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Comparison between aromatase inhibitors and sequential use☆

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Abstract

The biochemical efficacy of aromatase inhibitors and inactivators in vivo may be determined by two types of methods; by measuring plasma or tissue estrogen levels, or assessment of the conversion of the androgen substrate (in practice, androstenedione) into estrogens (estrone) by the use of tracer methods. While methods to determine plasma and tissue estrogens are limited through lack of sensitivity required to measure the very low concentrations recorded in postmenopausal women on treatment with these compounds, measurement of in vivo aromatization is an extensive procedure, applicable to a limited number of patients only. While we may correlate the mean level of aromatase inhibition achieved with different compounds to clinical efficacy, data correlating individual estrogen suppression to clinical outcome among patients treated with a specific compound is limited. The now well-characterized phenomenon of lack of cross-resistance between non-steroidal aromatase inhibitors and steroidal aromatase inactivators are likely due to biochemical effects not related to differences in total body aromatase inhibition.

Keywords: Aromatase; Inhibition; Estrogens; Suppression; Breast cancer

1. Introduction

Successful development of third-generation aromatase inhibitors and inactivators has made these compounds among the greatest achievements in contemporary breast cancer therapy. While the definite proof of clinical efficacy relates to well-conducted phase III studies in the metastatic and, more recently, in the adjuvant setting, what should be recalled is that the clinical success of these compounds, perhaps more than for any other group of compounds developed for cancer therapy, builds on the fundament of successful "translational research". The biochemical assessment of in vivo efficacy successfully conducted by different groups around the world has created a mandatory platform for the clinical trials subsequently to be conducted.

In this paper, I briefly cover three aspects: (1) methods for in vivo assessment of the biochemical efficacy of anti-aromatase compounds; (2) evidence suggesting a correlation between the degree of aromatase inhibition at (a) the individual level and (b) in-between compounds; and (3) discuss the issue of lack of cross-resistance between aromatase inhibitors and inactivators belonging to the different classes.

2. Methods for in vivo measurement of aromatase inhibition

The issue of in vitro evaluation and preclinical models are covered elsewhere (including this conference reports), and the readers are referred to other contemporary papers addressing these issues [1–3]. Here, I focus on biochemical evaluation of these compounds in vivo. Needless to say, the biochemical efficacy of a compound (and, in particular, rating between compounds) may be different in vivo from what is seen in an in vitro test system, as in vivo efficacy may depend on additional parameters like drug metabolism (half-life), as well as tissue distribution. This may be illustrated by comparing the "second-generation" compound fadrozole to letrozole; thus, while in vitro experiments have revealed a higher biochemical efficacy for fadrozole compared to letrozole [2], in vivo, letrozole shows a better suppression of plasma estrogens and more efficient inhibition of the aromatase reaction.

In vivo, aromatase efficacy may be determined either by measuring plasma or tissue estrogen levels or, alternatively, assessment of the conversion of androgens into estrogens, using double-tracer methods.

The relationship between plasma and tissue estrogen levels is complex, in particular in postmenopausal women. As has been shown by several groups over the years and will be covered by others in this issue (see papers by Miller and Geisler), in postmenopausal women breast tumour estradiol

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levels are at least one magnitude higher compared to plasma levels [4–6], and studies by different groups have revealed a significant inter-individual difference regarding local tumour estrogen production versus uptake from the circulation [7,8]. However, while several groups have determined either intratumor aromatase inhibition in vivo or tumour estrogen levels [5,6,9–11], different groups have applied different methods, and none of these groups have consistently evaluated the full spectrum of first-, second- and third-generation aromatase inhibitors available. Thus, I limit the discussion in this paper to plasma estrogen levels and assessment of total body aromatase inhibition.

We are facing three major problems considering use of plasma estrogen levels to compare the efficacy of different aromatase inhibitors.

