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Mini review

The pharmacology of letrozole

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Abstract

Recent clinical trials indicate that the third-generation aromatase inhibitors may be more effective than tamoxifen as first line endocrine therapy in ER+ metastatic breast cancer in postmenopausal women. This review will focus exclusively on the pharmacology of the non-steroidal inhibitor letrozole. Aromatase derived from a variety of sources is inhibited at low nM concentrations of the drug. In non-cellular systems, letrozole is 2–5 times more potent than anastrozole and exemestane in its inhibition of aromatase, whilst in cellular systems it is 10–20 times more potent. Anti-tumour effects of letrozole have been demonstrated in several animal models. In postmenopausal women, letrozole commonly suppresses circulating concentrations of estrone and estradiol to below the sensitivity limit of the assays used to measure them. In a recent randomized cross-over study, letrozole (2.5 mg daily) achieved a significantly greater suppression of the plasma concentrations of both estrone and estrone sulphate than anastrozole (1 mg daily) and a greater inhibition of in vivo aromatization. Letrozole appears to have a small effect on adrenal steroidogenesis such that a small number of patients exhibit an abnormal response to synthetic ACTH during letrozole therapy. This is unlikely to have any clinical significance. In short-term studies letrozole has been shown to increase markers of bone resorption indicating the need to monitor bone integrity when the drug is used for extended periods of time. A consistent effect of letrozole on serum lipids has not been demonstrated.

Keywords: Aromatase; Aromatase inhibitors; Letrozole; Anastrozole; Breast cancer; Exemestane

1. Introduction

1.1. General

Two-thirds of breast cancers in postmenopausal women are estrogen receptor (ER) positive indicating the hormone-dependent nature of the disease. Two main approaches have been used to prevent the stimulatory effects of estrogen in these women: antagonism of estrogen action by selective estrogen receptor modulators (SERMs) and inhibition of estrogen synthesis by aromatase inhibitors. Until recently tamoxifen was fully established as standard first line therapy in ER+ metastatic breast cancer while aromatase inhibitors were used as second-line treatment. However, this situation is currently being re-evaluated in light of results from recent clinical trials (see further) which indicate that the third-generation aromatase inhibitors, anastrozole and letrozole, may be more effective as first line therapy than tamoxifen.

Aromatase is a cytochrome P-450 enzyme complex found in many tissues including the ovary (in premenopausal women), fat, muscle, liver and breast. It is responsible for the final step in estrogen biosynthesis, catalysing the aromatization of androstenedione and testosterone into estrone and estradiol, respectively. The decline in ovarian steroid production at menopause means that the primary source of estrogens in postmenopausal women is the conversion of circulating androgens into estrogens by the aromatase enzyme in peripheral tissues. Administration of a third-generation aromatase inhibitor such as letrozole can inhibit this process by >99%.

Aromatase inhibitors (Fig. 1) can be divided into two main classes, steroidal compounds which bind to the substrate-binding site of the enzyme, and, depending upon the presence or absence of substrate lead to reversible or irreversible inhibition of the enzyme (type 1 inhibitors), and non-steroidal compounds which interact competitively with the heme group of the cytochrome P-450 component of aromatase (type 2 inhibitors). Non-steroidal compounds include aminoglutethimide (AG), the first aromatase inhibitor to be available clinically. Although this first generation inhibitor demonstrated good efficacy it suffered from a lack

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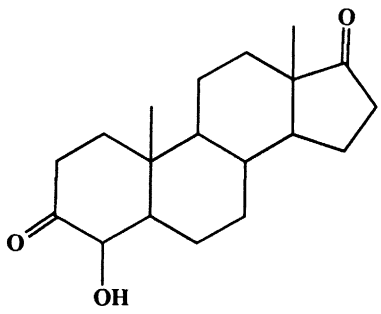
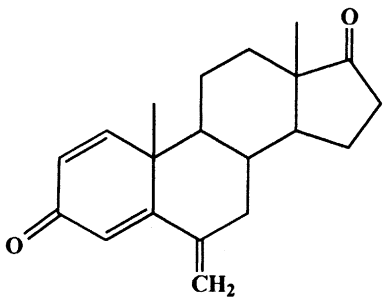
Steroidal aromatase inhibitors

