Introduction

Aminogluthethimide, the first aromatase inhibitor, was established in the 1970s as an active treatment for patients with advanced breast cancer, but its lack of specificity was associated with side effects. Since that time, a series of much more specific non-steroidal aromatase inhibitors has been developed which are up to 10 000 times as potent as aminogluthethimide in vivo with no evidence of inhibition of other steroid pathways at doses required to inhibit oestrogen. Two of these inhibitors, letrozole (Femara; Novartis) and anastrozole (Arimidex; Zeneca) are now well established in the treatment of advanced breast cancer and are under investigation as adjuvant therapy. These agents achieve 98-99% aromatase inhibition in patients, and reduce serum concentrations of oestrone and oestradiol beyond the limit of detection in many patients (Iveson et al. 1993). Until recently, it had been assumed that no clinical dose-response effect could exist beyond the achievement of maximum serum oestrogen suppression, but recent data have suggested this may not be the case. If a dose-response effect does indeed exist for modern aromatase inhibitors, then it has important implications for their future development.

Aminogluthethimide

Endocrine and clinical effects

After our initial clinical investigations with aminogluthethimide (Smith et al. 1978), we began to investigate the lowest dose of aminogluthethimide that would be effective clinically and, in a dose escalation study, we found that 62.5-125 mg twice daily was as effective as conventional doses at decreasing the serum concentrations of oestrone and oestradiol. Objective tumour responses were seen in 23% of patients given aminogluthethimide 125 mg twice daily (Stuart-Harris et al. 1984).

Subsequently, two randomised trials have compared aminogluthethimide 500 mg daily and 1000 mg daily. In the first (Bonnetterre et al. 1985), involving 149 patients, responses with the lower and higher doses were 19 and 24% respectively. Response including patients in whom there was no change was 58% for each group and there was no significant difference in survival. Likewise, in the second trial (Robustelli et al. 1993) involving 91 patients, objective response rates were 28 and 35% respectively for the lower and higher doses, 58 and 75% for response including patients in whom there was no change and median survival was 20 and 22 months. None of these differences was significant.

Fadrozole

Fadrozole is a non-steroidal imidazole aromatase inhibitor that is between 200 and 400 times more potent than aminogluthethimide when tested in microsomal preparations derived from rat ovaries and human placenta (Steele et al. 1987).

Endocrine effects

Fadrozole was studied in a phase I clinical study in 24 postmenopausal patients with advanced breast cancer treated for 4 weeks with doses of 0.3, 1.0 and 2.0 mg twice daily (Dowsett et al. 1990). The study was conducted in two parts which compared the two lower doses and the two higher doses separated in a crossover design protocol. All doses significantly suppressed serum oestradiol and oestrone concentrations below pre-treatment values. Crossover analysis indicated that the 2 mg twice daily dose achieved significantly greater suppression of oestradiol concentrations than 1 mg twice daily, but there were no significant differences between any of the doses in the suppression of oestrone.

No significant effects were noted in serum concentrations of luteinizing hormone, follicle-stimulating hormone (FSH), sex hormone-binding globulin, prolactin, testosterone, androstenedione, 17-hydroxyprogesterone or cholesterol. However, serum aldosterone concentrations were significantly suppressed by fadrozole 1.0 mg twice daily, and further suppressed by 2.0 mg twice daily.

These results demonstrated that the fadrozole was an effective suppressor of serum oestrone and oestradiol in postmenopausal patients, and that the most effective dosage appeared to be 2 mg twice daily for oestradiol suppression. In contrast to aminogluthethimide, there were no suppressive effects on the synthesis of cortisol, and no
significant suppression of cortisol. The partial suppression of androstenedione, however, showed that fadrozole was not totally selective in its effect.