First, it is difficult to compare results obtained by different groups due to methodological differences. Noteworthy, currently there are no international guidelines or quality control programs focusing on determination of low plasma estradiol (E_2) levels in postmenopausal women, and mean values reported by different groups vary considerably [12–15]. A general trend over time has been recording of subsequently lower E_2 levels in contemporary, compared to older, studies, with mean plasma E_2 levels of about 15–20 picomolar (pM) among groups currently focusing on aromatase inhibitors [14,15].

A second issue relates to potential drug interactions in the assay system. Currently, the only methods expressing sensitivity for measurement of hormone levels in the pM range are radiometric methods, in general done following a "crude" purification of the samples using LH20 Sephadex or Lipidex columns. Only a few methods involve use of HPLC. This problem is of particular relevance for compounds containing a steroidal structure, like formestane and exemestane. While cross-resistance to the mother compound and major identified metabolites may be identified, the fact that exemestane is administered at a dose of 25 mg daily while endogenous estrogen production in postmenopausal women may drop from about 50 μg [16] to perhaps 1 μg per day on treatment (based on expected degree of aromatase inhibition [17]) suggests that even a slight cross-reaction to a metabolite accounting for less than 1% of drug metabolism may create problems. Thus, in a recent phase I study evaluating endocrine effects of exemestane in escalating doses, we found a general suppression of plasma estrogen levels of about 70% which improved to about 85–90% in case the samples were purified by HPLC prior to RIA [18].

The third problem relates to lack of sensitivity of the RIAs in use for estrogen measurement in general. Based on mean plasma estrogen levels and the sensitivity limit in our own assays, we recently estimated that it may be possible to detect around 99% suppression of plasma estrone sulphate (E_1S) in 50% of the patient, while the similar figures in relation to estrone (E_1) and estradiol (E_2) turned out to be around 90 and 85% only [19]. In contrast, assessment of aromatase inhibition by use of tracer injection techniques [20] allows for assessment of >99% inhibition in the bulk of patients. The major limitation of such in vivo tracer methods, however, relates to their time- and resource-requirement; thus, they are applicable to studies evaluating the efficacy of a compound in a limited (example $n = 12$) group of patients, but cannot be applied to larger numbers.

Table 1
Aromatase inhibitors in current or previous use

Compound	Type	Generation (%)	Dose	Aromatase inhibition	Reference
Aminoglutethimide	Inhibitor	First	1000 mg per day	91	[23]
Fedrozole	Inhibitor	Second	2 mg per day	82.4	[21]
			4 mg per day	92.6	
Formestane (oral)	Inactivator	Second	125 mg per day	72.3	[25]
			125 mg bid	70.0	
			250 mg od	57.3	
Formestane (intramuscular)	Inactivator	Second	250 mg/2 wk	84.8	[22]
			500 mg/2 wk	91.9	
			500 mg/wk	92.5	
Anastrozole	Inhibitor	Third	1 mg per day	96.7	[26]
			10 mg per day	98.1	
Letrozole	Inhibitor	Third	0.5 mg per day	98.4	[28]
			2.5 mg per day	98.9	
Anastrozole/letrozole[a]			1 mg per day (anastrozole)	97.3	[27]
			2.5 mg per day (letrozole)	>99.1	
Exemestane	Inactivator	Third	25 mg per day	97.9	[17]

The figures for the percentage aromatase inhibition are all obtained in a joint programme involving the Royal Marsden Hospital and our own institution, using the same experimental design: bid, twice daily; od, once daily; wk, weeks; 2wk, every second week.

[a] Evaluated in the same 12 patients in a cross-over-study.

