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GENERAL REVIEW

POSTMENOPAUSAL ESTROGEN SYNTHESIS AND METABOLISM: ALTERATIONS CAUSED BY AROMATASE INHIBITORS USED FOR THE TREATMENT OF BREAST CANCER

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Summary—Inhibition of postmenopausal estrogen production by aromatase inhibitors is an established drug treatment modality for postmenopausal breast cancer. In this article postmenopausal estrogen disposition and the alterations caused by treatment with aromatase inhibitors are reviewed. Recent investigations have challenged the hypothesis that aromatization of androstenedione into estrone is the sole produciton pathway for estrogens in postmenopausal women. The finding that estrogens persist in the plasma of patients receiving aminoglutethimide treatment despite a near total inhibition of the aromatase enzyme suggests that alternative pathways for estrogen synthesis exist. While nonspecific actions of aromatase inhibitors may be disadvantageous, certain effects may also be beneficial. Recent findings that aminoglutethimide may induce estrone sulfate metabolism questions whether this "prototype" aromatase inhibitor might have a dual mechanism of action. The importance of investigating the possible influence of different aromatase inhibitors on all components of estrogen disposition is considered.

INTRODUCTION

Modern medical endocrine treatment of advanced, postmenopausal breast cancer may be achieved by one of three treatment modalities (1) antiestrogens, acting directly on the tumour cell, (2) aromatase inhibitors, reducing estrogen production, or (3) additive treatment with progestins in high doses, the mechanism of action of this last treatment being uncertain.

The major pathway of estrogen production in postmenopausal women is peripheral conversion (aromatization) of circulating androstenedione (A) into estrone (E_1) [1], adrenal and ovarian secretion of estrogens being minimal [2, 3]. Evidence that certain drugs might inhibit this aromatization *in vivo* was provided in 1978 when aminoglutethimide was found to reduce the conversion of A into E_1 by 95–98% [4]. Due to the successful clinical use of aminoglutethimide treatment the possibility of treating postmenopausal breast cancer patients with aromatase inhibitors has caused considerable interest [5]. The substantial number of side effects caused by aminoglutethimide treatment has stimulated research for the development of other aromatase inhibitors. Currently, several new drugs are undergoing phase I or II trials [6–8]. Animal *in vitro* and *in vivo* investigations have found some of these drugs to be much more potent aromatase inhibitors than aminoglutethimide [9, 10]. Such investigations, however, may provide information of limited value in relation to the clinical use of the drug.

While aminoglutethimide has generally been considered as a second-line endocrine treatment for use after tamoxifen [11], the increasing use of tamoxifen for adjuvant therapy may result in the use of aromatase inhibitors as first-line endocrine therapy of recurrent breast cancer. Because of the finding that long-term use of tamoxifen as adjuvant therapy may increase the risk of endometrial carcinoma [12], nontoxic aromatase inhibitors might be considered also for adjuvant therapy.

Despite aminoglutethimide causing a near total inhibition of peripheral aromatization of A into E_1 [4], plasma E_1 and E_2 are sustained at a level of 45–65% its control level in patients receiving aminoglutethimide treatment [5, 13–16]. During the last decade several investigations have challenged the hypothesis that aromatization of androstenedione accounts for the total estrogen production in postmenopausal women [17–19]. If alternative pathways do occur, this may have important implications for the development of estrogen ablative medication.

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In this review the present knowledge on estrogen production and metabolism is considered together with our understanding of the alterations in estrogen disposition caused by treatment with different aromatase inhibitors. Evidence suggests that different aromatase inhibitors may differ in their influence on estrogen dynamics. Aminoglutethimide has recently been found to stimulate estrogen metabolism in addition to inhibiting the aromatase enzyme [20]. As plasma hormone levels depend on metabolism as well as the production rate, it is possible that both mechanisms may be affected and thus act in concert to suppress plasma estrogen levels. 4-Hydroxyandrostenedione, one of the first selective aromatase inhibitors described has recently begun clinical investigation. This drug was found to be a "suicide inhibitor" *in vitro* which not only inhibited the aromatase enzyme but also irreversibly inactivated it [21]. New aromatase inhibitors may differ not only in their potency as aromatase inhibitors but also with respect to their inhibition of other enzymes or their influence on steroid metabolism. Such additional effects could be beneficial or detrimental to drug efficacy.

