity indicated that inhibition of up to 99.1% was detectable (Dowsett et al., 1995). In the present study, each patient had in vivo aromatisation determined before commencing treatment and at the end of period I and II by use of this urinary HPLC technique. The injections were administered on day −3, 25 and 53. On each occasion, the patient received a bolus injection of 500 μCi [3H]A and 5 μCi [13C]E1 dissolved in 50 ml saline containing 8% ethanol (w/v). Aliquots of the isotopes in the injection mixture were taken for calculation of the ratio of [3H]:[13C]. Urine was collected for a period of 96 h, pooled and kept frozen (−20°C) until time of processing.

Urine analysis
A detailed description of the analytical method and its reproducibility has been given previously (Jacobs et al., 1991) with slight modifications (Dowsett et al., 1995). In brief, the pooled urine samples were thawed and about two-thirds of the total sample used for analysis. Urinary steroid glucuronides were concentrated on an Amberlite XAD-2 column using water and methanol as mobile phase followed by Sep-pak C18 cartridges and a DEAE Sephadex A-25 column eluted by a salt gradient. The glucuronides were hydrolysed with 1 ml (144 000 units) β-glucuronidase (Sigma, C-8885) dissolved in 20 ml 0.1 M acetic acid buffer, pH 4, and incubated at 37°C for 48 h. The unconjugated steroids were separated from the water phase by ether extraction. The ether extract was subsequently washed with sodium bicarbonate (8%). The sodium bicarbonate was acidified by adding hydrochloric acid to a pH of about 2–4, and the oestrogens extracted with ether. The oestrogen fraction was purified on two column systems using DEAE Sephadex with acetic acid buffer (0.05 M, pH 12)–methanol (75:25, w/w) as mobile system and QA-E Sephadex in the boric acid form using methanol and acetic acid (0.05 M, pH 9–9.3) in methanol as mobile phase (Fotsis and Adlercreutz, 1987). Oestradiol (E2), E1 and E3 were separated by reverse-phase HPLC using Hypersil ODS 5 μm (Chrompack) 4.6 × 250 mm column and a mobile phase of acetonitrile/phosphate buffer 0.05 M, pH 3.

Because the amount of labelled E2 was much lower than the amount of E1 and E3 excreted in the urine, we used the mean value of the ratio between the amount of 3H-labelled and 14C-labelled E1 and H1-labelled and 14C-labelled E2 to calculate the ratio between H1-labelled and 14C-labelled oestrogens in the urine. Accordingly, we calculated the percentage aromatisation from the equation:

$$\%\text{ aromatisation} = \frac{[\text{H}]E2_{\text{ur}}/[\text{14C}]E2_{\text{ur}}}{[\text{H}]A_{\text{inj}}/[\text{14C}]E1_{\text{inj}}} \times 100$$

where [\text{H}]E2_{\text{ur}}/[\text{14C}]E2_{\text{ur}} is the mean value of the ratio of H1- to 14C-labelled E1 and E3 in the urine and [\text{H}]A_{\text{inj}} and [\text{14C}]E1_{\text{inj}} are the amounts of H1-labelled A and 14C-labelled E1 injected into the patient.

Plasma hormone measurements
Blood samples for E2, E1, E3 and A measurements were obtained between 08.00 and 10.00 after an overnight fast before daily drug intake and before tracer injections on days −3, 25 and 53. Blood was collected in sodium-heparinised vials, and plasma separated by centrifugation and stored at −20°C until analysis. Plasma levels of E2 and E1 were determined by methods reported elsewhere (Dowsett et al., 1987; Lenning et al., 1995). The sensitivity limit for E2 and E1 was 2.1 and 6.3 pmol l−1 respectively. Plasma levels of E3 were determined by a novel highly sensitive assay involving purification and derivatisation into E3 and RIA analysis using E2-6-carboxy-methylene-l-3H-iodohistamine as tracer ligand (Lenning and Ekse, 1995). The sensitivity limit for E3 using this method is 2.7 pmol l−1. Plasma A was measured by a commercial radioimmunoassay kit obtained from Diagnostic Systems Lab. (USA).