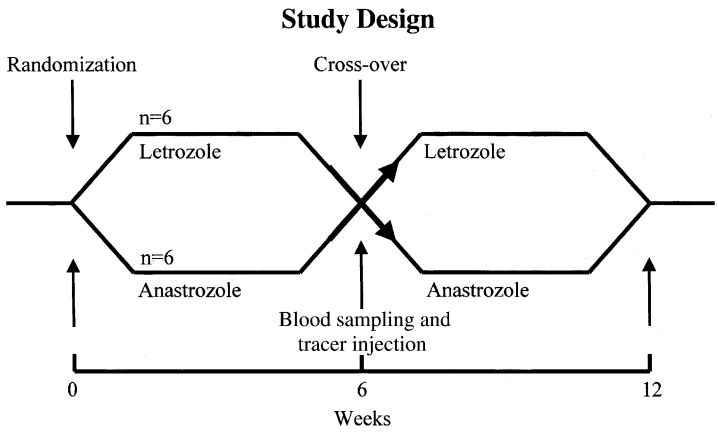

Fig. 1. Design of "cross-over-study" comparing in vivo aromatase inhibition of anastrozole and letrozole [27]. Reproduced with permission from [66].

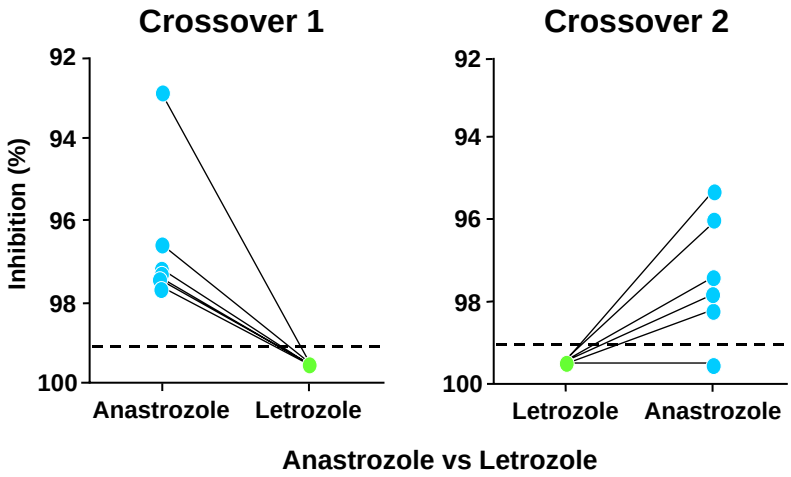

Fig. 2. In vivo aromatase inhibition of anastrozole and letrozole achieved in the study presented in Fig. 1 [27]. Notably, letrozole caused a more profound suppression of in vivo aromatisation compared to anastrozole independent of the sequence of administration. Reproduced with permission from [66].

In a joint program conducted between the Department of Academic Biochemistry, headed by Professor Mitch Dowsett, collaborating clinicians at the Marsden Hospital and our own group at the Section of Oncology, University of Bergen, we evaluated different first-, second- and third-generation compounds for their ability to inhibit in vivo aromatization (Table 1) [17,21–28]. While several other groups [10,29] have performed similar studies since the pioneering work of Richard Santen and colleagues in 1978 [30], none of these groups evaluated a panel of drugs. For method reasons, results obtained by different groups using different methods may not be compared.

The results presented in Table 1 show that aromatase inhibitors and inactivators may be divided into 3 main categories; those showing a poor biochemical efficacy (rogletimide and formestane when administered by the oral route), the "in-between" first- and second-generation compounds causing 85–90% inhibition, and the recent third-generation compounds (anastrozole, letrozole and exemestane), causing around 98% inhibition or better. Noteworthy, any comparison between compounds based on a modest difference with respect to mean level of aromatase inhibition should be interpreted carefully. While the method used in the different studies is similar, due to inter-individual variation in patient response minor differences may occur by chance.

The issue whether there may be a difference between anastrozole and letrozole merits particular attention, because these compounds were compared in a cross-over-study involving 12 patients (Fig. 1). In this study, letrozole was found consistently to cause more profound inhibition of in vivo aromatization compared to letrozole [27], independent of the sequence of administration (Fig. 2).