Second generation



Formestane

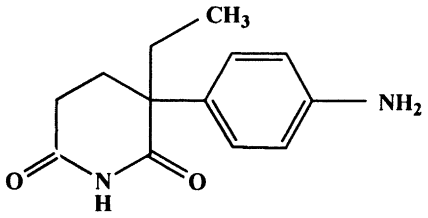
Third generation



Exemestane

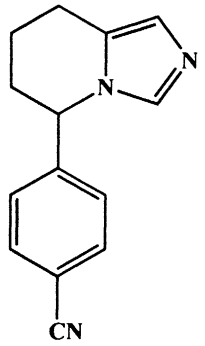
Non-steroidal aromatase inhibitors

First generation



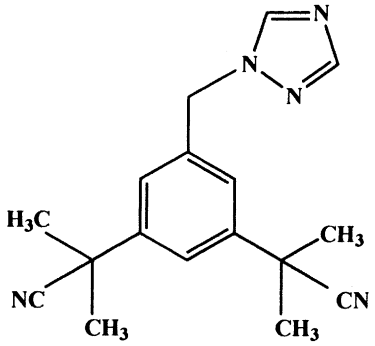
Aminoglutethimide

Second generation

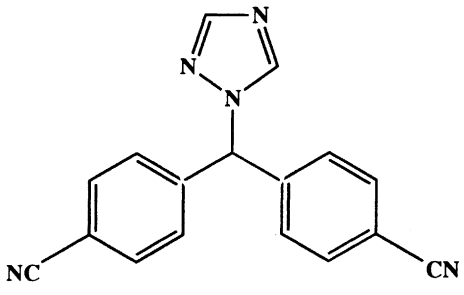


Fadrozole

Third generation



Anastrozole



Letrozole

Fig. 1. Structures of aromatase inhibitors.

of specificity (necessitating corticosteroid supplementation) and had a number of side-effects. The development of more potent, better tolerated and selective inhibitors of aromatase yielded a number of third-generation aromatase inhibitors, three of which have entered widespread clinical use: the non-steroidal competitive inhibitors letrozole and anastrozole and the steroidal irreversible inhibitor exemestane.

In randomised clinical trials all three of these compounds have been demonstrated to be superior to megestrol acetate as second-line therapy in postmenopausal women with advanced breast cancer [1–5]. In the neoadjuvant setting, a phase III randomised trial showed that letrozole was more active than tamoxifen in hormone receptor positive primary breast cancer [6]. Recently, letrozole and anastrozole have been compared to tamoxifen as first line therapy in prospective randomised trials. Both agents were shown to improve outcome [7–9] and have now been approved by the FDA as first line therapy for hormone receptor positive, metastatic breast cancer in postmenopausal women. A large randomised trial of exemestane in this setting is currently ongoing.

This review will focus exclusively on the pharmacology of letrozole. Comparative data with other aromatase inhibitors has been quoted only for published studies in which direct comparisons have been made with letrozole.

1.2. Letrozole

The binding characteristics of letrozole to the human aromatase enzyme have been investigated in site-directed mutagenesis studies. Thus, comparisons of the inhibitory profiles of letrozole and other aromatase inhibitors in a series of human aromatase mutants have provided a molecular basis for how these compounds bind to the active site of the enzyme [10,11] (Fig. 2).

In healthy volunteers letrozole is rapidly and completely absorbed after oral administration, has a large volume of distribution (1.87 l/kg) and moderate protein binding (60%) [12]. After a single dose its plasma terminal half-life has been reported as 42 h in healthy volunteers [13,14] and 82 h in breast cancer patients [15]. Breast cancer patients also had higher area under curve (AUC) values than those found