Measurement of plasma levels of anastrozole
Plasma levels of anastrozole were measured in fasting blood samples obtained before daily drug intake and before tracer injection on days −3, 25 and 53. Venous blood was taken into lithium heparinised tubes, centrifuged, and the plasma obtained stored at −20°C until analysis. All samples from each patient were analysed in the same batch. Anastrozole was determined using a gas liquid chromatography method with a sensitivity limit of 3.0 ng ml−1.

Statistical analysis
Percentage aromatisation and plasma hormone levels on treatment with 1 and 10 mg of anastrozole were compared
with pretreatment values by analysis of variance (ANOVA). Previous studies from our group have revealed plasma oestrone levels in post-menopausal breast cancer patients to be well fitted to a log-normal distribution (Lønning et al., 1995). Accordingly, all values are expressed as geometric means with 95% confidence intervals. The mean value of percentage suppression from baseline for a parameter was calculated as 100 minus X, where X is the geometric mean value of the individual parameters in the on-treatment situation expressed as percentage of pretreatment values.

Results

In vivo aromatase inhibition

Treatment with aromatase 1 and 10 mg reduced in vivo aromatisation from an initial value of 2.25% (95% confidence interval 1.73%–2.92%) to 0.074% (0.064%–0.083%) and 0.043% (0.021%–0.082%) respectively (Table I). This corresponds to a mean suppression of 96.7% and 98.1% (P < 0.005). Except for one patient (no. 9) who experienced a suppression of in vivo aromatisation of only 78.2% during treatment with aromatase 10 mg o.d., all patients had in vivo aromatisation suppressed by ≥93.7% during treatment with both doses of aromatase.

Comparing the percentage aromatisation during treatment with arimidex 1 and 10 mg, an arithmetic difference of 0.21% (95% confidence interval –4.99% to 4.57%) was found. However, if the analysis was repeated excluding the outlier patient (no. 9), in vivo aromatisation was suppressed by 96.0% during treatment with 1 mg and 98.6% during treatment with 10 mg, in which case the difference between the two situations became statistically significant (P < 0.01).

Calculating the ratio of the percentage aromatisation during treatment with arimidex 10 mg compared with 1 mg, this revealed a geometric mean value of 0.58 (95% confidence interval 0.29–1.15).

Plasma sex hormone levels

Many patients achieved plasma levels of E₂ and E₁ during treatment that were below the sensitivity limits of the assay, in which case the value was given as the sensitivity limit (Table II and Figure 2).

Mean plasma level of E₁ was suppressed from 73.0 pmol l⁻¹ to 9.7 pmol l⁻¹ (mean suppression of 86.8%) and 9.8 pmol l⁻¹ (mean suppression of 86.5%) during treatment with aromatase 1 mg and 10 mg respectively (P < 0.005). Plasma levels of E₂ fell from a mean value of 17.7 pmol l⁻¹ before treatment to 2.8 pmol l⁻¹ and 2.9 pmol l⁻¹ (mean suppression of 84.0% and 83.5% respectively, P < 0.005), and plasma levels of E₃ decreased from a mean value of 387.2 pmol l⁻¹ to 25.3 pmol l⁻¹ and 16.9 pmol l⁻¹ (mean suppression of 93.5% and 95.7% respectively, P < 0.005). No significant differences between plasma levels of any of the oestrogens during treatment with the two doses of aromatase were seen.

Treatment with aromatase had no significant influence on plasma levels of A (mean level of A before and during treatment with aromatase 1 and 10 mg o.d. 4.1 nmol l⁻¹, 3.3 nmol l⁻¹ and 3.1 nmol l⁻¹ respectively).

