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EXEMESTANE (FCE 24304), A NEW STEROIDAL AROMATASE INHIBITOR

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Summary—Exemestane (FCE 24304; 6-methylenandrosta-1,4-diene-3,17-dione) is a novel orally active irreversible aromatase inhibitor. Its *in vitro* and *in vivo* pharmacological properties have been compared to 4-hydroxyandrostenedione (4-OHA). In preincubation studies with human placental aromatase, exemestane, like 4-OHA, showed enzyme inactivating properties with a similar affinity (K_i 26 vs 29 nM) and a lower rate of inactivation ($t_{1/2}$ 13.9 vs 2.1 min). Conversely, when tested in pregnant mares' serum gonodatropin-treated rats, exemestane was more potent in reducing microsomal ovarian aromatase activity than 4-OHA, after both subcutaneous (ED_{50} 1.8 vs 3.1 mg/kg) and oral dosing (ED_{50} 3.7 vs >100 mg/kg). No interference of exemestane on desmolase or 5α-reductase activity was found. The compound did not show any relevant binding affinity to steroidal receptors, but slight binding to the androgen receptor (≈0.2% of dihydrotestosterone), like 4-OHA.

In the first phase I trial, healthy postmenopausal volunteers were given single oral doses of exemestane, ranging from 0.5 to 800 mg, and plasma [estrone (E_1), estradiol (E_2) and estrone sulphate (E_1S)] and urinary estrogens (E_1 and E_2) were measured up to 5–8 days. The minimal effective dose in decreasing estrogens was 5 mg. At 25 mg the maximal suppression was observed at day 3: plasma estrogens fell to 35 (E_1), 39 (E_2) and 28% (E_1S), and urinary estrogens fell to 20 (E_1) and 25% (E_2) of basal values, these effects still persisting on day 5. No effects on plasma levels of cortisol, aldosterone, 17-hydroxyprogesterone, DHEAS, LH and FSH, and no significant adverse events were observed up to the highest tested dose of 800 mg exemestane.

INTRODUCTION

The aromatization of C_{19} steroids (androstenedione and testosterone) to obtain C_{18} estrogens [estrone (E_1) and estradiol (E_2)] constitutes the last and one of the most important steps in the biosynthetic progression from cholesterol to estrogens. The aromatase system is an enzyme complex consisting of two components, a flavoprotein (NADPH–cytochrome *P*-450 reductase) that transfers electrons from NADPH to the terminal enzyme, a specific form of cytochrome *P*-450 (*P*-450_{AROM}) hemoprotein. The latter protein is involved in the binding of C_{19}-steroid substrates and catalyzes the multistep reaction leading to aromatization of the A ring of the steroid [1].

Specific inhibitors of this enzyme complex can be useful in controlling pathologic conditions associated with estrogens, such as breast cancer, endometrial cancer, and benign prostatic hyperplasia [2–4]. Compounds that interfere with the aromatase enzyme complex are usually classified as reversible or irreversible (mechanism-based) aromatase inhibitors [5]. Reversible aromatase inhibitors are competitive inhibitors of cytochrome *P*-450, and aminoglutethimide may be regarded as the pioneer drug of this type. Aminoglutethimide was initially introduced as an anticonvulsant and its endocrine properties became apparent only after extensive clinical use [6]. Although the drug has been of some benefit in postmenopausal breast cancer, it is a nonspecific aromatase inhibitor and presents problems of tolerability [7]. Several nonsteroidal, reversible aromatase inhibitors, more selective than aminoglutethimide, have recently been described and those currently under clinical investigation are listed in Table 1 [8–18]. The most advanced compound is fadrozole (CGS 16949A) [8–10], which is currently in phase III clinical trials in postmenopausal breast cancer patients. However, this compound has been reported to inhibit aldosterone secretion in patients at doses near the therapeutic ones [10].