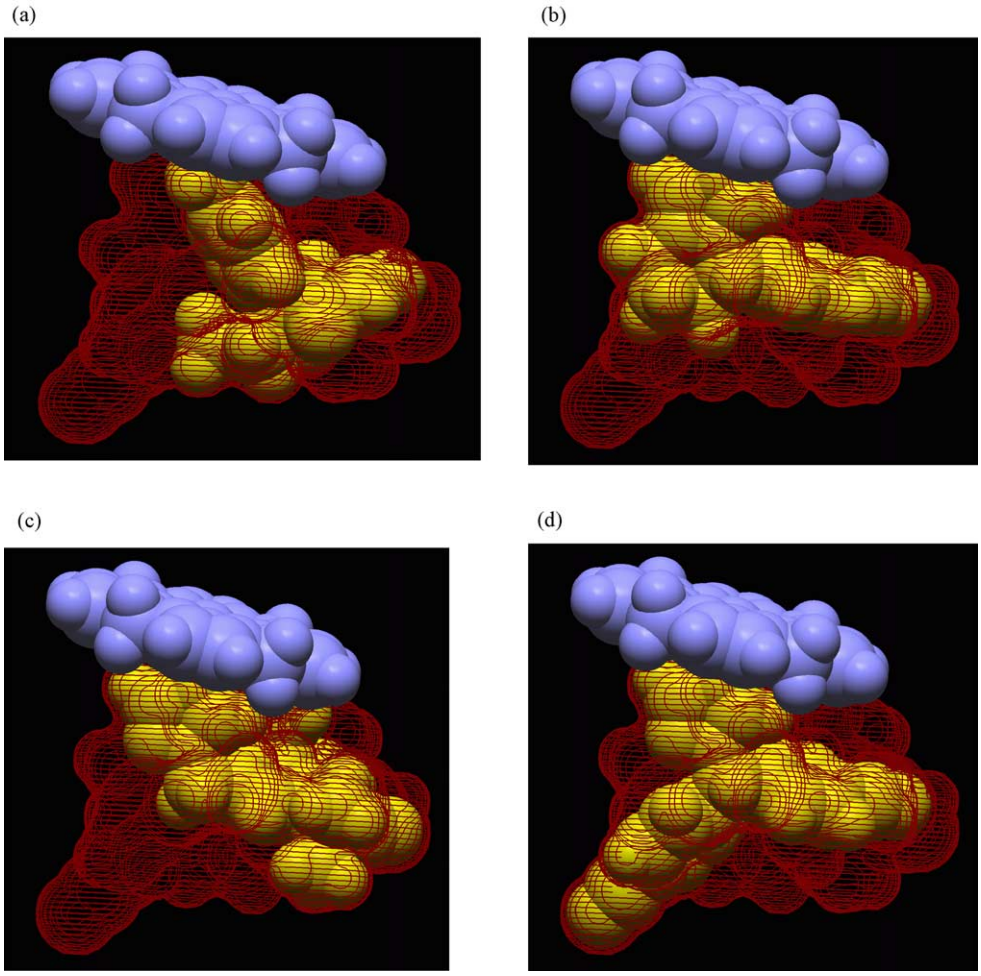


Fig. 2. Space-filled models showing the molecular basis of the binding of (a) aminoglutethimide; (b) fadrozole; (c) anastrozole and (d) letrozole to the active site of aromatase [11].

in healthy volunteers which suggests they have a reduced elimination rate most likely due to a reduction in metabolic clearance [15]. The pharmacokinetics of letrozole were not significantly different in a younger versus an older group of postmenopausal patients (median ages 61 and 71, respectively) [15].

The major route of elimination of letrozole is via metabolism by cytochrome P-450 isozymes (CYP3A4 and CYP2A6) into a pharmacologically inactive carbinol metabolite [13,16]. In early clinical studies there was some evidence of non-linearity in the pharmacokinetics of letrozole after single high doses (>10 mg) or repeated daily doses of 2.5 mg [17]. A recent study confirmed this observation in the clinical setting although the degree of deviation from linearity was small: at a 2.5 mg daily dose the steady state AUC was only 28% higher than that expected from the single dose AUC [15]. The non-linearity in the pharmacokinetics of letrozole has been attributed to an auto-inhibition or saturation of its own metabolism via CYP2A6 [16]. Steady state concentrations of letrozole are reached after about 2–6 weeks of daily dosing and are maintained over long periods of time [15,18] indicating that continuous drug accumulation does not occur. This suggests that the non-linearity may be restricted to an exposure range where CYP2A6 is auto-inhibited/saturated but before CYP3A4 becomes the dominant metabolising enzyme [15].

2. Efficacy

2.1. In vitro

Letrozole potently inhibits aromatase derived from a variety of different sources including human placental and rat ovarian microsomes, hamster ovarian cells, human adipose fibroblasts, JEG-3 human choriocarcinoma cells, MCF-7 cells transfected with aromatase (MCF-7Ca) and particulate fractions of human breast cancer (Table 1) [19–26]. In the non-cellular systems letrozole (IC50 1–13 nM) has similar potency to fadrozole, is 2–5 times more potent than anastrozole and is over two orders of magnitude more potent than the first generation inhibitor aminoglutethimide. Interestingly, in all the cellular systems letrozole and fadrozole are at least 10–20-fold more potent than anastrozole (Fig. 3).

2.2. In vivo

2.2.1. Animal models

2.2.1.1. Anti-endocrine effects. Inhibition of androstenedione-induced uterine hypertrophy in immature rats has been used as a standard method to assess the in vivo efficacy of aromatase inhibitors. In this system letrozole has

Table 1
Inhibitory concentrations of letrozole, anastrozole, exemestane, fadrozole, 4-hydroxyandrostenedione (4–OHA) and aminoglutethimide (AG) against the aromatase enzyme derived from various cellular and non-cellular sources