PLASMA ESTROGENS IN POSTMENOPAUSAL WOMEN

While several estrogen metabolites and conjugates may be detected in plasma at low levels, three different plasma estrogens are generally considered as possible sources of estrogenic stimulation for breast cancer cells: estrone (E_1) estradiol (E_2) and estrone sulfate (E_1S).

While E_2 is biologically the most potent estrogen [22], in postmenopausal women with advanced breast cancer its mean plasma level is only about 30–40 pM [23]. E_1 has a minor biological activity, but it may be converted to E_2 [24, 25]. Mean plasma E_1 levels are in the range of 100–150 pM in postmenopausal women [5, 26, 27]. There seems to be no difference in plasma E_1 and E_2 levels between women who have obtained a spontaneous menopause or women who have undergone radiological or surgical castration [28]. E_1S is a biologically inactive sulfate conjugate of E_1. Its plasma level is about 5–10 times the plasma level of E_1 [29–31], and there is evidence that this estrogen may be taken up by breast cancer cells, hydrolyzed to E_1 and reduced to E_2 inside the tumour cell [32–35].

Estrogen production

Estrogen production rates in postmenopausal women. Results obtained from different studies measuring postmenopausal E_1 production rate are given in Table 1, according to whether they were measured by the urine or plasma method. The variation between individual studies can be seen to be more extensive than any variation between results obtained by the two different methods. Accordingly, there is little evidence from these data to suggest that a major amount of E_1 is produced and metabolized in the same compartment before entering the circulation.

Estrogen production pathways in postmenopausal women. In premenopausal women, except for the first days of the menstrual cycle the major source of plasma estrogens is from direct ovarian secretion. After the menopause, while the ovary still produces androstenedione, ovarian estrogen production ceases [36, 37]. The adrenal cortex was believed at one time to be a major source of postmenopausal estrogens, but later studies have shown that while adrenal venous estrogen concentrations are found to be above arterial levels, direct adrenal estrogen excretion may account for only a few per cent of total estrogen production [2]. The first evidence that the administration of "male sex hormones" was followed by an increased urinary excretion of substances with estrogenic activity was obtained as early as 1937 [38]. In 1956 West and coworkers proved that these substances were true estrogens [39], and in 1962 Braun-Cantilo and coworkers demonstrated that tracer testosterone was converted to estrogens *in vivo* [40]. Later studies have revealed that aromatisation of circulating androstenedione (A) into E_1 is the major pathway of estrogen production [1, 41]. *In vitro* experiments have revealed the aromatase enzyme to be found in nearly every tissue examined [42–50]. Thus many tissues may contribute to this peripheral synthesis of estrogens.

Mean postmenopausal plasma androstenedione levels are about 2–4 nM [17, 26], about the same

Table 1. Estrone production rate in postmenopausal women

Author	Ref. No.	Patient group	No. of patients	E_1 prod rate; mean (range)
		(a) Measured by the urine method		
Kirschner *et al.*	147	Normal	6	19.7 μg/day (11.3–37.4)
Grodin *et al.*	1	Normal	6	46.3 μg/day (15.8–76.3)
Rizkallah *et al.*	56	Endometrial carcinoma	7	46.0 μg/day (9–110)
Reed *et al.*	148	Endometrial carcinoma	4	26.8 μg/day (20.2–33.5)
		(b) Measured by the plasma method		
Baird *et al.*	149	Normal	Not specified	115.0 μg/day not specified
Longcope	150	Endocrine dis.	2	16.5 μg/day (11–22)
Kirschner *et al.*	17	Normal	4	42.0 μg/day ± 15 (SD)
		Non-breast cancer	9	44.0 μg/day ± 16 (SD)
		Breast cancer	46	57.0 μg/day ± 32 (SD)
Judd *et al.*	66	Normal	10	49.0 μg/day (18.2–81.1)
Reed *et al.*	18	Normal	8	44.2 μg/day ± 15.7 (SD)
		Breast cancer	9	65.2 μg/day ± 39.5 (SD)

levels that are found in men [51, 52], but in premenopausal women the mean levels are about 2–3-fold higher [51]. About two thirds of the postmenopausal plasma androstenedione has been claimed to be of adrenal origin, the rest being considered to arise by ovarian synthesis [36].

