PERGAMON

Journal of Steroid Biochemistry & Molecular Biology 86 (2003) 255–263

The Journal of Steroid Biochemistry & Molecular Biology

www.elsevier.com/locate/jsbmb

Hormonal effects of aromatase inhibitors: focus on premenopausal effects and interaction with tamoxifen☆

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Abstract

Third generation aromatase inhibitors have excellent specificity. Some reports indicate that letrozole may have a minor effect on cortisol synthesis but these were not confirmed: valid comparisons with other aromatase inhibitors requires randomised study.

The putative use of a third generation inhibitor as a single agent in premenopausal women has been investigated using YM511. It was hypothesised that in this situation site-specific suppression of estrogens in breast carcinomas, without systemic effects, may lead to a down-regulation of tumour proliferation. Plasma levels of androstenedione and testosterone were significantly increased by 2 weeks treatment with YM511. Mean plasma estrone levels were suppressed, but some plasma estradiol levels were abnormally high and others abnormally low. These differential effects of YM511 on circulating estrogens supported the concept that peripheral synthesis of estrogens might be suppressed while ovarian production remained high. However, YM511 did not demonstrate anti-proliferative effects in hormone sensitive breast carcinomas.

Consideration of the pharmacology of the estrogen receptor during tamoxifen therapy indicates that tamoxifen effectively saturates the receptor (>99.94% occupancy) in postmenopausal women. The addition of an aromatase inhibitor in this situation would be very unlikely to affect the biological activity of the estrogen receptor. This provides a possible explanation why the clinical efficacy of tamoxifen combined with an aromatase inhibitor appears to be equivalent to that of tamoxifen alone.

Keywords: Hormonal effects; Aromatase inhibitors; Tamoxifen; Breast cancer; Estradiol; YM511; Premenopausal

1. Introduction

The comparative pharmacology of aromatase inhibitors has been widely described [1–3] and this is therefore only briefly summarised below. This article focuses on novel issues relating to the use of these agents in premenopausal breast cancer patients, the effects of non-steroidal compounds on glucocorticoid synthesis and the interaction of aromatase inhibitors with tamoxifen.

2. Main hormonal effects

All of the third generation aromatase inhibitors suppress plasma estradiol, estrone and estrone sulphate levels to or below the sensitivity limits of sensitive immuno-assays [4–6]. Detailed comparisons between these agents using plasma assays therefore has very limited value. Instead the use of isotopic analyses which measure the in vivo whole body conversion of androstenedione to estrone have been used as a benchmark of pharmacological effectiveness. In this regard anastrozole, exemestane and letrozole have been found to inhibit aromatisation by 96.7% [5], 97.9% [7] and 98.9% [8], respectively. A recent cross-over study [6] has confirmed that the differences between anastrozole and letrozole in their effects on aromatase inhibition are statistically significant although the biological and clinical significance remains unclear.

This profound suppression and the log-linear response curve between estrogen suppression and drug-dosage means that substantial estrogen suppression may be elicited by doses orders of magnitude below those used in the clinical treatment of breast cancer. This is of contemporary importance since it is possible that such doses might be useful in adjusting (rather than eliminating) the plasma concentration of estrogens during breast cancer prevention strategies. This is an attractive concept since it may result in a lesser degree of complicating side-effects such as osteoporosis. For example Trunet et al. [9] reported 34% suppression of plasma estradiol 24 h after a single dose of 0.02 mg of letrozole in male volunteers. Moreover, in postmenopausal women, a

☆ Presented at the VI International Aromatase Conference: AROMATASE 2002, Kyoto, Japan, 26–30 October 2002.

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doi:10.1016/S0960-0760(03)00365-0

single dose of 0.10 mg was found to provide nadir estradiol levels of about 5 pmol/l (77% suppression) 3 days after a single dose [10]. In contrast, the clinical dose of letrozole is 2.5 mg per day. The differential effects of these lower doses on surrogate markers of breast cancer risk and of metabolic complications merits study.