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Table 1. Aromatase inhibitors

Type of inhibition (type of compound)	Name of compound	Ref
Reversible (nonsteroid)	Aminoglutethimide	6, 7
	Fadrozole (CGS 16949A)	8–10
	R 76713/R 83842	11–14
	Pyridoglutethimide	15, 16
	CGS 20267	17, 18
Irreversible (steroid)	4-OHA	19–25
	Atamestane(SH 489)	26–28
	MDL 18962	29, 30
	Exemestane (FCE 24304)	31–34
	FCE 24928	34
	ORG 30958	35

As a follow-up compound, the new nonsteroidal derivatives CGS 20267, reportedly more selective than fadrozole, is now under clinical evaluation [17, 18].

Attention has also been directed towards the development of irreversible inhibitors of aromatase (Table 1) [19–35], which are steroidal compounds structurally related to the natural substrate androstenedione (Fig. 1). Since the enzyme becomes inactivated as a consequence of its own mechanism of action, such inhibitors have been referred to as mechanism-based or "suicide" inhibitors. 4-Hydroxyandrostenedione (4-OHA), initially described by Brodie *et al.* [19, 20], is the prototype compound of this class of inhibitors and is now in phase III clinical trials in postmenopausal breast cancer patients. The majority of the new steroidal aromatase inhibitors, including atamestane (SH 489; 1-methyl-1,4-androstadiene-3,17-dione) [26–28], MDL 18962 [10-(2-propynyl)estr-4-ene-3,17-dione] [29, 30], exemestane (FCE 24304; 6-methylen-androsta-1,4-diene-3,17-dione) [31–34] and FCE 24928 (4-aminoandrosta-1,4,6-triene-3,17-dione) [34], are now at various stages of clinical investigation.

4-OHA, the most advanced steroidal derivative, was first reported in 1984 to cause regression of breast cancer metastasis in postmenopausal women [21] and was later reported to cause breast cancer remission when used intramuscularly (i.m.) at different drug schedules [22, 23]. In these patients, a weekly or biweekly i.m. administration of 4-OHA was found to cause a clear and sustained suppression of serum E_2 [21–23], the long-lasting effect being attributed to the formation of a local depot from which the drug is released [24]. Suppression of estrogen synthesis was also shown with oral administration of 4-OHA in postmenopausal patients [24, 25]. However, a suppression comparable to that observed with a biweekly i.m. dose of 250 mg was only obtained after daily oral administration of 250 mg [25]. The lower potency of oral 4-OHA, also reported in animals [33, 36], has been attributed to a rapid inactivation of the compound, which is highly excreted in the urine as a glucuronide at the 4-hydroxyl-group [37].

In a programme to select a novel irreversible aromatase inhibitor with improved oral activity over 4-OHA, we have identified the steroidal derivative exemestane [31–33]. In the present paper we summarize the most relevant pharmacological properties of exemestane, in comparison with those of 4-OHA, in experimental animals. In addition, the results of the first phase I trial with oral exemestane in healthy postmenopausal volunteers are described. In both animals and humans the oral administration of exemestane resulted in marked and long-lasting inhibition of aromatase.