In vivo investigations on aromatization have been performed by giving tracer androstenedione and estrone in concert, either as steady-state infusions with measurement of labelled plasma steroids, or as tracer injections followed by measurement of the isotope ratio in 3–7 day urine samples [1, 41, 53]. Results reported by different investigators are given in Table 2. One group [1] found that aromatization measured by the urine method was twice the value they obtained by using the plasma method in the same subjects. Otherwise, the results in the literature do not suggest that there is any consistent difference between the results obtained by the two methods. In obese females, a slow entry of estrogens from peripheral tissue into the blood stream makes long-term infusions necessary to obtain plasma aromatization values similar to urine data [54]. In premenopausal women, peripheral aromatization seems to occur to a lesser extent than in postmenopausal women [55]. The extent of aromatization of circulating A correlates with body weight [56–59] as well as age [60], but the latter relationship may be caused by menopausal factors alone [61]. Aromatization seems to occur to a similar extent in previously oophorectomized and adrenalectomized patients to that in spontaneously postmenopausal women [41]. For reasons which are not clear aromatization seems to be increased in hyperthyroidism [62] and in certain conditions of liver disease [63, 64]. Glucocorticoids are known to stimulate aromatase *in vitro* [65], but its influence on aromatization *in vivo* is uncertain.

While most *in vitro* investigations on the aromatase enzyme have used A as substrate and measured its conversion to E_1, aromatization of testosterone (T) to E_2 has also been measured [66, 67]. One study found this reaction to be of small quantitative importance in its contribution to plasma E_2 [66]. Plasma T levels are only about 40% the A levels in postmenopausal women [26], and the aromatase enzyme has a lower affinity for testosterone than for androstenedione as substrate. The mean plasma T–E_2 transfer factor is only about 0.4–0.5%, but it should be recalled that considerable interindividual differences do occur [66, 67].

A major point of controversy is whether peripheral aromatization of A to E_1 approaches the total estrogen production rate with the main production pathway of E_2 and E_1S being from E_1 [1, 15, 16, 19, 55, 68]. This may be of importance for the endocrine treatment of advanced breast cancer with aromatase inhibitors, since inhibition of the aromatization of A into E_1 *in vivo* has been used to evaluate whether these drugs inhibit postmenopausal estrogen production effectively. Only testololactone has been tested for its inhibition of aromatization of testosterone [69]. These assessments may be ill-founded if alternative production pathways exist. When the relative contribution of different production pathways have been measured, significantly different results have been obtained in different centers:

Two studies found that the E_1 production rate was similar to the amount of E_1 produced from aromatization of A [1, 68], but two other studies were unable to account for total estrogen production by this pathway [17, 66].

Two studies found that the amount of E_2 produced from E_1 accounted for only 10–30% of the plasma E_2 [18, 66]. This is consistent with tracer studies reporting that 10% of E_1 is converted into E_2 [24, 70]. Plasma T is about half the level of plasma A in postmenopausal patients [67, 71], and the mean rate of aromatization of T into E_2 is about 0.4–0.5% [66, 67]. As the transfer factor for A into E_1 is 4–6-fold higher, this may indicate that production of E_2 from T and from E_1 account for a similar amount of plasma E_2. However, studies calculating the contribution of different production pathways

Table 2. *In vivo* aromatization of androstenedione into estrone in postmenopausal women

Author	Ref. No.	Patient group	No. of patients	pA-E_1 mean (range)
		(a) Measured by the urine method		
Grodin *et al.*	1	Normal	6	2.7% (1.6–3.6%)
MacDonald *et al.*	41	Pre/post/men	13	1.3% (1.0–1.7%)
Poortman *et al.*	68	Normal	8	2.5% (1.3–3.8%)
		Breast cancer	7	2.9% (2.4–3.7%)
Hausknecht *et al.*	151	Normal	12	1.7% (1.3–2.0%)
		Endometrial carcinoma	21	3.1% (2.1–6.0%)
Hemsell *et al.*	60	Normal	23	2.0% (0.7–3.4%)
Pelc *et al.*	152	Normal	37	1.8% (0.6–3.3%)
Reed *et al.*	148	Endometrial carcinoma	4	2.1% (1.1–2.5%)
Santen *et al.*	4	Breast cancer	2	1.0% (1.1–1.9%)
		(b) Measured by the plasma method		
Grodin *et al.*	1	Normal	6	1.35% (0.9–1.9%)
Judd *et al.*	66	Normal	10	1.9% (0.6–3.7%)
Kirschner *et al.*	17	Normal	4	2.74 ± 0.50% (SD)
		Breast cancer	46	2.13 ± 1.12% (SD)
		Non-breast cancer	9	2.35 ± 0.84% (SD)
Santen *et al.*	4	Breast cancer	5	1.65 ± 0.63% (SD)

have produced conflicting results [18, 66]. A contributory factor in these differences may be the difficulty of accurately measuring the very low plasma levels of postmenopausal E_2. Results obtained by RIA using ^{125}I-labelled E_2 with high specific activity show values considerably lower than those that were obtained previously with ^{3}H-labelled E_2 [6]. This is probably largely due to the greater than 10-fold increase in sensitivity which can be obtained with these reagents.