Similar findings were obtained in another phase I trial conducted by Lipton et al. (1990). Sixteen pretreated postmenopausal patients with metastatic breast cancer were treated with escalating doses of fadrozole from 0.6 to 16 mg total oral daily dose; endocrine data were available from 12 of these patients. Maximum inhibition of oestrogen biosynthesis was observed at a dose of 2 mg daily. Plasma oestrone decreased to 28% of basal values, oestrone sulphate to 30% and oestradiol to 67%. No significant changes in cortisol, adrenocorticotrophic hormone, androstenedione, testosterone or dehydroepiandrosterone sulphate were seen. At higher doses of 8 mg and 16 mg daily, however, a decrease in plasma aldosterone was seen. This was not associated with clinical symptoms, but criteria for orthostatic hypotension were met in two patients.

Subsequently, in a more detailed study by the Royal Marsden group, a double-blind, randomised endocrine study comparing fadrozole 0.5 mg twice daily with 1 mg twice daily and 2 mg twice daily was conducted in 80 postmenopausal patients with advanced breast cancer over a period of 3 months. Substantial decreases were seen in serum concentrations of oestradiol, oestrone and oestrone sulphate. For oestrone alone, there was a significant dose effect: mean concentrations during treatment were 38.0 pmol/l for the 0.5 mg dose, 25.0 pmol/l for the 1.0 mg dose and 23.9 pmol/l for the 2.0 mg dose ($P=0.048$). No significant differences were seen between doses for the other oestrogen measurements. There was a statistically non-significant decrease in aldosterone concentrations during treatment with both the 1 mg twice daily and 2 mg twice daily doses, with an associated non-significant decrease in the plasma sodium:potassium ratio. No clinical consequences were seen. Our conclusion from this study was that fadrozole achieved near maximal oestrogen suppression at 1 mg twice daily, and that its effect on aldosterone synthesis was unlikely to be of clinical significance (Dowsett et al. 1994).

**Randomised dose-finding: clinical effects**

Three dose-finding randomised trials on the clinical efficacy of fadrozole have been carried out. A South African trial randomly assigned 80 evaluable postmenopausal patients to receive fadrozole either 1 mg or 4 mg daily and found no significant differences in response (24% compared with 22%), stable disease (49% compared with 42%) or survival (Raats et al. 1992).

Miller et al. (1996) randomly assigned 56 evaluable patients to receive fadrozole 0.6 mg three times daily, 1 mg twice daily or 2 mg twice daily, and found no significant differences in response or no-change groups. It was of interest in this study that plasma fadrozole concentrations were shown to increase with dose, and yet this was not associated with any increase in endocrine or clinical effects.

Finally, at the Royal Marsden Hospital, we compared fadrozole 0.5 mg twice daily with 1.0 mg twice daily and 2.0 mg twice daily in 80 postmenopausal patients and found no difference in response (13%, 17% and 20%) or stable disease (9%, 38% and 16%, not significant) (Bonnefoi et al. 1996).

Side effects in all these studies were mild. For example, in our own study, nausea was reported in 15%, hot flushes in 5% and somnolence in 4% of patients.

**Phase III comparative trials against megestrol acetate**

Fadrozole 1 mg twice daily has been compared with megestrol acetate (Megace) 40 mg four times daily in two randomised double-blind multi-institutional trials (reported by Buzdar et al. 1996b) involving 672 patients. Objective response rates in the two trials (fadrozole data first) were 11% compared with 16% and 13% compared with 11%; no change was seen in 25% compared with 20% and 24% compared with 30% of participants. None of these differences was significant; likewise, there was no significant difference in time to treatment progression, response duration or survival.

**Anastrozole**

Anastrozole is a non-steroidal oral triazole derivative with high potency and specificity as an aromatase inhibitor. Serum oestradiol concentrations were suppressed below the lower limits of detection with daily doses of 1 mg and higher (Plourde et al. 1994). In phase II randomised trials anastrozole (Arimidex) 1 mg and 10 mg, and megestrol acetate 40 mg four times daily have been compared in the treatment of postmenopausal women with advanced breast cancer whose cancer had progressed after tamoxifen treatment. Each of these trials produced very similar results which have been reported as an overview analysis (Buzdar et al. 1996a).