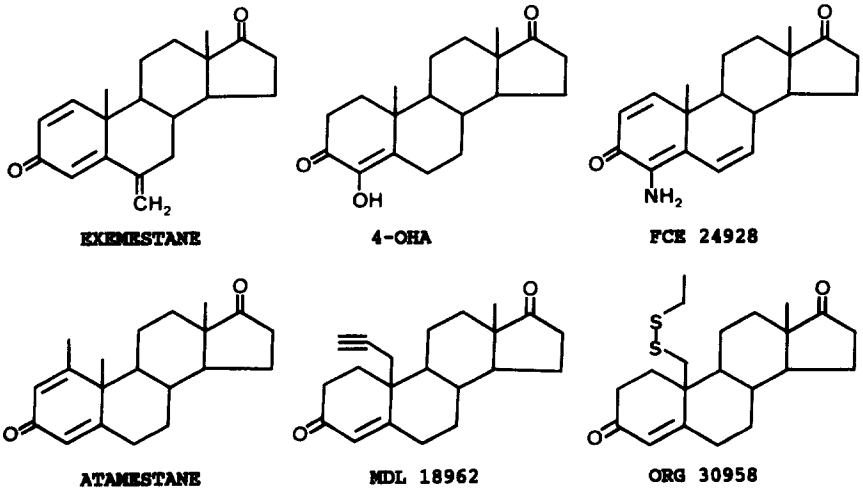

Fig. 1. Chemical structures of steroidal aromatase inhibitors.

ANIMAL PHARMACOLOGY

Aromatase inhibition

Microsomes from human placenta were used to assess the *in vitro* aromatase inhibitory effect. Aromatase activity was determined from the release of tritiated water from [1β,2β-^{3}H]-androstenedione converted to estrogens. Both co-incubation and preincubation studies (to measure enzyme inactivation) were carried out.

In co-incubation studies with the substrate (50 nM [^{3}H]androstenedione), exemestane was found to inhibit aromatase with an IC_{50} of 42.5 nM (Table 2), i.e. with a potency similar to 4-OHA (IC_{50} 43.7 nM) and about 40 times higher than for aminoglutethimide (IC_{50} 1754 nM) [31, 33]. In preincubation studies with the enzyme and in the presence of NADPH, exemestane, like 4-OHA, was shown to cause time-dependent enzyme inhibition, whereas no inactivation was observed with aminoglutethimide. Inactivation by 4-OHA was faster ($t_{1/2}$ 2.1 min) than that by exemestane ($t_{1/2}$ 13.9 min), whereas their affinity for the enzyme was similar (K_i 29 and 26 nM, respectively) (Table 2). The K_i value for aminoglutethimide, evaluated in a competitive inhibition study, was 1370 nM, whereas the K_m for the substrate androstenedione was 69 nM [33].

The *in vivo* efficacy of exemestane and 4-OHA has been tested in adult female rats in which ovarian aromatase activity was stimulated by the subcutaneous (s.c.) injection of pregnant mares' serum gonadotropin (PMSG), at 100 IU twice, 4 days apart. Three days after the second PMSG injection, the aromatase inhibitors were given s.c. or orally at various doses, and the animals were sacrificed 24 h later. Ovarian microsomes were prepared and the residual aromatase activity was determined by the ^3H_2O assay procedure. Exemestane reduced ovarian aromatase activity after both s.c. and oral treatment, showing ED_{50} values of 1.8 and 3.7 mg/kg, respectively (Table 3) [33]. 4-OHA was effective when given s.c. (ED_{50} 3.1 mg/kg) although with less potency than exemestane, whereas by the oral route it caused marginal enzyme inactivation, reaching only 28% inhibition even at 100 mg/kg.

Table 2. Inhibition of exemestane of human placental aromatase

Compound	IC_{50} (nM)[a]	$t_{\frac{1}{2}}$ (min)[b]	K_i (nM)[b]
Exemestane	42.5 ± 4.3	13.9 ± 0.7	26.0 ± 1.4
4-OHA	43.7 ± 1.6	2.1 ± 0.2	29.0 ± 7.5
Aminoglutethimide	1754.0 ± 81.0	NTD	

Mean ± SE of at least 3 assays. [a]Co-incubation studies with 50 nM [^{3}H]androstenedione. [b]Pre-incubation studies for 0–32 min with the enzyme in the presence of NADPH. NTD, no time-dependent inhibition.

Table 3. *In vivo* inhibition by exemestane of ovarian aromatase activity[a] in PMSG-stimulated adult rats

	Aromatase inhibition ED_{50} (mg/kg)	
Compound	s.c	Oral
Exemestane	1.8	3.7
4-OHA	3.1	>100.0

[a]Measured by the ^3H_2O procedure in ovarian microsomes from animals sacrificed 24 h after a single treatment.