The search for alternative estrogen production pathways has mainly been unsuccessful. There is no evidence to suggest that there is any direct secretion of E_1S. Studies so far have agreed that plasma E_1S seems to be produced by conjugation of free circulating steroids [72–74]. One study [19] suggested that the production of E_1 from DHEA without an A intermediate could account for 20–25% of the E_1 produced.

Estrogen metabolism

Estrogens are metabolized mainly by hydroxylations (and to some extent reductions and methylation) followed by glucuronidation. When tracer injections of E_1 or E_2 are administered, about 90% of radioactivity in the urine can be recovered as estrogen glucuronides [75]. The two major hydroxylation pathways are hydroxylation in the 2- and 16α-position of the steroid nucleus [76]. Hydroxylation of E_1 or E_2 in the 2-position will produce 2-OHE_1 and 2-OHE_2 respectively, the glucuronide of the former being a major urinary metabolite [77]. Similarly, hydroxylation of E_1 in the 16α-position will produce 16α-OHE_1, which may be reduced in the 17-position to yield estriol (E_3) [78]. Hydroxylation in the 16β- as well as 4-position occurs to a minor degree [79, 80]. Recent investigations have also indicated that several unidentified minor metabolites occur in urine [81].

Mean plasma clearance rates for E_1, E_2 and E_1S are reported to be 45–100, 40–70 and 5–8 l/h respectively, with somewhat higher values for males than females [18, 20, 24, 66, 70, 72–74, 82]. Thus, there is a major difference between the clearance rates of E_1 and E_2 on the one hand and E_1S on the other. E_1 and E_2 are "highly extracted compounds" [83] having a "high" clearance rate which exceeds hepatic plasma flow [74, 82], but E_1S has a slow turnover, with a mean terminal half-life of about 6 h [20]. While the high clearance rate of E_1 and E_2 may be partly explained by a high fraction of these estrogens undergoing metabolism in the extra-splanchnic compartment [24, 84, 85], oral administration of tracer E_1 and E_2 indicates that these estrogens have about a 90% splanchnic first pass extraction ratio [86].

LOCAL ESTROGEN PRODUCTION AND METABOLISM IN BREAST TUMOURS

Intracellular E_1 and E_2 concentrations in breast carcinomas are more than 10-fold higher than plasma levels [87–91]. The E_2/E_1 ratio in tumour tissue is in general higher than in normal surrounding tissue [92], possibly because of an increased concentration of 17β-hydroxysteroid dehydrogenase [93]. In contrast, the concentration of E_1S in tumours is somewhat lower than the plasma level [94]. This might be due to the hydrophilic nature of the conjugate or its rapid intracellular hydrolysis with conversion into E_1 and E_2. Tracer infusions of E_1 and E_2 have revealed tissue/plasma ratios of labelled estrogens of about 3–10 [25]. Unfortunately, labelled E_1S was not measured, and whether this estrogen could act as an intermediate for uptake of E_1 or E_2 into tumour cells is not known. However, the tumour cell contains a high content of sulfatase as well as estrogen dehydrogenase, and in theory cellular uptake and intracellular metabolism of E_1S may be a major source of unconjugated estrogens for the tumour cells [32, 33].

While the tumour cells may contain the aromatase enzyme, some *in vitro* investigations on tumour tissue have suggested that intracellular estrogen synthesis by aromatization of A into E_1 accounts for only a minor amount of the intracellular estrogen content [46, 95–97]. However, the conclusions of these studies depend on many assumptions, and recent *in vivo* tracer studies have indicated that *in situ* estrogen production may be more important in some breast tumours than uptake from the circulation [98]. Interestingly, some studies on a limited number of patients have indicated that patients with measurable intratumour aromatase activity may have a better response to treatment with aromatase inhibitors than patients with tumours lacking this enzyme [99, 100]. Further studies on a larger number of patients are warranted to confirm this finding. Other investigations have also raised the possibility that the fat and connective tissue surrounding breast cancer cells may also synthesise estrogens providing a further local estrogenic stimulus to the tumour cell [101]. Possible pathways of estrogen delivery to the tumour cell are illustrated in Fig. 1.

