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Comparison of the Effects of the Irreversible Aromatase Inhibitor Exemestane with Atamestane and MDL 18962 in Rats with DMBA-induced Mammary Tumours

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The antitumour activity of the steroidal aromatase inhibitors exemestane (FCE 24304), MDL 18962 and atamestane (SH 489) was evaluated on 7,12-dimethylbenzanthracene (DMBA)-induced mammary tumours in rats. The compounds were given subcutaneously at daily doses of 10 and 50 mg/kg for 4 weeks. Exemestane was also given orally, at daily doses of 100 and 200 mg/kg. Subcutaneous exemestane induced 30% (10 mg/kg) and 73% (50 mg/kg) regressions of established tumours and strongly reduced the appearance of new tumours. Conversely, atamestane, MDL 18962 and oral exemestane did not affect growth of established tumours nor influenced the appearance of new neoplasms. Aromatase activity of ovarian microsomes (OAA) was reduced by 85%–93% after subcutaneous exemestane and by 25%–59% after MDL 18962, and was unaffected after atamestane. Oral exemestane caused a reduction in OAA of 72%–74%. Serum luteinising hormone (LH) levels were reduced at both the subcutaneous doses of exemestane and at the higher dose of MDL 18962. Atamestane caused an increase in LH levels, while no effect was observed with oral exemestane. The LH-lowering effect of subcutaneous exemestane, the less marked effect of MDL 18962, and the ineffectiveness of oral exemestane were also observed after 10 days of treatment in ovariectomised rats. The antigonadotrophic effect of subcutaneous exemestane, which is probably due to its slight androgenic effect, could contribute to its antitumour activity in the DMBA tumour model in intact rats, through a counteraction of the negative feedback of oestrogens on gonadotropin secretion.
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INTRODUCTION

MUCH INTEREST in hormonal treatment strategies of breast cancer has recently focused on the blockade of oestrogen biosynthesis as a mean for inducing regressions of hormone-dependent tumours [1]. To this end, specific inhibitors of the enzyme aromatase—i.e. the enzyme system catalysing the conversion of androgens into oestrogens—have been developed. Besides the development of reversible non-steroidal aromatase inhibitors, resembling the mechanism of action of the pioneer drug aminoglutethimide, attention has also been directed towards the development of irreversible steroidal aromatase inhibitors, chemically related to the natural substrate androstenedione [1]. 4-hydroxyandrostenedione (4-OHA) may be regarded as the prototype compound of this class [1, 2]. In

studies carried out with 4-OHA in rats with hormone-dependent mammary tumour induced by the carcinogen 7,12-dimethylbenzanthracene (DMBA), the compound caused regression of the tumours and inhibition of ovarian aromatase activity [3]. In addition, because of its slight intrinsic androgenic activity, 4-OHA was also found to decrease the gonadotropins luteinising hormone (LH) and follicle-stimulating hormone (FSH), an effect which could contribute to its antitumoral effect in cycling rats [3, 4]. In fact, in intact rats the decrease in blood oestrogen levels due to aromatase inhibition stimulates gonadotropin secretion through feedback regulatory mechanisms; this effect in turn stimulates ovarian aromatase synthesis, thus counteracting the initial enzyme inhibition [3]. The additional direct inhibitory effect of 4-OHA on gonadotropins can therefore block the regulatory reflex increases. Whether this additional *in vivo* effect of 4-OHA represents an advantage in its use in premenopausal patients remains to be determined. However, in postmenopausal breast cancer patients, 4-OHA has been reported to have an interesting antitumoral effect [5] and phase III trials are now ongoing [1].

A number of novel irreversible, steroidal aromatase inhibitors have been described, including MDL 18962 (10-propargylestr-4-ene-3,17-dione) [6], SH 489 (atamestane; 1-methylandrosta-1,4-diene-3,17-dione) [7] and FCE 24304 (exemestane, 6-methylenandrosta-1,4-diene-3,17-dione) [8, 9], which are all currently undergoing clinical evaluation. The purpose of this study was to compare the antitumour effect, in the DMBA-induced mammary tumour in rats, of these three compounds, which, besides the specific aromatase inhibitory property, have different degrees of intrinsic androgenic activity. In fact, like 4-OHA, both MDL 18962 [10] and exemestane [11] have been reported to display androgenic activity in orchidectomised rats, whereas atamestane has been reported to be completely devoid of this property [12].

