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Evaluation of plasma and tissue estrogen suppression with third-generation aromatase inhibitors: Of relevance to clinical understanding?☆

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ABSTRACT

Development of aromatase inhibition and aromatase inhibitors as a therapeutic strategy was initiated through two different pathways. The one pathway went through systematic exploration of aromatase substrate analogues for enzyme inhibitions, subsequently leading to the development of steroidal agents for clinical use. The second involved clinical observation with an unsuccessful anti-epileptic compound named aminoglutethimide, attempting to achieve a "medical adrenalectomy". Endocrine studies on patients treated with aminoglutethimide lead to direct assessment of *in vivo* aromatase inhibition in patients on treatment, thus identifying a novel therapeutic strategy. As such, both research programs represent different examples of pioneering translational work leading towards a successful therapeutic strategy. Subsequent studies with respect to total aromatase inhibition have led to successful development of more potent strategies. Most importantly, these studies have revealed a correlation between aromatase inhibition and clinical outcome. Ongoing studies exploring tissue estrogen levels as well as gene expression profiles on therapy may further improve this important therapeutic area.

1. Introduction

Development of aromatase inhibition as a therapeutic strategy in breast cancer was triggered by the seminal work of two groups. Following the discovery that a non-successful anti-epileptic, aminoglutethimide, created toxic adrenal effects; the drug was implemented in a pilot experiment aiming at achieving a "medical adrenalectomy" in a breast cancer patient by Ralph Cash in the late 1960s [1]. The seminal work of Professor Santen and his team documented the clinical efficacy of this compound [2]. Moreover, through careful translational research, they revealed an endocrine profile inconsistent with the drug acting as a medical adrenal suppressor [3]. In a study reported in 1978, they showed aminoglutethimide to be a potent inhibitor of *in vivo* aromatization [4]. This observation introduced a new concept for endocrine therapy against breast cancer.

In parallel, Professor Angela and Harry Brodie's team worked on substrate analogues (androstenedione derivatives) as a therapeutic strategy blocking the aromatase enzyme in experimental systems [5,6]. Subsequently, they identified the steroidal compound 4-hydroxy- androstenedione, which was implemented for breast cancer treatment in the first pilot trial in collaboration with Charles Coombes' group in London in 1984 [7].

This finding provided the definite "proof of concept" that aromatase inhibition on its own could be an effective therapy strategy. Despite endocrine studies revealing plasma androstenedione levels to remain unchanged or even increased during aminoglutethimide treatment pending on the steroid dose administered in concert [8], other steroids, like dehydroepiandrosterone sulphate, was suppressed [3,8]. Aminoglutethimide also expressed additional biochemical effects like interacting with the disposition of prostaglandins [9]. Subsequent studies identified aminoglutethimide to be a potent inducer of mixed function oxidases [10]; in addition to severe drug interactions, this enzyme induction enhanced estrone sulphate metabolism [11,12].

While being effective therapeutic agents, aminoglutethimide as well as 4-hydroxyandrostenedione both suffered from limitations. As such, aminoglutethimide was associated with significant side effects [13]. In contrast, being non-toxic, 4-hydroxyandrostenedione had to be given by the parental route to achieve optimal aromatase inhibition and plasma estrogen suppression [14–17]. Aiming at developing less toxic compounds for oral use, the pharmaceutical industry implemented extensive research programs in this area.

The second-generation non-steroidal compound identified was fadrozole, or CGS16949A [18]. While fadrozole, similar to

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4-hydroxyandrostenedione, caused little toxicity compared to aminoglutethimide, fadrozole and formestane, similar to aminoglutethimide, revealed clinical efficacy resembling tamoxifen as first-line therapy and megestrol acetate in second-line treatment (see references in [19]).

Finally, the third-generation compounds anastrozole, letrozole and exemestane all improved clinical efficacy in metastatic disease (see references in [20]) and, more recently, in the adjuvant setting [21–25] in comparison to conventional therapy. Aiming at optimizing this therapeutic area for the future, it is imperative to explore how these clinical observations fit to contemporary translational data.

2. Third-generation aromatase inhibitors; effect on total body aromatization and plasma estrogen levels

Contrasting first- and second-generation compounds [26] for which the aromatase inhibition achieved was less than 90%, third-generation compounds; anastrozole, letrozole and exemestane each inhibit *in vivo* aromatization by 98% or better [27–29]. While these studies all were conducted on a limited number of patients, they were conducted by the same teams applying similar methods. Examining the results from these studies (Fig. 1) they clearly discriminate the biochemical efficacy of the third-generation compounds from the first- and second-generation ones. Comparing the results achieved with anastrozole, letrozole and exemestane, it may be tempting to speculate whether there are any differences in-between these compounds with respect to biochemical efficacy. Notably, while they all inhibit *in vivo* aromatization by at least 98%, based on formal assessment the assay is expected to detect around 99% inhibition in the majority of the patients [30]. As such, it is not possible to say whether a compound causing an average of 99% inhibition actually may cause 99.9% inhibition in certain patients. Realizing the effect of estrogen stimulation on cancer cells *in vitro* to be logarithmic [31], such a difference actually could be of significant importance. Interestingly, while no direct comparison between exemestane and any of the non-steroidal compounds have been performed, in a head-to-head comparison study we revealed letrozole to inhibit total body aromatization more effectively compared to anastrozole in each patient [29]. Making indirect comparison of the results from the different studies [27–29], exemestane 25 mg daily seems to be at least as potent as anastrozole 1 mg daily. In contrast, letrozole 2.5 mg daily seems to inhibit *in vivo* aromatization to a higher degree as compared to the other two compounds.