Aromatase inhibitor	IC50 values, nM (relative potency; letrozole = 1)								Reference
	Human placental microsomes	Particulate fractions of human breast cancer	Rat ovarian microsomes	MCF-7Ca cancer cells	JEG-3 cancer cells	CHO cells	Hamster ovarian tissue	Human breast	
Letrozole		2 (1)						0.8 (1)	[19]
Anastrozole		8 (0.25)						15 (0.053)	
Exemestane		15 (0.13)						5 (0.16)	
4-OHA		30 (0.07)						30 (0.027)	
AG		20000 (0.0001)						10000 (0.0008)	
Letrozole	11 (1)			0.07 (1)	0.07 (1)		20 (1)	0.8 (1)	[20–22]
Anastrozole	23 (0.48)			0.82 (0.085)	0.99 (0.071)		600 (0.033)	14 (0.057)	
Fadrozole	5 (2.2)			0.05 (1.4)	0.07 (1.0)		30 (0.67)	1 (0.80)	
4-OHA	62 (0.18)								
AG	1900 (0.0058)								
Letrozole	1.02 (1)			0.35 (1.0)	0.45 (1)			0.14 (1)	[23,24]
Anastrozole	5.35 (0.19)			3.62 (0.097)	5.66 (0.080)			17.17 (0.0082)	
4-OHA				0.59 (0.59)	1.6 (0.28)			0.72 (0.19)	
Letrozole			7 (1)						[25]
Anastrozole			25 (0.28)						
Fadrozole			7 (1)						
Letrozole						1.4 (1)			[26]
Anastrozole						27 (0.052)			
4-OHA						60 (0.023)			
AG						5500 (0.00025)			

Values quoted are IC50 values representing the concentration needed to achieve 50% inhibition of aromatase activity; the relative potency of each inhibitor compared to letrozole is shown in parentheses.

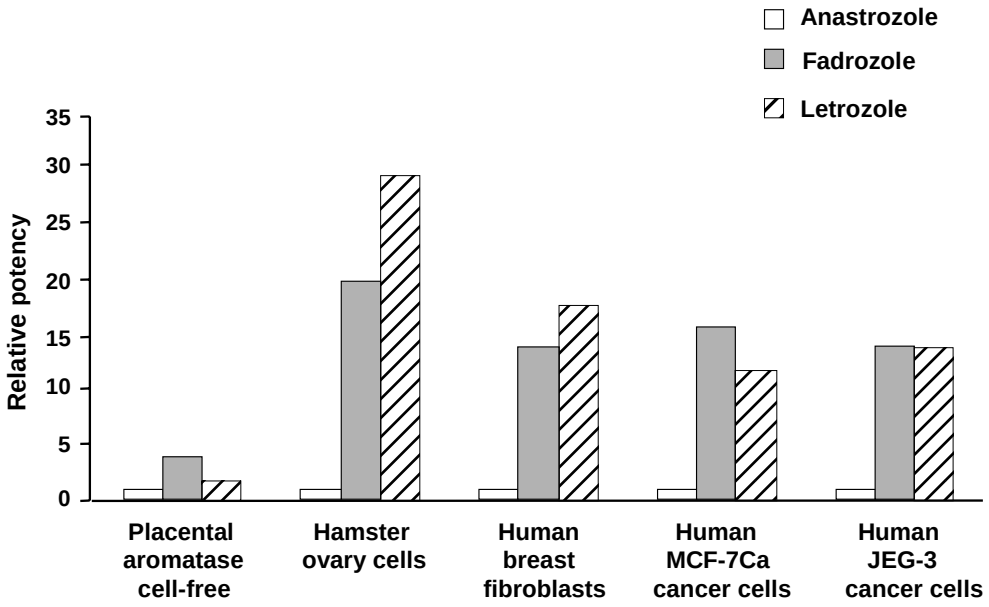


Fig. 3. Relative potencies with which letrozole, anastrozole and fadrozole inhibit aromatase from non-cellular and intracellular sources [22].

an estimated ED50 of 1–3 μg/kg and is over four orders of magnitude more potent than AG. In the adult female rat, letrozole (0.3–1 mg/kg daily p.o., 14 days) completely interrupted ovarian cyclicity and reduced uterine weight and serum estradiol (E2) concentrations to a similar extent to that seen after ovariectomy [20,27–29].

2.2.1.2. Anti-tumour effects. Letrozole causes almost complete regression of estrogen-dependent DMBA-induced mammary tumours in adult female rats at a dose of 100 μg/kg per day, with an ED50 of 10–30 μg/kg per day [20,27]. The anti-tumour effects of letrozole have also been assessed in a MCF-7 xenograft model in athymic nude mice. In this model, MCF-7 cells transfected with the human aromatase gene (MCF-7Ca/AROM-1) are inoculated into ovariectomized nude mice and tumour growth driven by the administration of the aromatizible substrate androstenedione [30,31]. After oral dosing, letrozole significantly decreased tumour growth at 2 mg/kg per day and completely inhibited growth at 20 mg/kg per day p.o. [31]. When administered sub-cutaneously doses as low as 5 μg per day of letrozole potently inhibited tumour growth [24]. In comparative studies, fadrozole was not as effective as letrozole at the same dose (60 μg per day) in reducing tumour weight [30]. Letrozole (10 μg per day) was also more potent in reducing tumour growth in this model than the pure antiestrogen ICI 182780 (5 mg per week), tamoxifen (60 μg per day) and anastrozole (60 μg per day) [32]. In order to test the hypothesis that combining an aromatase inhibitor and an antiestrogen might result in better anti-tumour efficacy Lu et al. [24] further investigated the effects of letrozole (5 μg per day) combined with either tamoxifen (3 μg per day) or ICI 182780 (70 μg per week). The combinations tended to be less effective than letrozole alone and although this was not significant [24] it indicated that there was no advantage in combination therapy in this animal model.