The selectivity of these agents for their aromatase target appears near complete in postmenopausal women at clinical dosages. One of the few areas in which incomplete selectivity has been suggested is in corticosteroid synthesis, as estimated by the response of plasma cortisol levels to stimulation by synthetic ACTH. While the response in patients treated with 10 mg anastrozole daily has been reported to be normal [11], a study by Bajetta et al. [12] indicated that a statistically significant ($P = 0.015$) reduced response to ACTH occurred during letrozole 2.5 mg daily treatment after both 1 and 3 months. However, it is important to note that the normality of response to Synacthen is determined on an individual patient basis such that patients should reach a stimulated level of 550 nmol/l or show an increment of 220 nmol/l between the pre-treatment and stimulated level [13–17]. In this regard the data on letrozole from two different studies are conflicting (Table 1).

At the 2.5 mg dose of letrozole in the study conducted by Bajetta et al., before treatment 1 of 23 patients showed an abnormal response after 60 min while none showed an abnormal response at 30 min. In comparison, after 3 months treatment, 5 of 22 patients showed an abnormally low response at 30 min but only 1 of 22 patients showed an abnormal response after 60 min, suggesting a slower but overall normal response to ACTH. In contrast, the study by Dixon [18] using a dose of 10 mg per day showed no abnormal responses in any of the 12 patients studied after 3 months treatment.

These apparent differences in the behaviour of letrozole and anastrozole and different doses of letrozole may be due to the different patient populations studied and, more importantly, the fact that the measurements have been made using different methodologies in different laboratories. There are no published data on exemestane. To know whether there are significant differences between any of the third generation aromatase inhibitors in their response to ACTH stimulation requires randomised studies in which all of the measurements are conducted in the same laboratory.

Table 1
Effect of letrozole treatment (0.5–10 mg daily for 3 months) on response to ACTH stimulation

Dose (mg daily)	Number of patients exhibiting abnormal cortisol response				Reference
	Pre-treatment		3 months		
	30 min	60 min	30 min	60 min	
0.5	1/22	0/22	2/19	0/19	Bajetta et al. [12]
2.5	0/23	1/23	5/22	1/22	Bajetta et al. [12]
10	0/12	0/12	0/12	0/12	Dixon [18]

3. Effects in premenopausal patients

Aromatase inhibitors are restricted in their use in breast cancer to the postmenopausal population. This is based on the inability of the first and second generation aromatase inhibitors aminoglutethimide and 4-hydroxandrostenedione respectively, to suppress estrogen levels in premenopausal breast cancer patients into the postmenopausal range on a consistent basis [19–22]. Additionally, pre-clinical studies with third generation aromatase inhibitors have indicated that the resultant increase in gonadal stimulation after aromatase inhibition can lead to the development of multiple ovarian follicles [23]. Thus, third generation aromatase inhibitors have been investigated in premenopausal women only in combination with a GnRH agonist.

It remains possible, however, that a low dose of a third generation inhibitor might have significant effects on the estrogen synthesis occurring in breast carcinomas and the surrounding tissues while any systemic effects might be compensated for by increased stimulation of gonadal synthesis. This could result in a site-specific suppression of estrogens in premenopausal women which would be particularly interesting in the preventive context in which systemic estrogen deprivation may not be desired or necessary. Estrogen suppression in the tumour alone would be of interest if this was sufficient to lead to a down-regulation of proliferation. We, therefore, undertook a multi-centre, placebo-controlled, double-blind study to evaluate the effects of the triazole aromatase inhibitor, YM511 (Fig. 1), on the proliferation of primary breast cancer cells in premenopausal women.