The antitumoral effect of the three compounds was studied at subcutaneous doses that in a preliminary study were found effective in lowering plasma oestradiol levels in pregnant mare's serum gonadotropin (PMSG)-stimulated female rats. The antitumoral effect of oral doses of exemestane was also studied. In addition, the effects of the various compounds on ovarian aromatase activity, on serum LH and prolactin levels and on endocrine organ weights of tumour-bearing rats were evaluated.

MATERIALS AND METHODS

Drugs

Exemestane, atamestane, MDL 18962 and 4-OHA were synthesised in the laboratories of Farmitalia Carlo Erba, Milan, Italy. Testosterone propionate was purchased from Sigma. Drugs were dissolved in benzyl alcohol and diluted in sesame oil (final concentration of benzyl alcohol 5%) for subcutaneous injections. Oral exemestane was suspended in 0.5% methocel (methylcellulose 400) containing 0.4% Tween 80 (polysorbate 80).

PMSG-stimulated rats

Immature female Sprague-Dawley rats (CD, supplied by Charles River, Italy) of 22 days of age, 40–45 g body weight, were injected subcutaneously with 30 IU of PMSG (Sigma). 48 h after priming, animals were treated subcutaneously with the aromatase inhibitors at 10 mg/kg, or with the vehicle alone (5 ml/kg). 6 h later they were killed, blood was collected in heparinised tubes and plasma stored at −20°C for oestradiol radioimmunoassay. The ovaries were removed, weighed and stored at −20°C for aromatase assay.

Mammary tumour model

At 50–54 days of age, Sprague-Dawley rats (IOPS-OFA, supplied by Iffa Credo, France) were dosed intragastrically with 20 mg DMBA (Sigma) dissolved in sesame oil (1 ml per rat). Starting 40 days after DMBA treatment, animals were examined weekly by palpation; when at least one tumour of 1 cm in diameter was found, the rats were placed sequentially into experimental groups (9–10 rats per test group). Animals without tumours by day 150 were discarded. The two perpendicular tumour axes were measured with calipers twice weekly during treatment. Tumour weight was calculated according to the formula $d^2 \times D/2$, where d is the minimal and D the maximal diameter [13]. At the end of the treatment period, tumour response was designated as CR (complete remission, i.e. complete disappearance of the tumour), PR (partial remission, i.e. > 50% reduction in tumour weight), NC (no change, < 50% increase or decrease) or P (progression, > 50% increase).

Treatments were given twice daily (at 1000 and 1600 h), 6 days a week for 4 weeks, subcutaneously (2 ml/kg) or orally (5 ml/kg). On the last experimental day, rats were dosed at 1000 h; 4 h later they were killed by decapitation, blood was collected and serum stored at −20°C for prolactin and LH radioimmunoassay. The pituitary, ovaries, adrenals and uterus were then removed and their wet weights recorded. Ovaries were stored at −20°C for aromatase assay.

Ovarian aromatase assay

Each pair of ovaries was processed for isolation of the microsomal fraction as described in Zaccheo *et al.* [14]. Aromatase activity of ovarian microsomes was assayed according to Thompson and Siiteri [15], by measuring the amount of ^3H_2O formed during 1 h incubation at 37°C with [1β,2β-^{3}H]androstenedione (specific activity 1.48-2.22 TBq/mmol, supplied by NEN, Boston, USA). Aromatase activity was expressed as the amount of oestrogens (E) formed by each pair of ovaries per hour.

LH-hypersecreting rats

Adult female Sprague-Dawley rats (CD, supplied by Charles River), weighing 125–150 g, were ovariectomised under light ether anaesthaesia; 10 days later they were treated once daily for 10 consecutive days with the test compounds. In the first experiment, exemestane was given either subcutaneously (3 and 10 mg/kg per day) or orally (30 and 100 mg/kg per day). In a further experiment, subcutaneous doses of 10 and 50 mg/kg per day of both exemestane and MDL 18962 were compared to 50 mg/kg per day of 4-OHA, or to 1 and 5 mg/kg per day of testosterone propionate. 4 h (first experiment) or 6 h (second experiment) after the last drug dose, rats were killed by decapitation and blood collected for serum LH determination.