Effects on desmolase and 5α-reductase

The possible interference of exemestane on desmolase (cholesterol side chain cleavage enzyme) and testosterone 5α-reductase was evaluated *in vitro* by using rat adrenal mitochondria or rat prostate particulate fraction, in the presence of 10 μM [^{14}C]cholesterol or 1 μM [^{14}C]testosterone as substrate, respectively. Like 4-OHA, exemestane was ineffective in inhibiting desmolase activity up to the concentration of 100 μM, whereas the reference standard aminoglutethimide caused enzyme inhibition with an IC_{50} of 32 μM (Table 4) [34]. No inhibition of 5α-reductase activity was observed with exemestane and 4-OHA up to 30 μM, whereas the reference standard 4-MA was very effective (IC_{50} 0.03 μM) [31, 34].

Steroid receptor binding affinity

Binding of the compounds to cytoplasmic steroid receptors was determined by standard dextran coated charcoal adsorption techniques. The sources for receptor preparations were: prostatic tissue from adrenalectomized and orchiectomized rats for androgen receptors; uterine tissue from ovariectomized rats for estrogen receptors; uterine tissue from estrogen-primed immature rabbits for progestin receptors; thymus and kidneys from adrenalectomized rats for glucocorticoid and mineralocorticoid receptors, respectively. Various concentrations of the compounds were incubated for 2–3 h at 0°C with the [^{3}H]-specific ligand at 1 nM (3 nM for

Table 4. Effect of exemestane on desmolase and 5α-reductase

Compound	Desmolase[a] IC_{50} (μM)	5α-Reductase[b] IC_{50} (μM)
Exemestane	>100	>30
4-OHA	>100	>30
Aminoglutethimide	32	—
4-MA	—	0.03

[a,b]Substrate 10 μM [^{14}C]cholesterol or 1 μM [^{14}C]-testosterone.

Table 5 Steroid receptor binding affinity of exemestane

Receptor	Standard	RBA[a] (%) Exemestane	4-OHA
Androgen	Dihydrotestosterone	0.22	0.25
Estrogen	Estradiol	⩽0.005	⩽0.005
Progestin	Progesterone	0.10	0 25
Glucocorticoid	Dexamethasone	0.075	0.03
Mineralocorticoid	Aldosterone	0.12	⩽0 09

[a]RBA calculated by considering the potency (IC_{50}) of the specific standard as 100% Results are the average of at least two experiments.

aldosterone). The relative binding affinity (RBA) of each compound was calculated by considering as 100 the potency of the specific unlabelled standard, according to the relation: RBA = (IC_{50} of standard/IC_{50} of test compound) × 100. Data summarized in Table 5 indicate that exemestane had no binding affinity to the estrogen receptor and only very low affinity for progestin, glucocorticoid and mineralocorticoid receptors (RBA ≈ 0.1%) and androgen receptors (0.22%). 4-OHA showed only very low binding affinity to androgen (0.25%) and progestin (0.25%) receptors [31, 34].

Androgenic activity

The possible intrinsic androgenic effect of exemestane, in comparison with testosterone propionate (TP), was tested in immature rats orchiectomized at 21 days of age. The compounds were given s.c. for 7 consecutive days, starting on the 7th day after orchiectomy. Twenty four hours after the last dose, ventral prostate and seminal vesicles were removed and weighed. Exemestane, at the s.c. doses of 3 and 10 mg/kg/day, did not induce any increase in seminal vesicle weight, whereas it caused a significant increase in prostate weight; however, its dose–response curve was flatter than that of TP at doses of 0.1 and 0.3 mg/kg/day (Fig. 2) [34]. At these doses TP caused a relevant increase in seminal vesicle weight.