3. Clinical relevance of biochemical efficacy

This important issue may be addressed by two different approaches: (a) to look for any potential correlation between degree of estrogen suppression among individuals exposed to the same drug compound in a clinical study and (b) by

Table 2
Third-generation aromatase inhibitors and inactivators compared to megestrol acetate (MA) in phase III studies

	Letrozole–MA		Vorozole–MA	Anastrozole–MA[a]	Exemestane–MA
RR (CR + PR)	Yes	No	No	No	No
"Benefit" (CR + PR + S.D. > 6 months)	No	No	No	No	No
Time to progression (TTP)	No	No	No	No	Yes
Time to failure (TTF)	Yes	No	–	No	Yes
Survival	No	No	No	Yes	Yes
Reference	[39]	[40]	[41]	[42]	[43]

Yes/no refers to whether there are statistical differences regarding endpoint parameters.
[a]Combined analysis of two studies.

comparing mean degree of aromatase inhibition to clinical response caused by the different compounds.

Considering the first issue, only two studies did compare the degree of estrogen suppression to clinical outcome among patients within a clinical trial [31,32]. During their pioneering studies establishing the biochemical as well as clinical rationale for aminoglutethimide (and, thereby, subsequent aromatase inhibitors) more than two decades ago, Professor Santen's group compared the degree of estrogen suppression among responders and non-responders to treatment with the first-generation aromatase inhibitor aminoglutethimide [31], revealing no difference between the two groups. However, as mentioned above, it may not be correct to compare these results to contemporary studies done more than two decades later. In a more recent study [32], the authors compared the degree of estrogen suppression among non-responders and responders to formestane, claiming a better estrogen suppression in the second group. Their general finding of a mean suppression varying between 35 and 60% in the different patient sub-groups contrasts our own finding of a 85–90% inhibition as assessed by tracer techniques (Table 1), suggesting cross-reactions from drug metabolites in their assay. For reasons unexplained, these authors found higher pretreatment levels of plasma estrogens among responders compared to non-responders. Finally, a study conducted by Dowsett et al. two decades ago [33] revealed a marginal increase in plasma estrogens (together with adrenal hormones) at time of relapse, suggesting that an increase in plasma estrogen levels ("escape phenomenon") may not be a major cause of relapse among patients becoming resistant to therapy over time.

What information may be achieved by comparing the degree of aromatization to clinical outcome among different compounds? Looking at the second-generation compounds fadrozole and formestane, both compounds were compared to either megestrol acetate (MA) (second-line) or tamoxifen (as first-line) therapy in advanced breast cancer [34–38]. While all these studies contained a limited number of patients by today's standards, notably none of them revealed superiority in favour of formestane or fadrozole regarding time to progression (TTP), response rate, or survival versus their comparators.

Considering studies comparing the third-generation compounds anastrozole, letrozole and exemestane to megestrol acetate [39–43], a somewhat mixed picture may be seen (Table 2). However, the general trend suggests some improvement regarding clinical outcome with use of the novel compounds. A more consistent picture emerges from the phase III studies comparing these compounds to aminoglutethimide or fadrozole, depicted in Table 3 together with the results from the study comparing anastrozole to letrozole [44–47]. Noteworthy, in these studies a dose of aminoglutethimide of 500 mg daily was used, while our tracer study assessing aminoglutethimide aromatase inhibition used a dose of 1000 mg (Table 1). Earlier studies may provide some conflicting evidence here; while Dowsett et al. found aminoglutethimide administered at a low dose of 250 mg daily to cause >90% aromatase inhibition [48], aminoglutethimide also enhances metabolism of estrone sulphate [49] in a dose-dependent manner in the range of 250–1000 mg daily [50]. The potential clinical implication of this effect remains unknown.