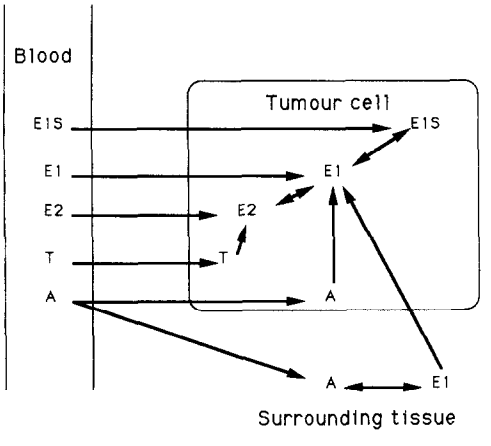

Fig. 1. Possible sources of intratumoural estrogens. E_1 is estrone, E_2 is estradiol, E_1S is estrone sulphate, A is androstenedione and T is testosterone.

PLASMA ESTROGEN DEPRIVATION AS TREATMENT OF ADVANCED BREAST CANCER

Plasma estrogen levels may be reduced by several types of treatment. In premenopausal women the levels of plasma estrogens are reduced to those in postmenopausal women following ablation of ovarian function by ovariectomy, ovarian irradiation or medical treatment with LH–RH analogues. In postmenopausal women, plasma estrogen levels are reduced by adrenalectomy, hypophysectomy, treatment with aromatase inhibitors or administration of glucocorticoids (Fig. 2), although the last of these has a smaller effect than the other treatment modalities [5, 102, 104].

The therapeutic effects of any type of ovarian deprivation in premenopausal women are well documented [105], but treatment with LH–RH analogues provokes a low response-rate among postmenopausal breast cancer patients [106, 107]. A slight suppression of plasma E_2 is seen in postmenopausal women receiving LH–RH agonists [71], but this is most probably due to a reduced ovarian secretion of T, the immediate precursor for aromatization to E_2 in peripheral tissue.

Surgical adrenalectomy is a well established treatment of advanced breast cancer in postmenopausal women [108]. Some studies have documented a significant reduction in plasma estrogens following bilateral removal of the adrenal glands or hypophysectomy. Randomized studies have suggested that the levels of plasma and urinary estrogens are reduced in breast cancer patients to a similar extent by aminoglutethimide treatment, surgical adrenalectomy or hypophysectomy [16, 103, 109]. Glucocorticoid administration decreases plasma estrogen levels by suppressing ACTH stimulation of adrenal steroidogenesis, but the reduction in plasma estrogens is of a smaller magnitude than that which is seen during aminoglutethimide treatment [102]. While certain breast cancer patients respond to glucocorticoid treatment, the overall response rate of about 15–20% is inferior to the 30–35% response rate seen in patients receiving aminoglutethimide treatment [104, 105, 110]. Thus, it seems that both glucocorticoids and LH–RH-analogues produce a slight suppression in plasma E_2 associated with a lower response rate than that to treatment with aromatase inhibitors.

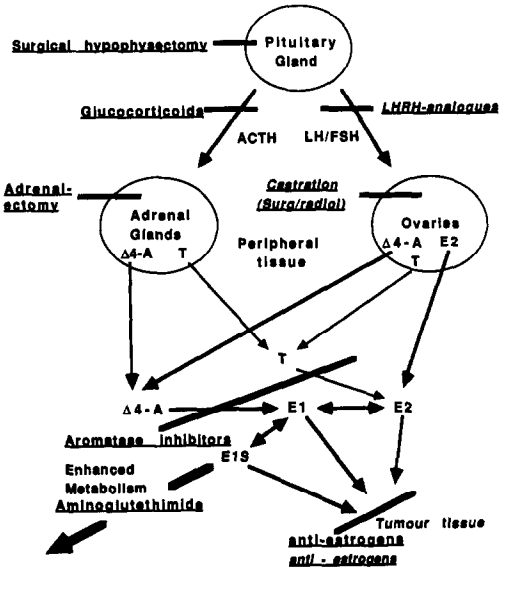

Fig. 2. Pre- and post-menopausal estrogen production pathways and therapeutic methods of estrogen deprivation in premenopausal patients. Different endocrine treatment modalities are underlined, the methods used for treatment of premenopausal patients are written in *italic*.