Thirty premenopausal women with primary breast cancer were randomised, 1:2 between placebo and YM511 (10 mg per day), with treatment starting irrespective of the date of their last menstrual period. A blood sample for hormonal analysis was obtained prior to starting treatment and at surgical excision 2 weeks later. A core-cut biopsy was taken prior to starting treatment for the estimation of estrogen receptor (ER), progesterone receptor (PgR) and the nuclear marker of proliferation, Ki67. Tissue was also taken from the excision biopsy for the immunohistochemical measurement

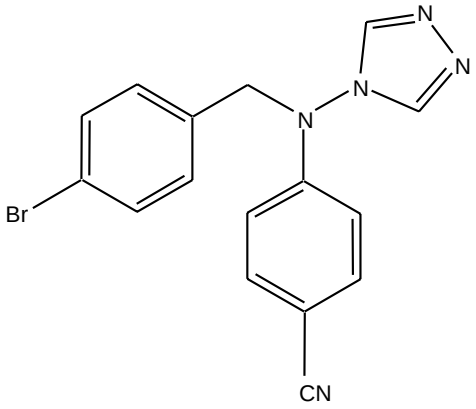

Fig. 1. Structure of YM511.

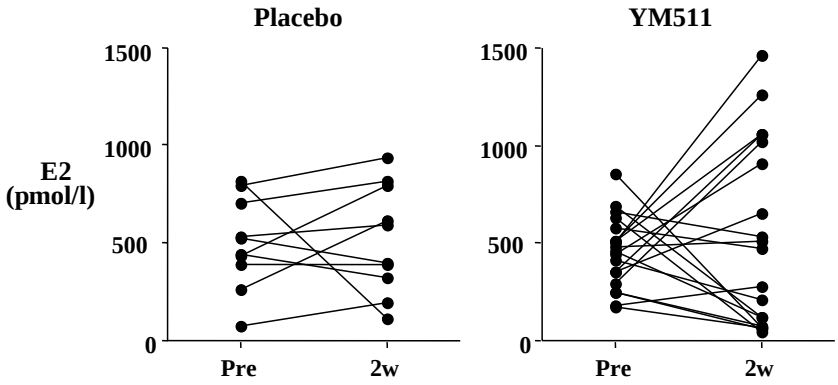

Fig. 2. Effect of YM511 on plasma E2 levels in premenopausal women. Each line represents an individual patient.

of the same three markers. The changes in plasma estradiol in the placebo and YM511 groups are shown longitudinally in Fig. 2. Highly variable effects are seen, with marked decreases in some patients and increases in others. The overall impact of this is better revealed when the data are plotted in Fig. 3a comparing the values after 2 weeks of YM511 with those pre-treatment in both groups or post-treatment in the placebo group (combined as a no-treatment group). After 2 weeks of YM511 5 of the 19 patients showed estradiol levels above 1000 pmol/l and 5 showed levels below 100 pmol/l. In contrast none of 38 values in the untreated group fell above 1000 pmol/l and only 1 below 100 pmol/l. This suggests that in some of the patients, a suppression to below normal premenopausal levels was achieved but in others a stimulation to supra-normal levels resulted. This bears comparison to the use of letrozole, administered on Days 3–7 of the menstrual cycle, as an ovulation inducer which, although associated with a significant fall in estradiol levels, is followed by a rapid recovery to levels high enough to trigger an endogenous LH surge around Days 12–14 [24,25]. There appeared to be no straightforward relationship between the day of the cycle on which YM511 treatment was started and the final level of estradiol achieved (Fig. 4).