There is also evidence that letrozole might be useful as a chemopreventive agent. Thus, in an aromatase-transgenic mouse model, letrozole has been shown to completely abrogate preneoplastic and neoplastic changes induced in mammary glands as a result of transgenic aromatase overexpression [33]. Letrozole has also been found to decrease the incidence of spontaneous mammary tumours and granular cell tumours of the distal female reproductive tract of Sprague–Dawley rats in carcinogenicity studies [34,35].

2.2.2. Human studies

2.2.2.1. Peripheral pharmacology. In a phase I trial letrozole (0.1, 0.5 and 2.5 mg daily) suppressed serum estrone (E1) and E2 levels by an average of 79 and 74% from baseline, respectively after 4 weeks in postmenopausal women with breast cancer [36]. All three doses were equally effective: estrogen levels fell by over 50% within 24 h and were maximally suppressed by day 14. Many serum samples had levels of E1 and E2 below the detection limit of the assays (<3 and <10 pmol/l, respectively) giving a maximum measurable estrogen suppression of 86% and making the average percent suppression values inherent underestimates. Lipton et al. [37] found similar results in women receiving 0.1–5 mg letrozole daily, observing greater than 90% suppression of plasma E1, E2, estrone sulphate (E1S) as well as urine E1 and E2 within 2 weeks with no dose dependency apparent.

Only two direct comparisons of letrozole with other aromatase inhibitors in their ability to suppress plasma estrogens have been carried out. In the first of these Demers [38]

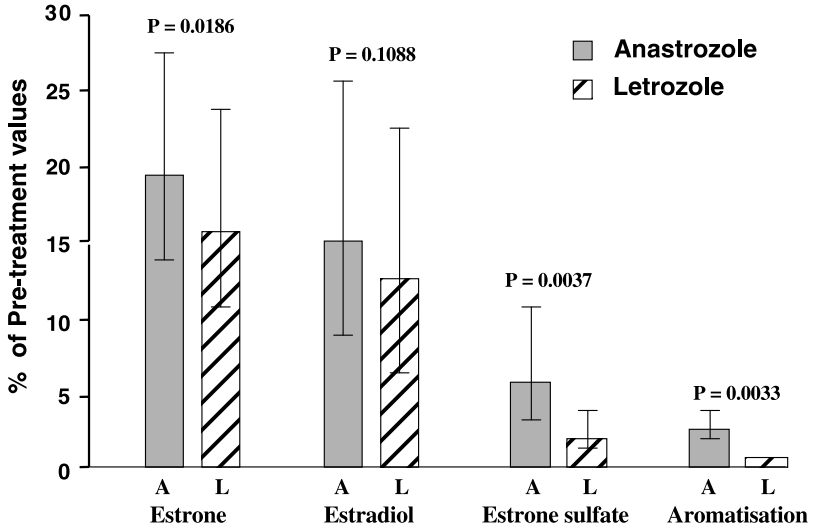


Fig. 4. Comparison of the suppression of plasma estrone, estradiol and estrone sulfate by anastrozole (A) and letrozole (L) in a randomized cross-over study. Geometric mean values with 95% confidence interval of the mean are shown. The *P*-values refer to the differences between the two treatment regimens [39].

directly compared the effects of letrozole and fadrozole although this was in a non-randomised setting. Letrozole was more effective, suppressing plasma estrogen concentrations to undetectable levels (>95% of baseline) at all doses investigated (0.1–5 mg per day) while fadrozole (2–4 mg daily) only achieved 68 and 70% suppression of plasma E1 and E2 concentrations, respectively.

The second direct comparison involving letrozole was in a recent randomized cross-over study where letrozole (2.5 mg daily) was compared with anastrozole (1 mg daily) in 12 patients [39]. The most accurate measure of plasma estrogen suppression in these patients is E1S, for which a maximum theoretical suppression of 99.4% (compared to 91.9 and 87.8% for E1 and E2, respectively) can be detected based on the ratio between pre-treatment levels and the sensitivity limit of the respective immunoassays [39]. Letrozole achieved a significantly greater degree of suppression of the plasma concentrations of both E1 (84.3% versus 81.0%; $P = 0.019$) and E1S (98.0% versus 93.5%; $P = 0.0037$) in this study. Any potential significant difference in the effect on plasma E2 concentrations could not be fully evaluated as values were suppressed below the sensitivity limit of the assay in all 12 patients during treatment with letrozole and 9 of the 12 patients during treatment with anastrozole (Fig. 4). This study also evaluated in vivo whole body aromatization (see further).