Objective tumour responses were 10.3% for anastrozole 1 mg, 8.9% for anastrozole 10 mg and 7.9% for megestrol acetate; respective figures for stable disease (at least 24 weeks) were 25.1%, 22.6% and 26.1%. None of these differences was statistically significant; likewise, no significant differences in response duration were seen. In this trial, there were likewise no significant differences in survival. In an update 1 year later, with a median follow-up of 31 months, median survival was 27 months for anastrozole 1 mg, 26 months for 10 mg and 23 months for megestrol acetate. Respective 2-year survival rates were 56%, 55% and 46%; anastrozole 1 mg now showed a
significant survival improvement compared with megestrol acetate \((P=0.02)\); there were no significant differences between the two doses of anastrozole.

**Letrozole**

Letrozole is a non-steroidal triazole derivative and one of the most potent aromatase inhibitors yet developed. It is around 10 000 times as potent as aminoglutethimide \textit{in vivo}, with no evidence of inhibition of other steroid pathways at doses required to inhibit oestrogen. In animal models, it achieves almost complete regression of oestrogen-dependent dimethyl-benz-[a]anthracene-induced mammary tumours (Bhatnagar \textit{et al.} 1990).

**Endocrine effects**

In a phase I Royal Marsden study, 21 postmenopausal patients were treated with letrozole in three successive groups of seven, receiving 0.1, 0.5 and 2.5 mg orally daily respectively. There was a statistically significant suppression from baseline of oestradiol, by 74%, and oestrone, by 79% \((P<0.0001)\). Suppression occurred in all three patient groups, with many patients having serum concentrations below the limit of detection of the assays \((3 \text{ pmol/l and } 10 \text{ pmol/l respectively})\), corresponding to a maximum measurable oestrogen suppression of 86\% (Iveson \textit{et al.} 1993). Letrozole had no significant effect on serum FSH, thyroid-stimulating hormone, cortisol, 17-hydroxyprogesterone, androstenedione or aldosterone.

In a subsequent phase I trial of letrozole specifically designed to measure aromatase inhibition \textit{in vivo} (see below), 13 patients were randomly allocated to receive 0.5 and 2.5 mg daily in a Royal Marsden study (Dowsett \textit{et al.} 1995). Oestrone and oestradiol concentrations decreased by 82\% and 84.1\% in response to letrozole 0.5 mg per day and by 81\% and 68\% with the 2.5 mg per day dose.

In a non-randomised comparative phase I clinical efficacy study, Demers (1994) compared the endocrine suppressing effects of fadrozole with those of letrozole, using the same high-sensitivity oestrogen radioimmunoassay. Letrozole appeared to be the more potent of the two. Fadrozole achieved a 68\% suppression of oestrone and 70\% suppression of oestradiol in all doses used; letrozole achieved greater than 95\% suppression at doses of 0.1-5 mg daily, with undetectable oestradiol concentrations in many patients.

**Aromatase inhibition \textit{in vivo}**

At the Royal Marsden Hospital, we measured \textit{in vivo} aromatase inhibition by letrozole in 13 postmenopausal patients with advanced breast cancer who were assigned randomly to groups to receive a daily dose of 0.5 mg or 2.5 mg for a treatment period of at least 6 weeks (Dowsett \textit{et al.} 1995). After 6 weeks, letrozole inhibited aromatisation by 98.4\% \((97.3 \text{ to } 99.1)\) and 98.9\% \((98.5 \text{ to } 99.1)\) \((\text{geometric means and ranges})\) at doses of 0.5 mg and 2.5 mg daily respectively. This was a greater degree of aromatase inhibition than that achieved with any other inhibitors that we had previously analysed.

**Phase III comparative trials**

Two pivotal multinational, multicentre randomised trials have been carried out comparing letrozole 0.5 mg against letrozole 2.5 mg and against megestrol acetate 40 mg four times daily (first trial) and aminoglutethimide 500 mg daily (second trial).