It was interesting to note that none of the patients on YM511 showed increased estrone levels and indeed the mean level of estrone after 2 weeks treatment was 145 pmol/l compared to 254 pmol/l in the untreated group ($P = 0.0006$, Mann–Whitney). There was only one value less than 100 pmol/l in the untreated group while in the YM511 group 10/19 values were below this level (Fig. 3b). Thus, there would appear to be a substantially greater effect on plasma estrone levels than on plasma estradiol. This may be a result of estradiol being a predominantly ovarian estrogen, which is subject to a greater degree of feedback stimulation than is estrone. This is reflected by the greater fluctuations for estradiol than estrone through the menstrual cycle and the greater change at the menopause and after ovarian oblation. In contrast the latter's greater level of synthesis from peripheral tissues may render it more susceptible to suppression by an aromatase inhibitor in premenopausal women. The differential effects of YM511 on the estrogens would in part substantiate the concept that peripheral

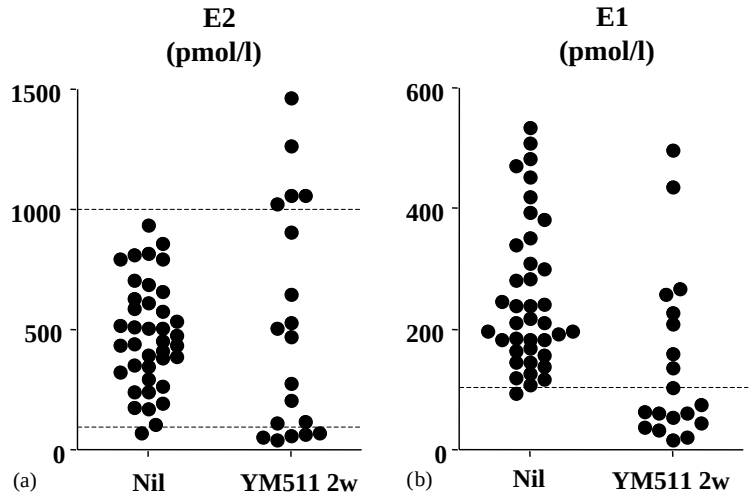

Fig. 3. Effect of YM511 on (a) plasma E2 and (b) E1 levels in premenopausal women. Nil: combination of pre-treatment values for YM511 and placebo and 2 week samples from placebo. Each point represents an individual patient.

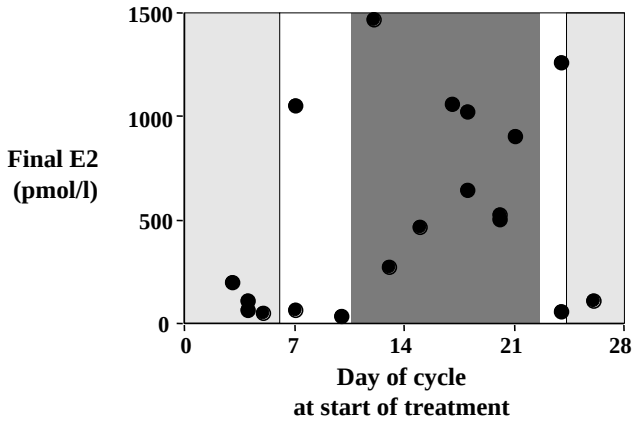

Fig. 4. Relationship between the day of the menstrual cycle that YM511 treatment (2 weeks) was started and the final level of E2 measured. The stippled areas show parts of the cycle where levels were either exclusively high or low.

synthesis of estrogens might be suppressed while the ovarian production remains high at least in some patients.

The changes in plasma estrogen levels were accompanied by highly significant increases in plasma testosterone (pre-treatment mean 1.14 nmol/l; 2-week mean 1.89 nmol/l; $P = 0.006$, Wilcoxon; Fig. 5) and androstenedione levels (pre-treatment mean 7.9 nmol/l; 2-week mean 10.6 nmol/l; $P = 0.013$; Fig. 6). Similarly, increases in LH and to a lesser degree FSH occurred. The substantial increases in testosterone and androstenedione are likely to result from the increased gonadal stimulation but decreased ability of aromatase to convert to oestrogens in the presence of YM511.