2.2.2.2. In vivo aromatization studies. The direct measurement of aromatase activity by isotopic analysis provides a more precise way of comparing between different aromatase inhibitors or different dosages of the same drug. Over the last 10 years we have assessed the potency of many aromatase inhibitors in this way. The very similar methodology used in all our studies and excellent reproducibility between the studies enables some general comparisons to be made between letrozole and other inhibitors (Table 2).

The first generation inhibitors AG and 4-hydroxyandrostenedione (4-OHA) achieved 90–92% inhibition of aromatization [40,41], with the second-generation inhibitor fadrozole only slightly better [42]. However, all the third-generation inhibitors tested suppressed in vivo aromatization by >96% [43–45].

A comparison of the effects of two different doses of letrozole (0.5 and 2.5 mg daily) in a randomised trial showed no significant difference in inhibition of aromatase (98.4% versus 98.9%, respectively) [43]. The very high potency of letrozole was emphasised by the fact that the degree of inhibition reached the sensitivity limit of the assay (99.1% inhibition) in 2 of 5 patients at the lower dose and 3 of the 5 patients at the higher dose.

The recent randomised cross-over study of letrozole and anastrozole [39] also examined their effects on the inhibition of in vivo aromatization. During treatment with letrozole, aromatization was suppressed below the detection limit (>99.1% inhibition) in all 12 patients. In contrast, during anastrozole treatment only 1 of the 12 patients was found to have >99.1% inhibition. The mean suppression of aromatization (97.3% for anastrozole versus >99.1% for letrozole) was significantly different ($P = 0.0022$). This corresponded to a 10-fold lower residual level of aromatization during letrozole treatment compared to anastrozole (0.006% versus 0.059%) (Table 3) The results from this study are very similar to those reported by us previously in separate studies of letrozole and anastrozole [43,44].

2.2.2.3. Normal breast and intra-tumoural pharmacology. Measurements of plasma estrogen levels and total body aromatization may not necessarily reflect alterations in

Table 2
Inhibition of whole body aromatization by aromatase inhibitors

Drug	Dose (mg)	Number of patients	Inhibition (%) (mean ± S.E.M.)	Reference
AG	250, p.o. qd	7	90.6 ± 1.8	[40]
4-OHA	250, i.m., 2 weekly	7	84.8 ± 1.9	[41]
	500, i.m., 2 weekly	7	91.9 ± 1.0	
Fadrozole	1, p.o. bd	8	82.4 ± 2.9	[42]
	2, p.o. bd	3	92.6 ± 1.6	
Exemestane	25, p.o. od	10	97.9 (96.3–98.8)[a]	[45]
Anastrozole	1, p.o. od	12	96.7 (95.6–97.5)[a]	[44]
	10, p.o. od	12	98.1 (96.1–99.1)[a]	
Letrozole	0.5, p.o. od	5	98.4 ± 0.3	[43]
	2.5, p.o. od	5	98.9 ± 0.2	
Anastrozole	1, p.o. od	12	96.9 ± 0.5	[39]
Letrozole	2.5, p.o. od	12	>99.1	

[a] Geometric mean (95% CI).

breast or tumour estrogen levels. Breast tumour concentrations of estrone and estradiol are 2–10 and 10–20-fold, respectively higher than plasma levels in postmenopausal women [46]. The source of intra-tumoural estrogens is probably a combination of active estrogen uptake [47] and local synthesis [48] although there appears to be large interindividual variation in these contributing factors [49,50].

In a neoadjuvant study, treatment with letrozole (2.5 or 10 mg daily for 3 months) was not associated with consistent effects on breast tumour estrogen uptake [51]. The majority of tumours (20/23) had evidence for in situ synthesis of estrogen and letrozole significantly inhibited this in almost all cases (19/20). This was reflected in a marked decrease in tumour E1 and E2 levels in these patients. There was no evidence for the 10 mg dose having greater effects than the 2.5 mg dose, the dose in general clinical use.

Letrozole is also associated with a consistent reduction in staining for Ki67 (a marker of cell proliferation) in the neo-adjuvant setting [52]. Tamoxifen had a similar effect on Ki67 in most cases, although 3 of 23 cases showed a paradoxical increase in score which may reflect clonal selection of tamoxifen-resistant highly proliferative cells. Treatment with letrozole also reduced the expression of PgR. This was in contrast to tamoxifen for which an increase in expression was the most common effect, consistent with its partial estrogen agonist properties.

