

Drug and hormone interactions of aromatase inhibitors

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Abstract

The clinical development of aromatase inhibitors has been largely confined to postmenopausal breast cancer patients and strongly guided by pharmacological data. Comparative oestrogen suppression has been helpful in circumstances in which at least one of the comparitors has caused substantially non-maximal aromatase inhibition. However, the triazole inhibitors, letrozole and anastrozole, and the steroidal inhibitor, exemestane, all cause >95% inhibition. Comparisons between these drugs therefore require more sensitive approaches such as the direct measurement of enzyme activity by isotopic means. None of these three agents has significant effects on other endocrine pathways at its clinically applied doses. Pharmacokinetic analyses of the combination of tamoxifen and letrozole have revealed that these drugs interact, resulting in letrozole concentrations approximately 35-40% lower than when letrozole is used alone.

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Introduction

Over the past 20 years, a large number of aromatase inhibitors have been studied in clinical pharmacological trials and the results from these have contributed to the clinical utilisation of the drugs, particularly in relation to the selection of dosage for widespread treatment. Some of these drugs are now accepted for use as the preferred second-line agent (after tamoxifen) for advanced breast cancer treatment and are also in large-scale trials for the adjuvant treatment of breast cancer. Consideration is also being given to their exploratory use for breast cancer prevention.

This review considers the comparative pharmacological effectiveness of the compounds and their selectivity and specificity. The majority of these data were derived from postmenopausal women, but data from their application in premenopausal women and in men is also considered. Recent data on pharmacokinetic interactions and illustrations of interactions at a pharmacodynamic level are also discussed.

Aromatase inhibitors are generally described as type 1 or type 2 inhibitors. Type 1 are steroidal compounds that may be purely competitive inhibitors, or more commonly in the case of those compounds that are used clinically, irreversible 'suicide substrates'. Type 2 inhibitors are non-steroidal and each of these binds to the haem group of the aromatase enzyme by co-ordination through a basic nitrogen atom. A series of these compounds has been

developed, with the most successful being the triazole group of inhibitors: letrozole, anastrozole, vorozole, and YM511. The different mechanisms of interaction of the two types of inhibitors with the enzyme are well described in the paper by Kao *et al.* (1996).

Pharmacological effectiveness

The degree to which an inhibitor reduces the activity of the aromatase enzyme can be measured in two ways: (i) directly by the *in vivo* measurement of the aromatase enzyme activity using isotopic tracer techniques, and (ii) by the measurement of plasma or urine oestrogen concentrations. Although the latter has been the most widely used approach in the clinical development and comparison of pharmacological efficacy between drugs/doses, its use is limited by the very low concentrations of unconjugated oestrogens in the plasma of postmenopausal women and the limited sensitivity of the respective immunoassays.

Aromatase inhibition

We have applied the isotopic technique to measure whole-body aromatisation, to compare the effectiveness of several aromatase inhibitors. Given that aminoglutethimide (AG) is viewed as the prototype aromatase inhibitor, this has been our reference point, with inhibition produced by the 1000 mg/day dose being approximately 90% (MacNeill *et al.* 1992). The structural derivative of

AG, rogletimide, was of greater specificity than AG, but it had only poor pharmacological efficacy, with inhibition less than 80% even at the dose of 800 mg twice daily. The steroidal aromatase inhibitor, 4-hydroxyandrostenedione (formestane), is used clinically at a dose of 250 mg every 2 weeks, to avoid the high incidence of local side effects that occurs with the 500 mg dose. This compromise dose results in a suppression of aromatase activity by a mean 85%, which is therefore less than that achieved by AG (Jones *et al.* 1992). More recently, we have been able to show that the orally active steroidal inhibitor, exemestane, at its clinically used dose of 25 mg/day suppressed activity by about 97% (Geisler *et al.* 1998), and this compares well with the suppression achieved by the triazole inhibitor, anastrozole, at the 1 mg/day dose (Geisler *et al.* 1996). We have also performed analyses of the other triazole inhibitor, letrozole (Dowsett *et al.* 1995). After treatment with its clinically used dose of 2.5 mg/day, three of five patients had undetectable aromatase activity (i.e. greater than 99% suppression) and activity was barely detectable in the other two. Very little difference was apparent between the 0.5 mg and 2.5 mg/day doses of letrozole, although it is of interest that substantial differences have been noted in the clinical activity of these doses (Dombernowsky *et al.* 1998, Gershanovich *et al.* 1998).

Oestrogen suppression

Measurement of the suppression of plasma oestrogens by aromatase inhibitors has been valuable when aromatase inhibition has been substantially incomplete. For example, we demonstrated several years ago that, with the 250 mg/2 weeks dose of formestane, there was a residue of approximately 10 pmol/l oestradiol, and that this partial suppression was poorer just before each injection (Dowsett *et al.* 1987). This was confirmed in a recent

multicentre study in which formestane was compared with anastrozole, and which confirmed the pharmacologically greater effectiveness of anastrozole over formestane (Kleeberg *et al.* 1997). More recently, we have found that vorozole is also a more effective oestrogen suppressant than formestane, as determined by plasma oestradiol, oestrone, and oestrone sulphate concentrations (Dowsett *et al.* 1999a) (Fig. 1).