There was no overall change in Ki67 levels in the breast cancers of patients treated with YM511 (Fig. 7a). During a similar 2-week interval, albeit in postmenopausal patients, the third generation aromatase inhibitor vorozole was able to achieve a suppression of 58% (Fig. 7b) [26]. Thus, it would appear that any suppression of intratumoural aromatisation by YM511 was not sufficient to cause a significant reduction in proliferation. Overall these data indicate that in some patients peripheral aromatisation may be suppressed but ovarian estrogen production maintained. The effects are very variable between patients and are likely to be difficult to control satisfactory. The lack of impact on tumour cell proliferation suggests that intratumoural estrogen deprivation was modest.

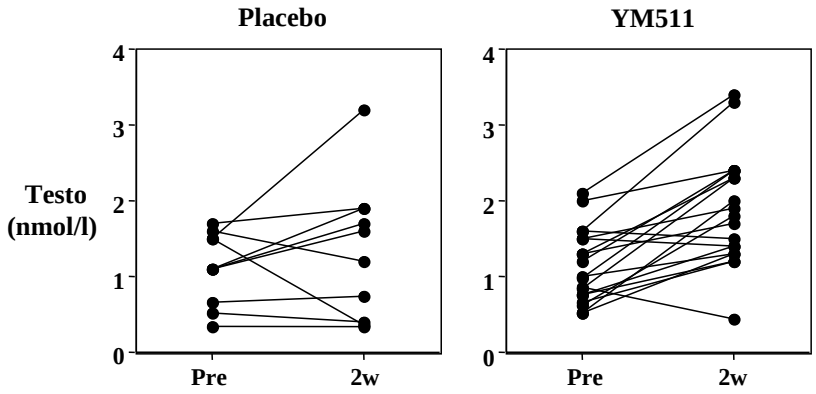

Fig. 5. Effect of YM511 on plasma testosterone levels in premenopausal women. Each line represents an individual patient.

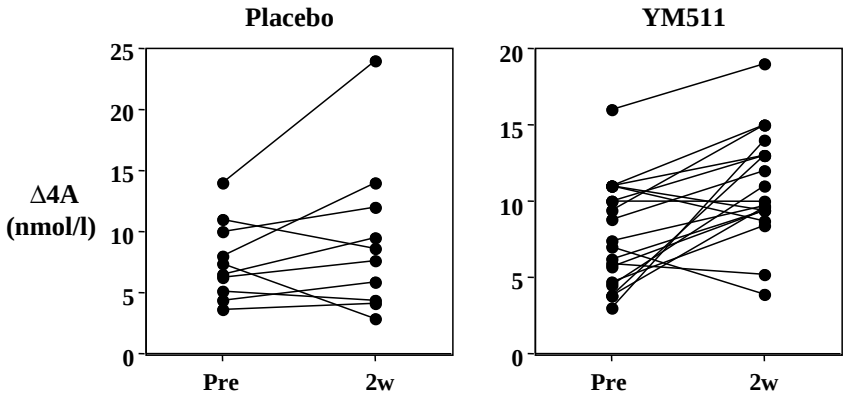

Fig. 6. Effect of YM511 on plasma androstenedione levels in premenopausal women. Each line represents an individual patient.