Plasma levels of anastrozole

The mean plasma level of anastrozole was 37.7 ng ml⁻¹ (range 22.0–83.9 ng ml⁻¹) during treatment with a drug dose of 1 mg and 341.4 ng ml⁻¹ (range 160.0–644.0 ng ml⁻¹) during treatment with a dose of 10 mg daily.

Discussion

Despite the fact that aminogluthethimide has been in clinical use for two decades and several other aromatase inhibitors for 5–10 years, many questions related to their biochemical action remain un-addressed. While the first study evaluating in vivo aromatase inhibition during treatment with aminoglutethimide reported the drug to inhibit the conversion of A to E₁ by about 98% (Santen et al., 1978) and contemporary studies by us (MacNeill et al., 1992; Jones et al., 1992; Lønning et al., 1991) and others (Reed et al., 1990) have found aminogluthethimide, as well as novel aromatase inhibitors such as formestane and fadrozole, to inhibit in vivo aromatisation by about 90%, plasma oestrogens have been found to be sustained at 30–50% of their control levels.

### Table I: Effects of treatment with aromatase 1 and 10 mg on peripheral aromatisation

| Patient | Pre-treatment | Aromatase 1 mg | Aromatase 10 mg | Anastrazole 1 mg | Anastrazole 10 mg |
|---------|---------------|----------------|----------------|----------------|-----------------|
| 3       | 3.06          | 0.074          | 97.6           | 0.028          | 99.1            |
| 4       | 1.99          | 0.085          | 95.7           | 0.021          | 98.9            |
| 5       | 1.79          | 0.064          | 96.4           | 0.023          | 98.7            |
| 6       | 4.72          | 0.067          | 98.6           | 0.066          | 98.6            |
| 7       | 1.77          | 0.068          | 96.1           | 0.033          | 98.1            |
| 8       | 2.85          | 0.080          | 97.2           | 0.025          | 99.1            |
| 9       | 1.94          | 0.061          | 96.8           | 0.422          | 78.2            |
| 10      | 1.46          | 0.058          | 96.1           | 0.023          | 98.5            |
| 11      | 1.60          | 0.100          | 93.7           | 0.082          | 94.9            |
| 12      | 2.83          | 0.093          | 96.8           | 0.034          | 98.8            |
| Geom.   | 2.25          | 0.074          | 96.7           | 0.043          | 98.1            |
| 95% L    | 1.73          | 0.065          | 95.6           | 0.022          | 96.1            |
| 95% U    | 2.92          | 0.084          | 97.5           | 0.083          | 99.1            |

*Percentage of aromatisation. *Percentage suppression from baseline. *Geometrical mean value. *Lower limit of the 95% confidence interval of the mean. *Upper limit of the 95% confidence interval of the mean.

### Table II: Percentage suppression of plasma oestrone levels during treatment with aromatase 1 and 10 mg

| Drug dose | Oestrone | Oestradiol | Oestrone sulphate |
|-----------|----------|------------|------------------|
|           | 1 mg     | 10 mg      | 1 mg             | 10 mg           |
| Patient   |          |            |                  |
| 1         | 93.1†    | 88.3       | 86.6             | 79.3            |
| 2         | 77.1      | 82.2       | 82.6             | 65.3            |
| 3         | 77.7      | 82.5       | 79.4             | 76.1            |
| 4         | 92.8†     | 92.8*      | 93.5*            | 96.4            |
| 5         | 91.7†     | 91.7*      | 85.0*            | 82.4            |
| 6         | 89.6*     | 89.6*      | 83.1             | 85.2*           |
| 7         | 83.8      | 82.0       | 89.5             | 93.4            |
| 8         | 76.5      | 80.2       | 74.1*            | 89.2            |
| 9         | 75.7      | 79.4       | 79.0*            | 86.0            |
| 10        | 83.6      | 75.7       | 71.8             | 58.8            |
| 11        | 93.2      | 93.4       | 93.5*            | 98.1            |