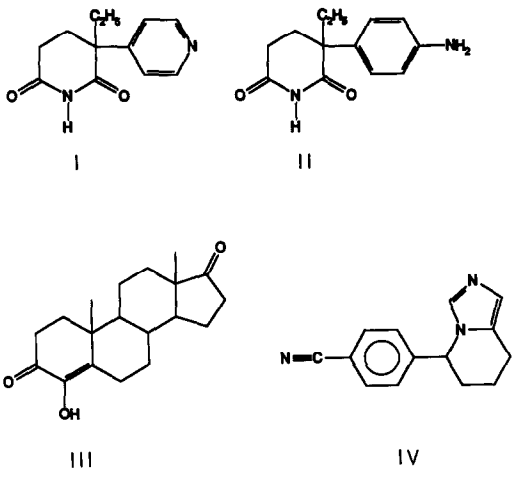

Fig. 3. The chemical structure of different aromatase inhibitors undergoing current *in vivo* investigations. I = pyridoglutethimide, II = aminoglutethimide, III = 4-hydroxyandrostenedione and IV = CGS 16949A.

IN VIVO EFFECTS OF AROMATASE INHIBITORS ON ESTROGEN DISPOSITION

Until recently, human investigations with aromatase inhibitors were limited to the two drugs aminoglutethimide and Δ^1-testololactone, but during the last few years several new aromatase inhibitors have undergone laboratory investigations. The drugs which have so far been administered to patients are the non-steroidal aromatase inhibitors aminoglutethimide, CGS 16949A and pyridoglutethimide, and the steroidal aromatase inhibitors testololactone and 4-hydroxyandrostenedione.

Investigations so far on patients receiving aromatase inhibitors have revealed persistent E_1 and E_2 levels about 45–65% their control values [5, 6, 13–15, 23] despite 95% inhibition of the aromatization of A to E_1 *in vivo*. There is considerable evidence to suggest that these persistent estrogen levels are not artefactual:

(a) Similar E_1 and E_2 levels are reported by several laboratories using different RIA methods.

(b) Circulating E_1S about 30–35% of the pretreatment levels are found in patients receiving aminoglutethimide treatment [29, 30, 74]. Quantitation of this estrogen involves isolation procedures different from isolation of E_1 and E_2. Also, because the plasma level of E_1S is about 5–10 times the plasma level of E_1 technical RIA artefacts are less likely to develop.

(c) In a recent study, plasma E_1 was measured by mass spectometry in patients receiving treatment with 4-hydroxyandrostenedione (4-OH-A), a potent aromatase inhibitor [111]. E_1 levels were found to be reduced to a mean of 40% of their pretreatment levels [23], which is consistent with findings obtained by RIA assay in patients receiving aminoglutethimide.

There is indirect evidence to suggest that these plasma estrogens do not have a dietary origin. As discussed previously under estrogen metabolism, E_1 and E_2 have first pass extraction ratios of about 90%. Therefore, only about 10% of a dose of E_1 or E_2 ingested will appear in plasma in an unmetabolized form. If about 50% of postmenopausal blood estrogens have a dietary origin, the estrogen production rate measured from urinary metabolites would be considerably higher than the production rate measured by a plasma method. The absence of a discrepancy between these two measurements was discussed above (Table 1).

Non-steroidal aromatase inhibitors

Aminoglutethimide. This drug was the first compound shown to inhibit the conversion of A into E_1 *in vivo* [4]. When aminoglutethimide was administered in the dose of 250 mg q.i.d. it was reported to cause a 95–98% inhibition of the aromatization of A to E_1 when measured by the plasma as well as urine methods. Later, patients receiving aminoglutethimide as a "low dose treatment" (125 mg b.d.) were found to aromatize A to E_1 at only about 8% of the rate found in untreated patients [112].

Despite this efficient inhibition of *in vivo* aromatization, E_1 and E_2 are sustained at about 45–65% their control levels [5, 13–15] and E_1S levels are reduced to a mean of 30–35% their control value [29, 30, 74]. As discussed above, most evidence suggests that the finding of persistent estrogens is not an artefact. In our opinion, the possibility that there are estrogen production pathways in addition to the aromatization of Δ^4-A to E_1 must be considered.