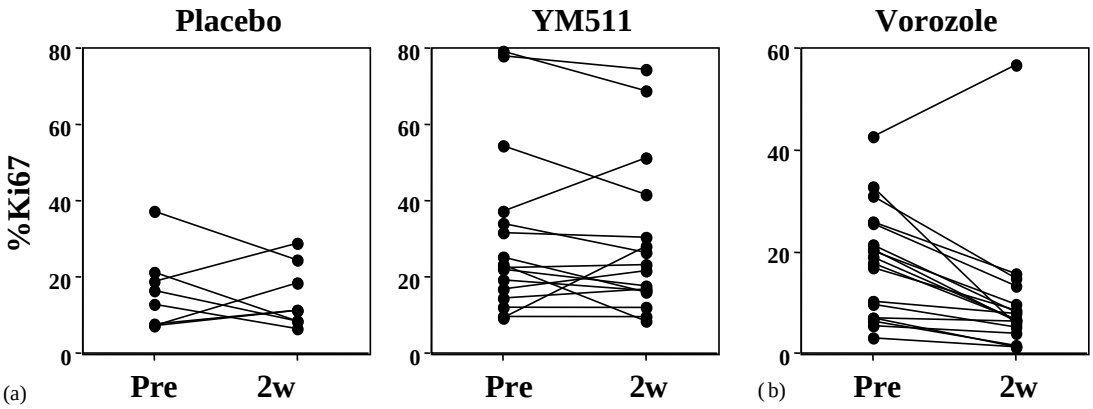

Fig. 7. Effect of (a) YM511 (in premenopausal patients) and (b) vorozole (in postmenopausal patients) on % Ki67 in breast carcinomas. Each line represents an individual patient.

4. Pharmacokinetic and pharmacodynamic interactions of tamoxifen and aromatase inhibitors

The recently reported anastrozole versus tamoxifen alone and combined (ATAC) trial, which compared the relapse-free survival in patients with primary breast cancer, found that the aromatase inhibitor was not only more effective than tamoxifen but was also more effective than the combination of tamoxifen with the aromatase inhibitor. Moreover, the efficacy of the combination was essentially equivalent to that of tamoxifen alone [27]. This equivalence of tamoxifen and the combination was true, not only for efficacy end-points but also for a large number of tolerability end-points. Thus, the effects of the tamoxifen were dominant above those of the aromatase inhibitor in all circumstances. The evidence discussed below indicates that there are pharmacokinetic interactions between the compounds but that these are unlikely to explain this result, rather it seems more likely that a pharmacodynamic explanation exists.

Aminoglutethimide was found several years ago to enhance the metabolism of tamoxifen such that circulating levels of tamoxifen decreased by a mean 73% [28]. Anastrozole has no impact on tamoxifen levels or on the levels of the major metabolite desmethyltamoxifen [29]. However, in the presence of tamoxifen, anastrozole levels are 27% reduced [30]. This is similar to the effects seen with letrozole where there is a mean 38% reduction in drug levels during combined usage with tamoxifen [31]. At first sight this might be seen to be a potential explanation for the poorer efficacy of anastrozole and tamoxifen than anastrozole alone in the ATAC trial. However, it is clear that the estrogen suppression achieved by anastrozole even in circumstances of reduced drug levels is near complete and efficacy at least close to that seen with anastrozole alone might have been anticipated on the basis of estrogen suppression.

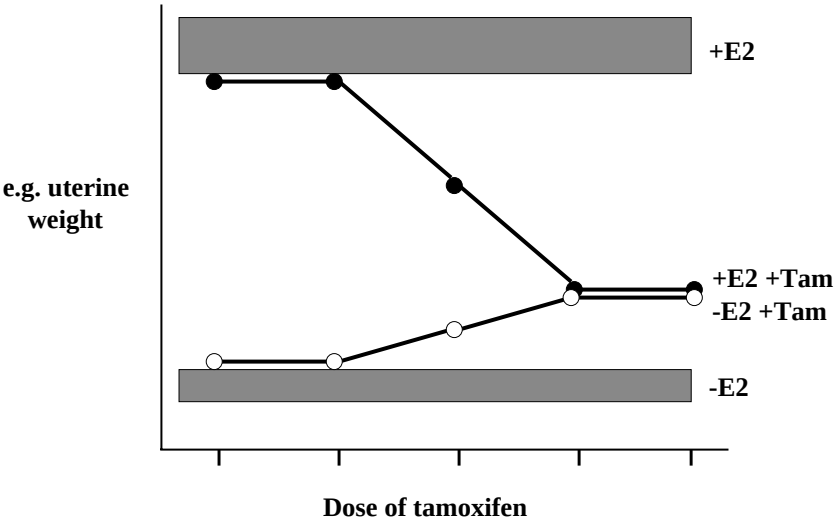

Fig. 8. Stylized demonstration of mixed estrogen agonist and antagonist effects of tamoxifen in the immature rat uterine weight model: see [32] for original data.