*Values < sensitivity limit of the method. *Geometrical mean value. *Lower limit of the 95% confidence interval of the mean. *Upper limit of the 95% confidence interval of the mean.
Figure 2 Plasma levels of oestrone (a), oestradiol (b) and oestrone sulphate (c) in individual patients before treatment and following 4 weeks of treatment with anastrozole 1 mg and 10 mg. Dashed line gives the sensitivity limit of the assays.

in patients treated with these drugs (Santen et al., 1982a; Vermeulen et al., 1983; Dowsett et al., 1989, 1990). Recent studies found the triazole drugs letrozole and vorozole to inhibit in vivo aromatisation by a mean of 98.5% and 93% respectively (Dowsett et al., 1995; Wall et al., 1993). While one group found letrozole to suppress plasma and urinary oestrogens by 90–95% (Masamura et al., 1994; Demers et al., 1993), others reported vorozole and letrozole to suppress plasma oestrogens by 55–90% (Iveson et al., 1993; Johnston et al., 1994), again revealing an internal inconsistency between the degree of aromatase inhibition and percentage of plasma oestrogen suppression. Such a difference could be due either to alternative (non-aromatase-dependent) oestrogen sources or lack of sensitivity of the radioimmunoassays used for plasma oestrogen measurement. Thus, there is a need to compare in vivo aromatase inhibition and plasma oestrogen suppression to develop the concept of aromatase inhibition in breast cancer treatment further.

This study was designed to determine in vivo aromatase inhibition and plasma oestrogen suppression during treatment with anastrozole, a novel aromatase inhibitor. Animal investigations (Plourde et al., 1995) suggest this drug to be a highly potent aromatase inhibitor, and preliminary studies in post-menopausal healthy women and breast cancer patients suggest the drug to be effective in suppressing plasma levels of E2 (Plourde et al., 1994). To determine in vivo aromatisation, we used a sensitive and specific HPLC assay previously used by our group to evaluate different aromatase inhibitors (Jacobs et al., 1991). Plasma levels of E2 and E1 were measured with sensitive methods previously validated in our laboratories (Dowsett et al., 1987; Lenning et al., 1995). However, owing to low levels of these oestrogens (mean concentration of plasma E2 and E1 of about 20 and 75 pmol l⁻¹) in post-menopausal women (Lenning et al., 1995), it remains difficult to detect >90% suppression of these oestrogens from pretreatment levels. On the other hand, the oestrogen conjugate E1S is found in much higher concentrations than E2 and E1 in post-menopausal women. Plasma E2, E1 and E1S are at equilibrium (Lenning et al., 1990). Thus, as long as an aromatase inhibitor does not influence enzymes involved in the interconversion of these oestrogens (sulphatase or sulphotransferase) or interacts with oestrogen metabolism (Lenning and Kvinnsland, 1988), plasma E1S and the unconjugated oestrogens should be expected to be suppressed by the same percentage during treatment with aromatase inhibitors. To measure E1S, we used a highly sensitive assay recently developed to determine plasma levels of this oestrogen in the very low range (Lenning and Ekse, 1995). Assuming a mean concentration of plasma E1S of about 400 pmol l⁻¹ in post-menopausal women, with a sensitivity of 2.7 pmol l⁻¹ this assay should be able to detect a 98–99% suppression of this plasma oestrogen conjugate.

This study reveals two important findings. First, it shows anastrozole, given as 1 mg or 10 mg o.d., to inhibit in vivo aromatisation by a mean value of 96.7% and 98.1%, respectively, and so to be one of the most potent aromatase inhibitors investigated so far. Secondly, treatment with anastrozole 1 and 10 mg o.d. suppressed plasma E1S by a mean value of 93.5% and 95.7% respectively. Therefore, our results revealed anastrozole at both doses administered to suppress plasma levels of E1S by a percentage close to the percentage aromatase inhibition. Of note, although without statistical significance, eight out of ten patients experienced a greater degree of E1S suppression when the higher dose of anastrozole was given. While we did not see a similar suppression of plasma E2 and E1, it is notable that many patients achieved plasma values of these oestrogens that were below the sensitivity limit of the methods. Thus, it is likely that we underestimated the percentage suppression of plasma E1 and E2. These data indicate that with the application of sufficiently sensitive assays, oestrogen suppression and aromatase inhibition are closely parallel in post-menopausal women.