Table 3
Comparison of the effects of letrozole and anastrozole on total body aromatization in a cross-over study [39]

Patient	Aromatization (%)		
	Pre-treatment	Anastrozole	Letrozole
1	1.942	0.092	0.013
2	3.025	0.075	0.004
3	1.767	0.047	0.013
4	1.675	0.038	0.007
5	2.366	0.062	0.009
6	3.854	0.091	0.006
7	1.687	0.031	0.002
8	2.029	0.144	0.001
9	2.952	0.100	0.012
10	4.270	0.011	0.005
11	2.238	0.058	0.005
12	2.384	0.096	0.010
Geometric mean	2.399	0.059	0.006
95% CI	1.966–2.927	0.038–0.092	0.004–0.009

2.2.2.4. Pharmacokinetic interactions. The possible use of the clinical combination of an aromatase inhibitor with tamoxifen has led to the assessment of potential pharmacokinetic interactions between these agents. Such studies were prompted by the finding that aminoglutethimide increases the clearance of tamoxifen, resulting in an approximate 70% decrease in the plasma concentration of tamoxifen [53]. Studies of the combination of letrozole (2.5 mg daily) and tamoxifen (20 mg daily) have shown that letrozole has no effect on the pharmacokinetics of tamoxifen [54]. However, in a separate study the steady state plasma levels of letrozole were reduced significantly (35–40%) during combination therapy with tamoxifen [18]. The most likely explanation for this unexpected finding is that tamoxifen induces enzyme(s) responsible for the metabolism of letrozole (possibly CYP3A4). Although the reduction in plasma levels of letrozole was not reflected by any change in the suppression of plasma estrogen levels in this study, minor alterations would be difficult to detect especially as many estrogen measurements were below the limit of detection of the assay. These data suggest that sequential use of letrozole and tamoxifen would seem to be preferable unless combination therapy is shown to provide synergistic benefits. A similar interaction between anastrozole and tamoxifen has recently been reported [55].

2.2.2.5. Induction of ovulation. The antiestrogen clomiphene is commonly used for the induction of ovulation in

infertility patients and the potential for the use of an aromatase inhibitor in this setting was noted over 10 years ago [56]. In a study performed on Bonnet monkeys, letrozole was highly efficient in inducing multiple mature follicles, which could then be easily ovulated using HCG [57]. In a recent study the ability of letrozole to induce ovulation was evaluated in women in whom clomiphene treatment had previously been unsuccessful [58]. Promisingly, letrozole caused ovulation induction in 75% of patients with anovulatory infertility, increased follicle recruitment in ovulatory infertility and maintained endometrial thickness above 0.5 cm in the majority of cases in contrast to clomiphene.

3. Selectivity/specificity

3.1. In vitro

The selectivity of letrozole has been assessed in vitro in two animal model systems: the luteinizing hormone (LH)-stimulated production of estrogen and progesterone in hamster ovarian slices and the adrenocorticotrophic hormone (ACTH)-stimulated production of corticosterone and aldosterone in rat adrenal tissue. While letrozole potently inhibited estrogen production (IC50 0.02 μM), it completely lacked any inhibition of progesterone or corticosterone at the highest tested concentration (350 μM) and had an IC50 for inhibition of aldosterone production of 210 μM [20]. Calculation of a selectivity index (the ratio of the IC50 for inhibition of estradiol production to the IC50's for inhibition of the production of the other three hormones) showed that letrozole was over three orders of magnitude more selective than AG in its effects on progesterone and corticosterone production. Moreover, letrozole was over 300-fold more selective against aldosterone than fadrozole [21].

3.2. In vivo

The in vitro selectivity of letrozole was confirmed in an in vivo animal model employing ACTH stimulation analogous to the Synacthen® test used clinically to assess adrenal reserve [20]. Adult male rats were given a single sub-cutaneous dose of ACTH 16 h prior to treatment with an aromatase inhibitor and serum corticosterone and aldosterone measured 2 h after treatment. Fadrozole was shown to suppress significantly aldosterone levels (at doses as low as 0.04 mg/kg) and corticosterone levels (at highest dose level of 4 mg/kg). In contrast letrozole had no significant effect on either aldosterone or corticosterone levels at the highest dose tested (4 mg/kg). This dose is over 1000 times higher than the ED50 for inhibition of aromatase in vivo (1–3 μg/kg).

In most cases endocrine measurements in clinical studies have confirmed the selectivity of letrozole observed in the above animal models. No change in the plasma level of cortisol has been observed during treatment with letrozole [36,38,59] except in one study in which a 20–25% fall was seen after 3 months although all values still remained within the normal range [60]. Similarly, plasma levels of aldosterone appear to be unaffected by letrozole; no change was observed in 3 studies [36,38,60] while Bajetta et al. [59] reported a small increase that was within the normal range.

Changes in the circulating levels of cortisol and/or aldosterone will be quickly corrected for by a compensatory rise in pituitary ACTH secretion in the presence of an intact endocrine feedback system. Therefore to assess if letrozole had effects on adrenal steroidogenesis which were not reflected in baseline values, its selectivity has also been investigated using the Synacthen® (cortrosyn) test. This test is commonly used to investigate adrenal insufficiency and involves the measurement of the plasma cortisol response after a single injection (i.v. or i.m.) of 250 μg Synacthen® ($ACTH_{1–24}$).