Table 2
Calculation of consensus steady state plasma concentrations of tamoxifen (TAM), desmethyl-tamoxifen (DMT), didesmethyl-tamoxifen (DDMT) and 4-hydroxytamoxifen (OHT) in breast cancer patients receiving a daily dose of 20–40 mg

Reference	Dose (mg per day)	No. of patients	Duration of treatment	Steady state plasma concentration (Css, ng/ml)							
				Tamoxifen		DMT		DDMT		OHT	
					20 mg[a]		20 mg[a]		20 mg[a]		20 mg[a]
ATAC trial [30]	20	111	>3 months	95	95	265	265	–	–	–	–
Ingle et al. [33]	20	27	6 weeks	107	107	200	200	–	–	3.0	3.0
Peyrade et al. [34]	30	316	>4 weeks	129	86	204	136	37	25	<3.8	<3.8
Buzdar et al. [35]	20	57	3 months	122	122	348	348	–	–	–	–
De Vos et al. [36]	30	8	>3 months	148	99	280	187	60	40	7.0	4.7
Langan-Fahey et al. [37]	20	35	>4 years	148	148	290	290	–	–	2.7	2.7
Lien et al. [38]	30	5	>4 weeks	141	94	220	147	51	34	3.5	2.3
Etienne et al. [39]	30	78	>4 weeks	173	115	378	252	46	31	1.9	1.3
Stevenson et al. [40]	30	12	>3 months	113	75	242	161	33	22	<5.0	<5.0
Slee et al. [41]	30	12	Steady state	130	87	245	163	–	–	–	–
Milano et al. [42]	30	6	> 4 weeks	145	97	343	229	55	37	6.5	4.3
McVie et al. [43]	40	14	2 months	202	101	257	128	–	–	–	–
Bratherton et al. [44]	20	77	>8 weeks	159	159	–	–	–	–	–	–
	40	75	>8 weeks	273	136	–	–	–	–	–	–
Kemp et al. [45]	40	15	Steady state	310	155	481	240	–	–	–	–
Daniel et al. [46]	40	7	>4 weeks	309	154	495	247	–	–	7.2	3.6
Patterson et al. [47]	40	15	Steady state	285	142	477	238	–	–	–	–
Mean ± S.D.					116 ± 28		215 ± 62		32 ± 7		3.1 ± 1.2

[a] Data were normalised to a 20 mg daily dose based on the assumption that tamoxifen exhibits linear pharmacokinetics.

It has been clear for many years that tamoxifen is not a pure anti-estrogen. Rather it has mixed agonist and antagonist effects. These are well demonstrated in the immature rat uterine weight model in which tamoxifen, in the absence of estradiol, exerts a partial estrogenic agonist effect, but in the presence of estradiol acts as an antagonist (Fig. 8) [32]. The observed biological effects of tamoxifen will thus depend on its competition with endogenous estrogen for occupancy of the estrogen receptor.

To predict how tamoxifen might behave in post-menopausal breast cancer one needs to know the degree to which tamoxifen saturates the estrogen receptor at its most widely used dose of 20 mg per day. Table 2 summarises and produces a consensus from data from published papers in which the steady-state serum concentrations of tamoxifen and its major metabolites, desmethyl-tamoxifen, didesmethyl-tamoxifen and 4-hydroxy-tamoxifen in breast cancer patients have been reported [33–47]. A similar consensus for the relative binding affinity for the estrogen receptor is calculated in Table 3 [48–55]. Multiplication of these consensus steady-state concentrations and relative binding affinities gives a value of 3,944,327 for the overall biological activity of tamoxifen at the estrogen receptor (Table 4). In comparison, the activity of estradiol, for which a steady-state concentration 25 pmol/l has been used [56], is 2500. The contribution of estrone and estriol to this activity is likely to be insignificant due to either their much lower concentrations or lower ER binding affinities. Overall, the data indicate an activity ratio of 1558:1 of tamoxifen (and metabolites) to estradiol at the estrogen receptor. This