While all patients experienced a suppression of in vivo aromatisation by >93.7% during treatment with anastrozole ≥1 mg o.d., one patient experienced an inhibition of 78.2% only during treatment with 10 mg o.d. No definite explanation for this observation was found. It is noteworthy that this patient was the one experiencing the lowest plasma concentration of anastrozole (160 ng ml⁻¹) when treated with a dose of 10 mg daily, but this concentration was considerably higher than the highest plasma concentration observed among our patients when they were treated with a
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drug dose of 1 mg daily. All medication received in this trial was accounted for, and it is not likely that the patient may have failed to take her medication as prescribed.

The difference in aromatase inhibition between anastrozole given as 1 and 10 mg o.d. (arithmetic difference of 0.21%, difference of 1.4% between geometrical mean values) was not of statistical significance. However, eight out of ten patients achieved a better aromatase inhibition during treatment with the 10 mg dose compared with 1 mg. Excluding the one outlier patient (no. 9) from the analysis revealed a difference in aromatisation of statistical significance. Thus, there is evidence that most patients may achieve a somewhat better aromatase inhibition on 10 mg compared with 1 mg of anastrozole. However, the small magnitude of this difference and the fact that it was not accompanied by any significant difference in plasma oestrogen levels suggest that this difference may be of little clinical importance. Thus, our results and previous observations by others (Yates et al., 1992), evaluating plasma E1 and E2 suppression with anastrozole single doses up to 60 mg, suggest that a dose escalation above 1 mg anastrozole once daily may not enhance plasma oestrogen suppression any further.

In conclusion, this study showed anastrozole given as 1 mg or 10 mg o.d. to be highly potent in inhibiting in vivo aromatisation and suppressing plasma oestrogens in postmenopausal breast cancer patients. Our results revealed anastrozole to suppress plasma levels of E1,S by a percentage approaching the percentage aromatase inhibition, providing an internal consistency between alterations in the in vivo aromatisation and plasma oestrogen levels in these patients. The findings support the concept that effective aromatase inhibition is accompanied by a profound suppression of plasma oestrogens in post-menopausal breast cancer patients and refute a hypothesis of alternative sources of plasma oestrogens in such patients.

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References

CASH R, BROUGH AJ, COHEN MNP AND SATOH PS. (1967). Aminoglutethimide (Elipiten-CIBA) as an inhibitor of adrenal steroidogenesis. Mechanisms of action and therapeutic trial. J. Clini. Endocrinol. Metab., 27, 1239–1245.

COOMBS RC, GOSS P, DOWSETT M, GAZET JC AND BRODIE A. (1984). 4-hydroxyxysterolane in treatment of postmenopausal patients with advanced breast cancer. Lancet, 2, 1237–1239.

DEMERS LM, LIPTON A, HARVEY HA, KAMICIK KB, GROSSBERG H, BRADY C AND SANTEN R. (1993). The efficacy of CGS 20267 in suppressing estrogen biosynthesis in patients with advanced stage breast cancer. J. Steroid Biochem. Mol. Biol., 44, 687–691.

DOWSETT M, GOSS P, POWLES TJ, HUTCHINSON G, BRODIE AHN, JEPSON IT AND COOMBES RC. (1990). Use of the aromatase inhibitor 4-hydroxyxysterolane in postmenopausal breast cancer: optimisation of dose and route. Cancer Res., 47, 1957–1961.