The question is frequently asked whether these very small differences in plasma oestrogen concentrations could be of biological or clinical importance. It has become clear over the past 2 years, however, that breast cancer cells *in vitro* that are deprived of oestradiol in the long term have enhanced oestrogen sensitivity such that, whereas wild-type cells are maximally stimulated with 10^{-11} - 10^{-10} M oestradiol, the deprived cells are maximally stimulated with 10^{-14} - 10^{-13} M (Masamura *et al.* 1995). These data also indicate that concentrations of oestradiol well below that which can be measured in conventional immunoassays may be of biological relevance: it is therefore important to recognise that biologically significant differences in oestrogen concentrations may be undetectable by standard pharmacology.

Specificity/selectivity of aromatase inhibitors

The aromatase enzyme is a member of the cytochrome P450 superfamily, which has over 100 members. For steroidal inhibitors, there is concern that they or their metabolites may have the potential for some hormonal activity. The non-steroidal inhibitors are unlikely to have hormonal activity but, as they actively inhibit the action of the haem prosthetic group of the enzyme, there is a possibility that they could interact similarly with other family members. The latter is illustrated by AG, which

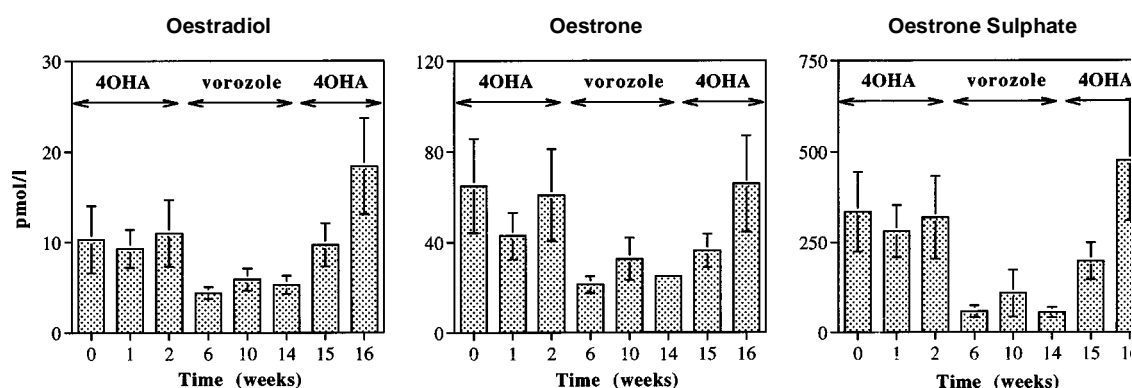


Figure 1 Comparison of plasma oestrogen concentrations (mean±S.E.M.) during formestane (4OHA) and vorozole treatment of postmenopausal breast cancer patients. Patients had received at least 3 months of 4OHA treatment before measurements were made during a 2-week period of 4OHA. Patients then received vorozole 2.5mg/day for 12 weeks before restarting 4OHA.

inhibited the 20/22-desmolase, the 11 β -hydroxylase and 18-hydroxylase enzymes, all of which led to complications in corticosteroid synthesis. The very potent inhibitor, fadrozole, also inhibits 18-hydroxylase and leads to the suppression of aldosterone at daily doses of the magnitude required for maximal oestrogen suppression (Dowsett *et al.* 1990). None of the triazole inhibitors, letrozole, anastrozole, vorozole, and YM511, has had selectivity issues identified at clinical dosages.

Formestane, when given by the intramuscular route, leads to no significant changes in luteinizing hormone (LH), follicle-stimulating hormone (FSH), or sex hormone-binding globulin (SHBG) concentrations, which are indices of androgenic or oestrogenic activity. It is of only academic interest that SHBG concentrations are decreased when formestane is given by the oral route (Dowsett *et al.* 1989). A decrease in SHBG is also seen with exemestane but, again, this is of little clinical relevance, as the change is noted only at drug dosages greater than the clinically used dose of 25 mg/day (Johannssen *et al.* 1997).

Aromatase inhibitors in premenopausal women

AG has been studied in three sets of premenopausal women (Santen *et al.* 1980, Harris *et al.* 1982, Wander *et al.* 1986). In none of these were oestrogen concentrations suppressed to postmenopausal values, although some menstrual irregularities were noted and two of the three patients in the study by Harris *et al.* showed no response to treatment with exogenous gonadotrophin. These observations, together with those from the study by Santen's group, which showed increased gonadotrophin concentrations during AG therapy, indicate a partly compensated inhibition of ovarian oestrogen synthesis. Studies by Stein *et al.* (1990) of the steroidal inhibitor, formestane, at a four times higher dose than that given clinically to postmenopausal women, again demonstrated no significant impact on oestradiol and no effect on LH or FSH concentrations. The only study published to date on the application of the new potent aromatase inhibitors in premenopausal women is that on vorozole, which showed that, after 24 h, plasma oestradiol concentrations could be reduced from approximately 400 to 200 pmol/l (Wonters *et al.* 1989).