Table 3
Calculation of consensus estrogen receptor relative binding affinities (RBA) for tamoxifen (TAM), desmethyl-tamoxifen (DMT), didesmethyl-tamoxifen (DDMT) and 4-hydroxytamoxifen (OHT)

Reference	ER source (uteri)	RBA (E2 = 100)			
		TAM	4-OHT	DMT	DDMT
Lyman and Jordan [48]	Mouse	2.5	131	–	–
Foster et al. [49]	Rat	0.9	175	–	–
Jordan et al. [50]	Rat	6.0	280	4.0	–
Jordan et al. [51]	Rat	3.0	252	–	–
Kemp et al. [45]	Rat	1.8	187.8	1.1	0.51
Katzenellenbogen et al. [52]	Rat	2.0	185	–	–
Robertson et al. [53]	Rat	2.0	285	3.0	2.0
Fabian et al. [54]	Rat	5.0	100	1.33	–
Jordan et al. [55]	Rat	1.8	36	–	–
Mean ± S.D.		2.8 ± 1.7	181 ± 84	2.4 ± 1.4	1.3 ± 1.1

Table 4
Comparison of the biological activity at the estrogen receptor (RBA × steady state concentration) of estradiol vs. tamoxifen (and its major metabolites) in postmenopausal women on a daily dose of 20 mg tamoxifen and a plasma estradiol level of 25 pmol/l

Compound	Css (ng/ml)	Css (pmol/l)	RBA (ER)	Activity (RBA × Css)
Estradiol		25	100	2500
Tamoxifen	116	312248	2.8	874294
DMT	215	601399	2.4	1443358
DDMT	34	98981	1.3	128675
OHT	3.1	8000	181	1448000
Total				3944327

corresponds to almost total saturation of the estrogen receptor by tamoxifen (99.94% occupancy). In these circumstances the partial estrogen agonist effects of tamoxifen would be expected to far outweigh the effects of the residual 0.06% occupancy of the receptor by estradiol. The addition of an aromatase inhibitor would therefore be very unlikely to affect the biological activity of the estrogen receptor. Rather the pharmacology of tamoxifen would be expected to be dominant and therefore the clinical efficacy of tamoxifen combined with an aromatase inhibitor to be equivalent to that of tamoxifen alone. This has indeed been the case in the ATAC trial [27] and also in previously reported studies of tamoxifen plus aminoglutethimide [57–59].

The above calculations are based on steady-state serum levels of tamoxifen and its metabolites as these have been published much more extensively on, and show far less variability, than tumoural concentrations. It is well known that breast tumour concentrations of estradiol are 10–20-fold higher than plasma concentrations in postmenopausal women [60–63]. However, it is also known that the concentrations of tamoxifen and its metabolites in breast tumours are 3–7-fold higher than in serum [64]. Thus, the effect of using serum concentrations rather than tumour concentrations in these calculations is unlikely to be a major one.

5. Conclusions

Third generation aromatase inhibitors have excellent specificity. Valid comparisons between agents on cortisol synthesis require randomised study. The effects of YM511 in premenopausal women are insufficient to lead to antiproliferative effects in hormone sensitive breast carcinomas. Clinical doses of tamoxifen effectively saturate the receptor in postmenopausal women and lead to tamoxifen-dominant pharmacology when combined with aromatase inhibitors.

Acknowledgements

We are grateful to the investigators of the YM511 trial and to the technical staff of the Biochemistry Department that contributed to the data presented.

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