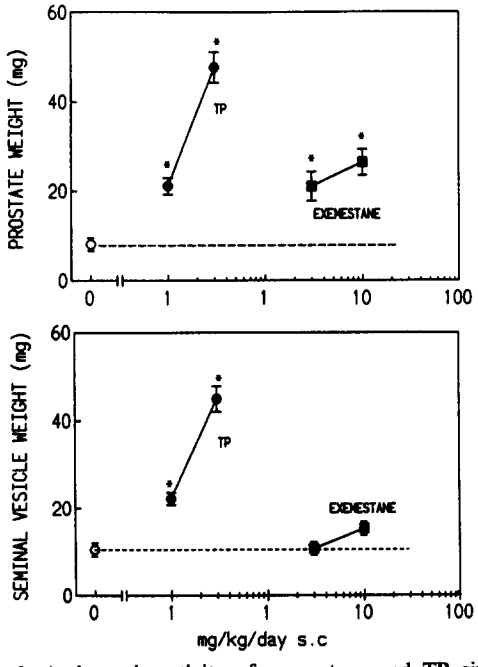

Fig. 2. Androgenic activity of exemestane and TP given s.c. for 7 days in prepubertal castrated rats. Animals were treated starting on the 7th day after orchiectomy. Mean ± SE of 8 animals per group. *$P < 0.01$ vs control group.

CLINICAL PHARMACOLOGY

A phase I trial in healthy postmenopausal volunteers has been conducted to assess the tolerability and aromatase inhibitory effect of exemestane after a single oral dose. Reduction in plasma E_1, E_2, and estrone sulphate (E_1S) and in urinary total E_1 and E_2 was taken as evidence of aromatase inhibition. Estrogens were measured by specific radioimmunoassays, after extraction with ether (and a preliminary enzyme hydrolysis for conjugated estrogens) and separation by celite column chromatography.

In a first part of the study, exemestane was administered at the dose of 25, 50, 200, 400 and 800 mg, respectively, in five successive cohorts of three subjects each. Plasma and urinary estrogens were assayed at baseline and for 48 h after drug administration; in the 800 mg cohort the sampling period was extended to day 7. In the 25 to 400 mg dose range, for which endocrine measurements were performed for only 48 h, a clear, nondose-related, suppression of estrogens was initially observed at 8–24 h in plasma and at 24–48 h in urine; at 48 h the effect on plasma levels was still increasing, estrogen suppression ranging between 60–23% of basal values for E_2, 52–38% for E_1, and 38–22% for E_1S. In general, the endocrine effects seen at 400 mg were not more evident than found with a dose of 25 mg. At the highest tested dose of 800 mg, at which endocrine measurements were extended up to 7 days, the maximal reduction in estrogen levels was observed at day 4, reaching 10% of basal values for E_1S and undetectable levels for E_1 and E_2; a similar degree of suppression was still present on day 7.

To determine the threshold dose for endocrine efficacy, the study was subsequently enlarged to doses of 0.5, 5, 12.5 and 25 mg in 14 additional volunteers (3–4 subjects per dose), with an extended sampling period, up to 5–8 days. The dose of 0.5 mg was ineffective. Suppression of

all plasma estrogens (to 60–47% of basal values on day 3) was observed starting from 5 mg, and was more pronounced and longer lasting with increasing doses. At 25 mg the maximal suppression was observed at day 3: plasma estrogens fell to 35 (E_1), 39 (E_2) and 28% (E_1S) and urinary estrogens fell to 20 (E_1) and 25% (E_2) of basal values, these suppressions still persisting on day 5 posttreatment.

No modifications were observed in other hormonal parameters (cortisol, aldosterone, DHEAS, 17-hydroxyprogesterone, FSH and LH), which were determined in the highest dose group of each part of the study (800 and 25 mg). No clinically relevant side effects or alterations in laboratory parameters were observed up to the maximum exemestane dose of 800 mg.