Table 3
Third-generation aromatase inhibitors and inactivators compared to first- and second-generation compounds; aminoglutethimide (AG) or fadrozole (FA) in phase III studies and the study comparing anastrozole to letrozole second-line

	Letrozole–AG[a]	Letrozole–FA	Vorozole–AG[a]	Anastrozole–letrozole
RR (CR + PR)	No	Yes	No	Yes
"Benefit" (CR + PR + S.D. > 6 months)	No	Yes	Yes	–
Time to progression (TTP)	Yes	No	No	No
Time to failure (TTF)	Yes	–	Yes	No
Survival	Yes	–	No	No
Reference	[44]	[45]	[46]	[47]

Yes/no refers to whether there are statistical differences regarding endpoint parameters.
[a]AG dose 500 mg daily.

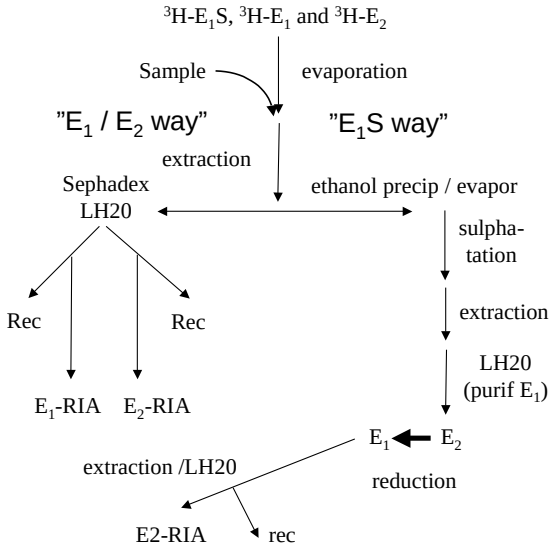

Fig. 3. Schematic outline of the assays for analysing plasma E_2, E_1 and E_1S currently used in our laboratory [52].

The data from first-line phase III studies comparing anastrozole, letrozole and exemestane to tamoxifen are reviewed elsewhere in this issue, and will not be further addressed here. Sufficient to say, they suggest superiority for these novel compounds compared to tamoxifen in the metastatic setting.

In conclusion, we lack data addressing whether there may be a significant correlation between the degree of aromatase inhibition and clinical outcome among individual patients treated with the same compound in a controlled clinical setting. Considering the multitude of potential mechanisms of resistance to endocrine therapy in general (reviewed in [51]), it may be foreseen that such studies (if to be conducted) may need a large number of patients. The only endocrine parameter at this stage that may be validated for such comparisons should be plasma E_1S. However, the methods for E_1S measurements harbouring the sensitivity for such evaluations are work- and resource-consuming, involving several sample purification steps, hydrolysis and reduction to E_2 prior to RIA (Fig. 3) [52].

4. Lack of cross-resistance between aromatase inhibitors and inactivators; due to enhanced biochemical efficacy regarding aromatase inhibition?

Following the pioneering work of Murray and Pitt [53] showing that patients failing on aminoglutethimide may subsequently respond to treatment with formestane, several publications (Fig. 4) have addressed the issue of lack of cross-resistance to compounds belonging to the “aromatase inhibitor type II” (non-steroidal, or reversible, inhibitors) and the “type I inhibitors” (steroidal compounds, currently named “aromatase inactivators”). With one exception [54] these studies all address the response to a steroidal compound following failure on non-steroidal agents. However, a study evaluating use of anastrozole or letrozole in patients failing exemestane is currently conducted [55]. The differences considering their biochemical effects on the aromatase enzyme is addressed elsewhere in this issue as well as in previous publications by others [1]. Based on evaluation of total body aromatase inhibition (Table 1), there is currently no evidence pointing to any difference regarding their efficacy on total body estrogen synthesis. While responses to exemestane following aminoglutethimide failure [56,57] or benefits to anastrozole after formestane [54] may be due to enhanced aromatase inhibition, this may not explain the fact that patients may achieve an objective response or durable stable disease when treated with formestane after failing on aminoglutethimide [53,58] or exemestane after failing on one of the novel, third-generation, non-steroidal inhibitors [57]. In a recent study, Carlini et al. further revealed durable stable disease on formestane in patients failing anastrozole or letrozole [59]. One explanation to these observations may be differences with respect to intratumor pharmacology (effects on breast cancer aromatase) or tissue