DOWSETT M, CUNNINGHAM DC, STEIN RC, EVANS S, DEHENVIN L, HEDGELA A AND COOMBS RC. (1989). Dose-related endocrine effects and pharmacokinetics of oral and intramuscular 4-hydroxyxysterolane in postmenopausal breast cancer patients. Cancer Res., 49, 1306–1312.

DOWSETT M, STEIN RC, MEHTA A AND COOMBS RC. (1990). Potency and selectivity of the non-steroidal aromatase inhibitor CGS 16949A in postmenopausal breast cancer patients. Clin. Endocrinol., 32, 623–634.

DOWSETT M, JONES A, JOHNSTON SRD, JACOBS S, TRUNET PJ AND SMITH HE. (1995). In vivo measurement of aromatase inhibition by letrozole (CGS 20267) in post-menopausal patients with breast cancer. Clin. Cancer Res., 1, 1511–1515.

EVANS TRJ, SALLE ED, ORNATI G, LASSMU S, BENEDEITI MS, PIANEZZOLA E AND COOMBS RC. (1992). Phase I and endocrine study of exemestane (FCE 24304), a new aromatase inhibitor, in postmenopausal women. Cancer Res., 52, 5933–5939.

FOTIS S AND ADLERCREUTZ H. (1987). The multicomponent analysis of oestrogens in urine by ion exchange chromatography and GC–MS–I. Quantitation of oestrogens in urine. J. Steroid Biochem. Mol. Biol., 28, 203–213.

GRODIN JM, SIETERI PK AND MCDONALD PC. (1973). Source of estrogen production in postmenopausal women. J. Clin. Endocrinol. Metab., 36, 207–214.

IVESON TJ, SMITH HE, AHERN J, SMITHERS DA, TRUNET PF AND DOWSETT M. (1993). Phase I study of the oral nonsteroidal aromatase inhibitor CGS 20267 in healthy postmenopausal women. J. Clin. Endocrinol. Metab., 77, 324–331.

JACOBS S, LÖNNING PE, HAYNES B, GRIGGS L AND DOWSETT M. (1991). Measurement of aromatisation by a urine technique suitable for the evaluation of aromatase inhibitors in vivo. J. Enzyme Inhib., 4, 315–325.

JOHNSON SRD, SMITH HE, DOODY D, JACOBS S, ROBERTS H AND DOWSETT M. (1994). Clinical and endocrine effects of the oral aromatase inhibitor vorozole in post-menopausal patients with advanced breast cancer. Cancer Res., 54, 5875–5881.

JONES AL, MACNEILL F, JACOBS S, LÖNNING PE, DOWSETT M AND POWLES TJ. (1992). The influence of intramuscular 4-hydroxyxysterolane on peripheral aromatisation in breast cancer patients. Eur. J. Cancer, 28A, 1712–1716.

LIPTON A, DEMERS LM, HARVEY HA, KAMICIK KB, GROSSBERG H, BRADY C, ADLERCREUTZ H, TRUNET PF AND SANTEN R. (1995). Letrozole (CGS 20267). A phase I study of a new potent oral aromatase inhibitor of breast cancer. Cancer, 75, 2132–2138.

LÖNNING PE AND EKSE D. (1995). A sensitive assay for measurement of plasma oestrone sulphate in patients on treatment with aromatase inhibitors. J. Steroid Biochem. Mol. Biol., 55, 409–412.

LÖNNING PE AND KVINNSLAND S. (1988). Mechanisms of action of aminoglutethimide as endocrine therapy of breast cancer. Drugs, 35, 685–710.

LÖNNING PE, DOWSETT M AND POWLES TJ. (1990). Postmenopausal oestrogen synthesis and metabolism: alterations caused by aromatase inhibitors used for the treatment of breast cancer. J. Steroid Biochem. Mol. Biol., 35, 355–366.

LÖNNING PE, JACOBS S, JONES A, HAYNES B, POWLES T AND DOWSETT M. (1991). The influence of CGS 16949A on peripheral aromatisation in breast cancer patients. Br. J. Cancer, 63, 789–793.