DISCUSSION

The selection of exemestane as a candidate for clinical development was based on its pharmacological properties, which have characterized the compound as a very potent, selective and orally active aromatase inhibitor [31, 33]. The irreversible nature of exemestane inhibitory effect on aromatase, as demonstrated in preincubation studies of the compound with human placental aromatase in the presence of NADPH, has been attributed to the presence of the double bond at C_1—C_2 [31]. In fact, the correspondent C_1—C_2 saturated derivative was found to be a reversible aromatase inhibitor. Although the inactivation rate of the enzyme was lower for exemestane ($t_{1/2}$ 13.9 min) than for 4-OHA ($t_{1/2}$ 2.1 min), their affinity to the enzyme was similar (K_i 26 vs 29 nM, respectively) and was higher than that of the natural substrate androstenedione (K_m 69 nM). The selectivity of exemestane was shown by its ineffectiveness on desmolase and 5α-reductase. In addition, the compound was found to have no relevant binding affinity to the estrogen, androgen, progestin, glucocorticoid or mineralocorticoid receptor. Although *in vitro* exemestane showed a very low binding affinity to the androgen receptor (RBA 0.22% of dihydrotestosterone), the compound was found to have a slight androgenic activity *in vivo*, when given s.c. in castrated rats. However, at exemestane doses of 3 and 10 mg/kg/day, the dose–response curve on prostate weight (the seminal vesicles were not stimulated) was flatter than at TP doses of 0.1 and 0.3 mg/kg/day, and the androgenic potency of exemestane was estimated as being approx. 1% that of TP. We have found that 4-OHA has a binding affinity to the androgen receptor comparable to that of exemestane and, in addition, 4-OHA, given s.c. in castrated rats, has been shown by Brodie *et al.* [19] to have approx. 1–4% of the androgenic potency of testosterone. The androgenic activity of both exemestane and 4-OHA has been shown to result in suppression of gonadotropin secretion in rats [38–40]. This gonadotropin suppressive effect by aromatase inhibitors can be of relevance when considering their antitumor efficacy in the situation of functional ovaries, as in intact rats with DMBA-induced mammary tumors or in premenopausal breast cancer patients. On the contrary, in the postmenopausal condition where peripheral aromatase, which is not regulated by gonadotropins, is the main source of estrogen synthesis, gonadotropin inhibitory activity is not expected to be an advantage for aromatase inhibitors [2, 38, 41].

The very potent oral activity of exemestane in rats has been confirmed in the first phase I study in healthy postmenopausal volunteers. The potency and the duration of the effect of a single oral dose of exemestane in women resulted even higher than initially expected. In fact, the study was initially conducted at doses starting from 25 mg and with an observation time up to 48 h. Only when it became apparent that all the doses were effective even at the last sampling time, a second part of the study was started with lower doses (from 0.5 and 25 mg) and with a sampling time up to 5–8 days. In this study the dose of 5 mg was found to be the lowest dose at which an effect in decreasing both plasma and urinary estrogens is observed. At the dose of 25 mg a very marked estrogen suppression was observed, which peaked at day 3 and persisted for at least 5 days after exemestane administration.

The long-lasting inhibitory effect of oral exemestane on estrogen synthesis is likely due to the irreversible nature of its enzyme inhibitory property, rather than to its pharmacokinetic properties. In fact, the measurement of exemestane plasma levels in the same postmenopausal volunteers has demonstrated that the drug reaches peak levels at approx. 2 h after oral intake and rapidly disappears thereafter [42].

In humans, aromatase inhibition by exemestane was very specific, as shown by the absence of any effect even at the very high dose of 800 mg, on plasma levels of cortisol, aldosterone, 17-hydroxyprogesterone and DHEAS.

In addition, no modifications in plasma levels of LH and FSH were detected.

These endocrine results together with the absence of any relevant side effects, render exemestane a very interesting aromatase inhibitor for testing in postmenopausal breast cancer patients.

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