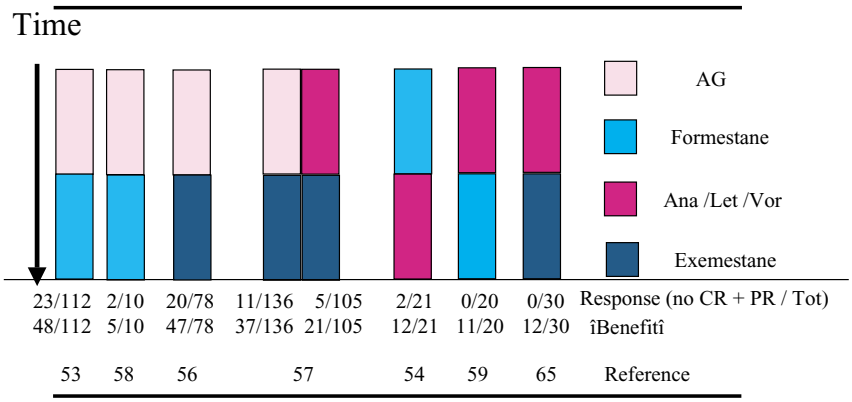

Fig. 4. Schematic presentation of studies evaluating different aromatase inhibitors and inactivators sequentially [53,54,56–59,65]. Red colours are the inhibitors, while blue represents the inactivators; dark colours illustrate the third-generation compounds of each class.

pharmacokinetics. A second possibility may be additional endocrine effects of the steroidal aromatase inactivators. Interestingly, exemestane, represented by its major metabolite 17-hydroexemestane, express androgenic activities in vivo, as documented by a dose-dependent suppression of plasma sex-hormone binding globulin [18]. A similar effect is seen when formestane is administered by the oral but not the parenteral route [60]. In a large study containing more than 1000 tumour samples, androgen receptor levels >10 fmol/mg protein was recorded in more than 80% of the cases [61].

In conclusion, we currently do not know the reason for lack of cross-resistance between compounds of the different classes. A potential strategy to explore this important issue may be to look at gene transactivation in tumours sampled on different therapies by global strategies like cDNA microarrays [62] concentrating on estrogen- as well as androgen-related genes.

5. Summary and conclusions

While we lack evidence confirming a dose-response relationship between aromatase inhibition and clinical outcome among individual patients on treatment with aromatase inhibitors, current evidence (albeit somewhat conflicting) suggest an improved efficacy with third-generation compounds (anastrozole, letrozole and exemestane) compared to conventional therapies like megestrol acetate and tamoxifen but also when compared to less potent aromatase inhibitors of the first- and second-generation class. Based on in vitro data, one mechanism of resistance towards estrogen deprivation may be development of "estrogen hypersensitivity" [63] instead of insensitivity; indirect support for this hypothesis was achieved in a recent study in which we found responses to high-dose estrogen therapy in patients failing on aromatase inhibitors [64]. While letrozole clearly is more potent inhibiting in vivo aromatization compared to anastrozole [27], the exact magnitude of the difference is difficult to assess, based on the fact that each patient achieved aromatase inhibition below the sensitivity limit during letrozole therapy. Clinical data are warranted. However, it may be recalled that the "adaptor process" of sensitization in vitro spanned a ratio of 10^4; thus, a potential lack of difference in clinical outcome between patients exposed to anastrozole and letrozole may not exclude the hypothesis that more enhanced estrogen suppression (below what is achieved by today's drugs) may cause additional responses in patients harbouring hormone-sensitive tumours. To address this issue is one of tomorrow's challenges in the field of endocrine therapy for breast cancer.

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