Three studies have used Synacthen® tests to investigate the selectivity of letrozole. In a short-term study (6 weeks) Demers [38] found no change in the cortisol or aldosterone response in patients taking doses up to 5 mg per day. In two longer term studies (3 months) differing results have been obtained. Bajetta et al. [59] reported a significant decrease in the mean peak cortisol levels after stimulation with Synacthen® at both 0.5 and 2.5 mg daily. The aldosterone peak response was also decreased significantly in the high dose group in this study. In contrast, Dixon et al. [61] found a higher dose of letrozole (10 mg daily) to have no effects on either the cortisol or aldosterone response in their study. However, it is important to note that, for diagnostic purposes, the clinical normality of response to Synacthen® is based on individual responses falling within prescribed limits: the response is considered normal if a peak value of >20 μg/100 ml (550 nmol/l) or an increment of >8 μg/100 ml (220 nmol/l) in cortisol is observed [62–66]. Using these criteria, an analysis of the data from the Bajetta and Dixon studies indicates a normal response to Synacthen® in the large majority of cases (46/53 at 30 min and 52/53 at 60 min; Table 4). It is particularly notable that no abnormal responses were seen at the 10 mg dose which is four times the clinical dose. Thus, although a few individual patients exhibited an abnormal response to synthetic ACTH, this result, when compared to the vast majority of normal responses, is very unlikely to have any clinical significance. Indeed there have been no recorded clinical problems with letrozole with regard to steroid insufficiency.

Table 4
Effect of letrozole treatment (0.5–10 mg daily for 3 months) on response to ACTH stimulation

Dose (mg daily)	Number of patients exhibiting normal cortisol response				Reference
	Pre-treatment (min)		3 months (min)		
	30	60	30	60	
0.5	21/22	22/22	17/19	19/19	[59]
2.5	23/23	22/23	17/22	21/22	[59]
10	12/12	12/12	12/12	12/12	[61]

Letrozole has been shown to have no effect on the plasma levels of 17α-OH progesterone, TSH, LH, FSH or androstenedione [36,59] and does not affect normal urine electrolyte excretion or thyroid function [38].

4. Other effects

The systemic effects of letrozole, particularly on other estrogen-dependent systems (e.g bone and cardiovascular) are very important to assess in light of the proposed use of third-generation aromatase inhibitors in the chemopreventive setting.

4.1. Bone

In a randomized clinical trial in healthy postmenopausal women, letrozole (2.5 mg daily, 6 months) did not affect bone formation markers but increased urinary markers of bone resorption [67]. Harper-Wynne [68] also found a significant increase in bone resorption (25% increase in serum CTx) after 3 months letrozole therapy (2.5 mg daily) in healthy postmenopausal women. These studies suggest that an increase in bone mineral loss may occur. The clinical significance of this requires further study.

4.2. Serum lipids

A consistent effect of letrozole on serum lipids has not been demonstrated. In a specific investigation of the effect of letrozole on the lipid profile in 20 postmenopausal women with advanced breast cancer, an increase in serum total cholesterol (8%), LDL cholesterol (15%) and apolipoprotein B (17%) was observed after 8–16 weeks of therapy [69]. This led to significant increases in the artherogenic risk ratios total cholesterol/HDL cholesterol (14%), LDL cholesterol/HDL cholesterol (21%) and a significant decrease in the artherogenic risk ratio ApoA1/ApoB (15%). These findings are generally unfavourable but they are in contrast to the studies of Harper-Wynne et al. [68] and Heshmati et al. [67] both of which found letrozole to have no significant effect on serum lipids (total, HDL or LDL cholesterol) after 3 and 6 months, respectively. Further studies are therefore needed to assess the long-term effects of aromatase inhibitors on lipid metabolism and its consequences.

4.3. IGF-1

A small randomised comparison of 0.5 and 2.5 mg daily doses of letrozole in breast cancer patients indicated a significant 24% increase in IGF-1 after 3 months therapy [70]. However, this appeared to be almost exclusively due to an increase in IGF-1 in the low dose group with very little change at the higher dose. No change in IGF binding protein 3 (IGFBP-3) was apparent in this study. In the only other study that has looked at the effect of letrozole on IGF-1 levels, Harper-Wynne et al. [68] found letrozole (2.5 mg daily, 3 months) to have no effect on IGF-1 levels in healthy postmenopausal women in contrast to the widely reported suppressive effect of tamoxifen treatment.

5. Summary

Letrozole is a highly potent and selective inhibitor of aromatase with anti-mammary tumour effectiveness in pre-clinical models. Its efficacy in human breast cancer is now well established, recent studies showing it to be better than the current gold standard for endocrine therapy, tamoxifen, in postmenopausal advanced disease. The absence of major pharmacological changes (other than on bone resorption markers) suggests that letrozole is well suited to play a role in early breast cancer or on non-oncological applications including ovulation induction.

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