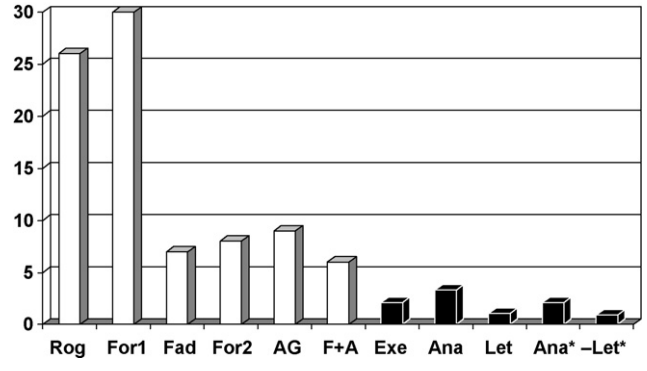

Fig. 1. *In vivo* aromatase inhibition with different first-, second- and third-generation aromatase inhibitors evaluated in the Royal Marsden–Bergen research program. Values presented as mean residual aromatase activity during therapy given as percentage of pre-treatment values. First-/second-generation compounds: Rog 0 rogletimide, For1 = formestane administered by the oral route, Fad = fadrozole, For2 = formestane administered by intramuscular injections, AG = aminoglutethimide, F + A = formestane and aminoglutethimide administered in concert. Third-generation compounds: Ana = anastrozole, Let = letrozole; *data from the study in which anastrozole and letrozole was administered in a cross-over design to the same patients. Data obtained from [14,15,18,27–29,75,76].

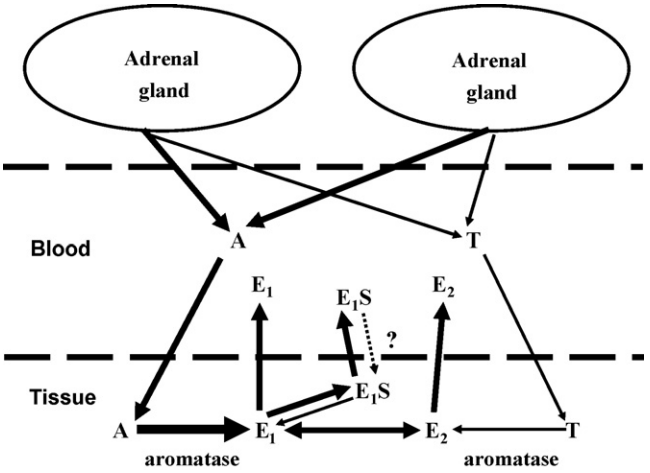

Fig. 2. Pathways of estrogen synthesis and elimination in postmenopausal women. A = androstenedione, T = testosterone, E_2 = estradiol, E_1 = estrone, and E_1S = estrone sulphate.

Considering plasma estrogen level measurements, results should be interpreted carefully. Thus, results obtained 2 decades ago with respect to drugs like aminoglutethimide cannot be compared to contemporary standards with highly sensitive assays [32,33]. Yet, even with the most sensitive assays we have today, we still observe plasma estradiol levels suppressed below detection limit in many patients during treatment with the most potent compounds [34]. Interestingly, regarding the study in which we revealed letrozole to be a more potent *in vivo* aromatase inhibitor compared to anastrozole [29], re-analyzing plasma samples with an improved methodology we now confirm better estrogen suppression with respect to all plasma hormones during letrozole therapy compared to treatment with anastrozole [34]. Similar findings have been confirmed in another study [35].

A particular problem related to estrogen measurements in plasma and tissue from patients treated with steroidal compounds as exemestane is the risk of having non-specific cross-reactions in the radioimmunoassay. Thus, to measure plasma estrogen levels with use of radioimmunoassays in patients on treatment with exemestane, samples need pre-purification with use of HPLC [36] or related methods.

One issue should be clarified. Sometimes, investigators speak about plasma estrogens as a pool separate from tissue estrogens. In postmenopausal women, estrogens are synthesized in most tissue compartments from circulating androgens, mainly produced by the adrenal glands [37]. Subsequently, tissue estrogens enter into the plasma compartment by diffusion (Fig. 2), finally to be eliminated by liver conjugation (sulphation) and renal glucuronidation with secretion into the urine. The fact that plasma levels of E_2 and E_1 are significantly lower compared to tissue levels contrasting E_1S, for which plasma and tissue levels are in the same range [38], is consisting with the finding that plasma clearance rates for E_2 and E_1 are in the range of 25–50 L/h, while plasma E_1S has a slow turnover rate (half-life 3–10 h, clearance rate 3–6 L/h) in postmenopausal women [11,12].

3. Tissue estrogen levels in postmenopausal women

The fact that tissue estrogen levels exceed plasma concentration, in particular with respect to estradiol, has been known for more than 2 decades [39–41]. The reason for this has been a subject of debate. One explanation is the potential for local estrogen syn-