A disadvantage of aminoglutethimide is the drug's lack of specificity. In addition to being an aromatase inhibitor it is also an inhibitor of the adrenal desmolase, the 11β- and 18-hydroxylases and possibly the 21-hydroxylase [113–119]. Contrary to previous beliefs, the inhibition of adrenal enzymes caused by aminoglutethimide treatment has no suppressive effect on plasma A. Adrenal A excretion is sustained or increased during aminoglutethimide treatment (presumably because of increased ACTH secretion), depending on whether glucocorticoids are co-administered [13, 120]. However, since it is possible that glucocorticoid reserve may be depleted [121] and that the high plasma A levels resulting from aminoglutethimide used alone may be detrimental to E_1 depression [129] glucocorticoids (and sometimes mineralocorticoids) should be added to aminoglutethimide treatment.

In addition to being a multiple enzyme inhibitor, aminoglutethimide is also a potent inducer of certain hepatic mixed function hydroxylases. While this may cause drug interactions [123–125] it could also be beneficial by increasing estrogen metabolism. In recent studies aminoglutethimide treatment was found to enhance E_1S clearance by more than 100% [20, 74]. This effect could explain why this estrogen is suppressed to a larger extent than plasma E_1 and E_2. As discussed above E_1S might be an important source of intratumoural estrogens, and stimulation of E_1S metabolism might be partly responsible for aminoglutethimide's mechanism of action. It is noteworthy that while low dose aminoglutethimide (250 mg/day) seems to be nearly as efficient as the conventionally used high dose (1 g/day) in inhibiting the aromatase [4, 112] and lowering plasma E_1 and E_2 levels [13, 122, 126], alterations in E_1S metabolism (similar to alterations in warfarin metabolism [20, 127]) are probably dose-dependent. It is not clear from the literature whether aminoglutethimide low dose treatment results in the same response rates as the high dose drug schedule [121, 128, 129]. Glucocorticoids suppress the ACTH-stimulation of androstenedione levels, and it has been claimed that any possible difference in response rate seen on different drug schedules could be related to whether glucocorticoids were co-administrated rather than to the dose of aminoglutethimide [129].

While aminoglutethimide is an efficient endocrine treatment with a response rate similar to tamoxifen [130–132], aminoglutethimide treatment causes significant side effects [11]. Therefore, there is much interest in evaluating alternative non-toxic aromatase inhibitors for clinical use.

CGS 16949A. In vitro investigations of CGS 16949A® (Ciba–Geigy, 4-(5,6,7,8-tetrahydroimidazo-[1,5-alpyridin-5-yl]-benzonitrile) monohydrochloride) have shown the drug to be between 200 and 400 times more potent than aminoglutethimide *in vitro* [10]. Also, CGS 16949A has been demonstrated to cause regression of DMBA-induced tumours in rats [133]. While this drug has been claimed to be a much more specific enzyme inhibitor than aminoglutethimide, there is some evidence that at a dose of 1 mg b.d. or greater (doses required to achieve maximal suppression of plasma estradiol) the drug may also inhibit the 18-hydroxylase since plasma aldosterone levels are suppressed in breast cancer patients [134]. Early results suggest that CGS 16949A may depress plasma E_1 and E_2 levels to a similar extent to that seen in patients receiving aminoglutethimide treatment [7, 134, 135]. The finding that E_1S is reduced by

about 50% in patients receiving CGS 16949A [135] compared to a 65–70% reduction in patients receiving aminoglutethimide treatment [29, 30] suggests that CGS 16949A in contrast to aminoglutethimide may not stimulate plasma E_1S metabolism. Further endocrine and clinical investigations on CGS 16949A are in progress.

Pyridoglutethimide. Pyridoglutethimide [3-ethyl-3-(4-pyridyl)-piperidine-2,6-dione] belongs to a group of aminoglutethimide derivatives synthesized with the purpose of developing a "pure" aromatase inhibitor [136, 137]. *In vitro* investigations have shown pyridoglutethimide to be slightly weaker than aminoglutethimide as an aromatase inhibitor, and that it lacks the effect of aminoglutethimide on 20,22 desmolase [136]. Investigations are in progress to evaluate its influence on plasma estrogen levels. An important question is whether pyridoglutethimide may stimulate estrogen metabolism in a manner similar to aminoglutethimide. If it does, comparison of endocrine and clinical effects of pyridoglutethimide and other aromatase inhibitors may provide a better understanding of the importance of different plasma estrogens to breast cancer growth.