In the megestrol trial (Dombernowsky \textit{et al.} 1998), involving 551 postmenopausal patients with locally advanced metastatic breast cancer previously treated with anti-oestrogens, the results were as follows. The objective response rate for letrozole 2.5 mg of 23.6\% was significantly greater than for letrozole 0.5 mg \((12.8\%\); \(P=0.04)\) or for megestrol acetate \((16.4\%\); \(P=0.02)\). The median duration of objective response was not reached for letrozole 2.5 mg, but was 18 months both for letrozole 0.5 mg and for megestrol acetate \((P=0.02)\).

Clinical benefit (including patients both with objective response and with stable disease for at least 6 months) was 35\% for letrozole 2.5 mg, 27\% for letrozole 0.5 mg and 32\% for megestrol acetate \((\text{no significant difference})\). Median duration of clinical benefit was 23.5 months for letrozole 2.5 mg, 18 months for letrozole 0.5 mg and 14.5 months for megestrol acetate.

Median survival was 25 months for letrozole 2.5 mg, compared with 21.5 months for letrozole 0.5 mg; this difference was statistically significant \((P=0.03)\). Median survival for megestrol acetate was also 21.5 months.

In the second trial, which involved aminoglutethimide (Gershovanovich \textit{et al.} 1998), 555 post-menopausal women with advanced breast cancer, previously treated with anti-oestrogens, were randomly allocated to groups to receive letrozole 2.5 mg or 0.5 mg once daily, or aminoglutethimide 250 mg twice daily with corticosteroid support. Objective response rates were 19.5\% for letrozole 2.5 mg, 16.7\% for 0.5 mg and 12.4\% for aminoglutethimide. These differences just failed to attain significance. The respective median durations of response were 24 months vs 21 months and 15 months.

Median duration of clinical benefit (response+stable disease for at least 6 months) was 21 months for letrozole 2.5 mg, 18 months for letrozole 0.5 mg and 14 months for aminoglutethimide. Overall survival was 28 months for letrozole 2.5 mg and 21 months for 0.5 mg; this difference was statistically significant \((P=0.04)\). Letrozole 2.5 mg was likewise superior to aminoglutethimide, with a median survival of 20 months \((P=0.002)\).
Discussion

For aminoglutethimide, fadrozole and anastrozole, the evidence suggests that there is no further clinical dose-response effect once each of these agents has achieved its maximum plasma oestrogen suppression. This is a comfortable conclusion and in line with what would be predicted. Against this backdrop, the results in from two pivotal letrozole trials are unexpected. In both trials there was a small but significant increase in clinical efficacy with 2.5 mg compared with 0.5 mg, even though no further plasma oestrogen suppression (or indeed in vivo aromatase inhibition) has been demonstrated with the higher dose. The benefit is unlikely to be artefactual or coincidental, as it was seen with survival, a hard endpoint, and in both trials.

How can these contradictory results be explained? It has been postulated that the letrozole data may indicate the role of intratumoural aromatase inhibition as a more important and sensitive parameter than plasma inhibition or oestrogen suppression. If this is the case, then why is a further dose-response effect not seen with other aromatase inhibitors? The answer to this may lie in the structure-function relationships between drug and enzyme. Letrozole is certainly a more powerful aromatase inhibitor than aminoglutethimide or fadrozole, and non-comparative data suggest that it is probably biochemically more active than anastrozole. There is a similar suggestion from non-comparative clinical data: response rates in the two phase III letrozole trials were significantly higher than those for anastrozole, and this may not simply be based on patient selection. The hypothesis would therefore be that letrozole, because of the better ‘fit’ with the aromatase enzymes complex, can achieve further intratumoral inhibition, beyond that attained with anastrozole, at a level below that at which further plasma biochemical changes are detectable. Non-comparative data suggest that, in this respect, vorozole is more similar to letrozole than to anastrozole, and it is thus regrettable that similar dose-response studies have not been carried out with vorozole.

The dilemma with intratumoral aromatase is that its assay is highly complex to perform, particularly at high levels of inhibition. The results with letrozole nevertheless are compelling enough to encourage further research in this important area.

References


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