We have shown, however, that aromatase inhibitors are effective in some premenopausal women after the application of a gonadotropin-releasing hormone agonist which itself results in postmenopausal plasma oestrogen concentrations (Stein *et al.* 1990). The demonstration that this occurred with goserelin plus formestane has now been confirmed with the addition of vorozole to goserelin: plasma oestradiol concentrations were suppressed from

approximately 50 pmol/l with goserelin alone to values less than 10 pmol/l with the combination (Dowsett *et al.* 1999a).

Aromatase inhibitors in men

The effect of aromatase inhibition on male gonadotrophin and sex steroid concentrations is illustrated in the paper by Trunet *et al.* (1993): 2.5 mg letrozole suppressed plasma oestradiol concentrations to less than 50% of pretreatment after 2 days, with recovery to approximately pretreatment values after 6 days. These decreases were accompanied by increased gonadotrophin concentrations, with resultant increases of approximately 50% in plasma testosterone. These results, and those previously published (Bhatnagar *et al.* 1992) on the effects of fadrozole in men, indicate that the aromatization pathway is of major importance in the regulation of gonadotrophin secretion by aromatisable androgens.

Pharmacokinetic interactions

The combination of an aromatase inhibitor with tamoxifen is a possible route whereby improved efficacy of endocrine therapy might be achieved. The consideration of such clinical combinations requires assessment of potential pharmacokinetic interactions. This was stressed by the study of Lien *et al.* (1990), who demonstrated that combination with AG led to decreases in tamoxifen concentrations of about 70%. We have now performed an analogous study with anastrozole, and found that this inhibitor does not lead to decreased tamoxifen concentrations (Dowsett *et al.* 1999b). In this issue of *Endocrine-Related Cancer*, Ingle *et al.* (1999) also report that letrozole has no impact on tamoxifen concentrations. Thus these two compounds may be added to tamoxifen with no concern that the pharmacokinetics of the antioestrogen will be compromised. However, unexpectedly, we have noted that, conversely, in combination with tamoxifen, the plasma concentrations of letrozole are reduced by between 35 and 40% (Dowsett *et al.* 1997). The mechanism underlying this highly unexpected finding is at present unknown. No other drugs are known to interact with tamoxifen in this manner, but it is of concern that systematic pharmacokinetic interaction studies with tamoxifen have been relatively limited, despite the millions of women years of its usage. Decreases in AG concentration did not occur when it was combined with tamoxifen. The question of whether the anastrozole-tamoxifen combination will demonstrate this difference is currently under study. As a result of this pharmacokinetic interaction, the plasma concentrations of letrozole are equivalent to those predicted to be achieved by 1.5-2.0 mg/day when given alone. It is possible that this

may be of clinical importance because, as mentioned above, there is a dose-response relationship between letrozole 0.5 mg/day and 2.5 mg/day (Dombrowsky *et al.* 1998, Gershanovich *et al.* 1998). Thus, with the combination, the complete additive efficacy of letrozole may not be achieved.

Pharmacodynamic interactions

Unexpected pharmacological interactions may also occur with aromatase inhibitors. One example of this is the adverse interaction of AG and danazol (Dowsett *et al.* 1985). As described above, AG markedly suppresses aromatase activity and plasma oestradiol concentrations in postmenopausal women. Danazol has little effect on total oestrogen concentrations, but its marked suppression of SHBG levels leads to an approximate doubling of percent-free oestradiol. These effects were shown to be maintained when the two drugs were combined and the impact of this was that the concentration of free oestradiol was sustained, despite the reduction in total oestradiol concentrations exerted by AG. The pharmacological mechanism of AG being compromised by the combination with danazol was reflected in a poor clinical outcome (Murray & Pitt 1984).

Conclusions

Steroidal and non-steroidal aromatase inhibitors are available which achieve effects that approximate to complete aromatase inhibition. However, the minor differences between them may be of significance, because of the extreme sensitivity that breast cancer cells can achieve when they are deprived of oestrogenic stimulation. The specificity of these inhibitors is also essentially complete so far as those pathways are concerned that have been examined in patients. The application of these new inhibitors in premenopausal women has yet to be extensively examined, but their use in men, in whom high concentrations of oestrogen or low concentrations of testosterone are a problem, may be worthwhile. An unexpected pharmacokinetic interaction between letrozole and tamoxifen has led to the discontinuation of a clinical study using these two agents in combination. Together with the pharmacological interaction of AG with danazol, this stresses the need for pharmacokinetic and pharmacological studies before the conduct of large-scale clinical combination trials.

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