Steroidal aromatase inhibitors

Testololactone. This drug is a weaker aromatase inhibitor than aminoglutethimide *in vitro* [138]. However, when administered at the conventional dose of 1 g a day it was found to cause about 90% inhibition of the *in vivo* aromatization of A into E_1 [69, 139]. Plasma E_1 levels were found to be depressed but those of plasma E_2 were not [139]. The reason for this latter finding is unclear, since the authors were unable to exclude the possibility of a cross-reaction of the drug in their E_2 assay. The response rate to testololactone is inferior to that seen with aminoglutethimide; in a group of 241 patients receiving this drug at a dose of 1000–2000 mg/day, a response rate of only 13.5% was recorded [140].

4-Hydroxyandrostenedione. This compound belongs to a group of androstenedione derivatives which were the first compounds reported as selective aromatase inhibitors, and 4-hydroxyandrostenedione has been shown by *in vitro* investigations to be a potent aromatase inhibitor [141]. 4-Hydroxyandrostenedione is also the first of these compounds to be used for human investigations. *In vitro* studies have found this compound is a much more potent inhibitor of the aromatization reaction than aminoglutethimide [142]. Not only does it cause a near total inhibition in test-systems at a much lower concentration than aminoglutethimide, but it also behaves as a so-called suicide inhibitor, binding irreversibly to the aromatase enzyme and causing a much more prolonged action *in vitro* [21, 143]. Investigations performed so far have found that plasma E_2 is depressed to the same magnitude to or more efficiently than that seen during aminoglutethimide treatment [144]. While plasma E_1 is difficult to measure by immunoassay in 4-hydroxyandrostenedione-treated patients (due to the interference of metabolites of 4-hydroxyandrostenedione in the assay), mass spectrometry studies have revealed that plasma E_1 is depressed to a mean of 40% during 4-hydroxyandrostenedione treatment [23]. So far E_1S has not been determined in patients receiving 4-hydroxyandrostenedione treatment.

4-Hydroxyandrostenedione was found to inhibit *in vivo* aromatization by about 90% in two monkeys investigated by A/E_1 infusions prior to and during 4-hydroxyandrostenedione treatment [145]. Unpublished results (M. Reed, personal communication) suggest that 4-hydroxyandrostenedione is an efficient inhibitor of *in vivo* aromatization in postmenopausal breast cancer patients.

Phase I/II investigations of 4-hydroxyandrostenedione administered by i.m. injections [6] and by the oral route [146] indicated that the response rate in postmenopausal women was not much different from that seen in most studies of aminoglutethimide.

CONCLUSION

Several aromatase inhibitors will be undergoing phase I and phase II trials in the next few years. The information obtained will provide a basis upon which drugs will be selected for phase III trials and comparison with aminoglutethimide.

While several investigations on *in vitro* effect and drug potency have been undertaken, the revelance of much of this information for *in vivo* drug use is uncertain. When the future role of aromatase inhibitors in the treatment of breast cancer is evaluated, the following points should be amongst those considered.

(1) To what degree does aromatization of A to E_1 account for the total estrogen production in postmenopausal women?
(2) If alternative estrogen pathways do occur, might any of the known aromatase inhibitors have an ability to inhibit such pathways too?
(3) Is there evidence of a correlation between the magnitude of plasma estrogen suppression and patient response? If so: is such a correlation related to any particular plasma estrogen? Or may lack of correlation between plasma estrogen suppression and treatment response indirectly suggest that a local influence of the drug on estrogen disposition is more important than alterations in plasma estrogen levels?
(4) How can the low 10–14% reponse rate to testololactone treatment be explained when this drug caused a 90% inhibition of A to E_1 aromatization *in vivo?*
(5) Aminoglutethimide influences plasma E_1S by stimulating its metabolism. Is it possible that different aromatase inhibitors may have (partly) different mechanisms of action?

Plasma steroid levels are influenced not only by the hormone production rate, but by its elimination rate. Accordingly, dynamic studies are required to assess the influence of different drugs on estrogen production and clearance. Such information might have clinical implications by providing a basis from which drugs may be chosen as first-line treatments and a better understanding of postmenopausal estrogen disposition may be achieved. Differences in the mechanism of action between different aromatase inhibitors may indicate that combined or sequential drug treatment with different aromatase inhibitors could be beneficial.

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