LÖNNING PE, HELLE SI, JOKKANSSON ED, ADLERCREUTZ H, LIEN EA, TALLY M, EKSE D, FOTIS S, ANKER GB AND HALL K. (1995). Relations between sex hormones, sex hormone binding globulin, insulin-like growth factor-I and insulin-like growth factor binding protein-I in post-menopausal breast cancer patients. Clin. Endocrinol., 42, 23–30.

MACNEILL FA, JONES AL, JACOBS S, LÖNNING PE, POWLES TJ AND DOWSETT M. (1992). The influence of aminoglutethimide and its analogue rolegitmidine on peripheral aromatisation in breast cancer. Br. J. Cancer, 66, 692–697.

MASAMURA S, ADLERCREUTZ H, HARVEY H, LIPTON A, DEMERS LM, SANTEN RJ AND SANTNER SJ. (1994). Aromatase inhibitor development for treatment of breast cancer. Breast Cancer Res. Treat., 33, 19–26.

PLOURDE PV, DYROFF M AND DUKES M. (1994). Arimidex: a potent and selective fourth-generation aromatase inhibitor. Breast Cancer Res. Treat., 30, 103–111.

PLOURDE PV, DYROFF M, DOWSETT M, JACOBS S, HAYNES B AND WEBSTER A. (1995). Arimidex: a new oral, once-a-day aromatase inhibitor. J. Steroid Biochem. Mol. Biol., 53, 175–179.
REED MJ, LAI LC, OWEN AM, SINGH A, COLDHAM NG, PUROHIT A, GHILCHIK MW, SHAIKH NA AND JAMES VHT. (1990). Effect of treatment with 4-hydroxyandrostenedione on the peripheral conversion of androstenedione to estrone and in vitro aromatase activity in postmenopausal women with breast cancer. Cancer Res., 50, 193–196.

SANTEN RJ, SANTNER S, DAVIS B, VELDHUIS J, SAMOJLIK E AND RUBY E. (1978). Aminoglutethimide inhibits extraglandular estrogen production in postmenopausal women with breast carcinoma. J. Clin. Endocrinol. Metab., 47, 1257–1265.

SANTEN RJ, SANTNER SJ, TILSEN-MALLET N, ROSEN HR, SAMOJLIK E AND VELDHUIS JD. (1982a). In vivo and in vitro pharmacological studies of aminoglutethimide as an aromatase inhibitor. Cancer Res., 42 (Suppl.), 3353s–3359s.

SANTEN RJ, WORGUL TJ, LIPTON A, HARVEY H AND BOUCHER A. (1982b). Aminoglutethimide as treatment of postmenopausal women with advanced breast carcinoma. Ann. Intern. Med., 96, 94–101.

SANTEN RJ, DEMERS LM, ADLERCREUTZ H, SANTNER S, SANDERS S AND LIPTON A. (1989). Inhibition of aromatase with CGS 16949A in postmenopausal women. J. Clin. Endocrinol. Metab., 68, 99–106.

VERMEULEN A, PARIDAENS R AND HEUSON JC. (1983). Effects of aminoglutethimide on adrenal steroid secretion. Clin. Endocrinol., 19, 673–682.

WALL EVD, DONKER TH, FRANKRIJKER ED, NORTIER HWR, THIJSSEN JHH AND BLANKENSTEIN MA. (1993). Inhibition of the in vivo conversion of androstenedione to estrone by the aromatase inhibitor vorozole in healthy postmenopausal women. Cancer Res., 53, 4563–4566.

YATES RA, DUKES M, DOWSETT M, DEBERARDINIS M, WILKINSON DM AND WILLIAMS AJ. (1992). Tolerability, pharmacokinetics and effect on serum oestradiol of ICI D 1033, a new aromatase inhibitor. Clin. Pharm. and Ther., Abstract of the Fifth World Conference on Clinical Pharmacology and Therapeutics, Yokohama.