Oestradiol radioimmunoassay

Oestradiol plasma levels were analysed in duplicate, after ether extraction, with the radioimmunoassay kit ER-155, supplied by Baxter, Düdingen, Switzerland. The plasma sample (0.5 ml) was extracted twice by shaking with 1.5 ml of diethylether (Merck). Crossreaction of the inhibitors with the antibody was

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Table 1. Effect of exemestane, atamestane and MDL 18962 (10 mg/kg subcutaneously) on plasma oestradiol levels and microsomal ovarian aromatase in PMSG-stimulated rats

Treatment	No. of rats	Plasma oestradiol (% of controls)	Ovarian aromatase: pmol oestrogens/pair of ovaries/h	Ovarian aromatase: % of control value
Vehicle	15	100.0 (17.0)*	22.0 (2.2)	100
Exemestane	10	27.9 (6.5)†	9.6 (1.4)†	43.6
Atamestane	11	49.3 (14.1)§	21.1 (2.0)	96.0
MDL 18962	11	30.7 (5.6)†	9.2 (1.0)†	41.8

Mean (S.E.).
*The mean value of the plasma oestradiol level of the vehicle-treated group was 792 pg/ml.
§$P < 0.05$, † < 0.01 vs. vehicle-treated group (Dunnett's test).

found negligible, i.e. less than 0.00002% for exemestane and atamestane and approximately 0.0001% for MDL 18962. The sensitivity of the assay was 8 pg/ml.

Prolactin and LH radioimmunoassay

Prolactin and LH were assayed by a double antibody radioimmunoassay, using reagents supplied by the National Pituitary Agency (Baltimore, Maryland). Results are expressed as ng/ml NIADDK-Rat PRL RP-3 (PRL = prolactin) or NIADDK-Rat LH RP−2. ^{125}I-PRL (specific activity, 740–1850 kBq/mg) was supplied by NEN, and ^{125}I-LH was obtained by iodination with the chloramine T method. The sensitivity of the assay was 0.6 ng/ml for prolactin and 0.2 ng/ml for LH.

RESULTS

Effect on plasma oestradiol levels and on ovarian aromatase activity

The effects of exemestane, atamestane and MDL 18962 on plasma oestradiol levels and microsomal ovarian aromatase activity in PMSG-primed prepuberal rats are presented in Table 1. After a single dose of 10 mg/kg subcutaneously, all three compounds were effective in reducing plasma oestradiol levels as measured 6 h after treatment. Ovarian aromatase activity was significantly reduced in the animals treated with exemestane and MDL 18962, but not in rats treated with atamestane.

Antitumour activity

As shown in Table 2, exemestane given subcutaneously was clearly effective in reducing tumour growth. In fact, this compound induced 30% tumour regressions (20% CR + 10% PR) at the dose of 10 mg/kg per day, and 73% (55+18) at 50 mg/kg per day in comparison with 8% (0+8) spontaneous tumour regressions seen in the control group. The number of new tumours that appeared during the treatment period was also markedly lower in both exemestane-treated groups. Subcutaneous treatment with this compound was also associated with a clear increase in body weight gain over controls at both doses. Atamestane and MDL 18962, at doses of 10 and 50 mg/kg per day subcutaneously by the same treatment schedule as for exemestane, had no effect on tumour growth nor on body weight (Table 2). Oral doses of 100 and 200 mg/kg per day exemestane did not appreciably affect tumour growth and the number of new tumours was reduced only at the higher dose (Table 2). Additionally, oral treatment with this compound was not associated with detectable changes in body weight gain compared to controls.

Effect on ovarian aromatase activity (OAA) and on serum LH and prolactin levels

OAA and serum hormone levels were evaluated in tumour-bearing rats killed 4 h after the last drug dose. As shown in Table 3, exemestane given subcutaneously at 10 mg/kg per day inhibited total OAA by 85%, whereas at the 50 mg/kg per day dose level OAA inhibition was 93%. Atamestane had no effect on OAA at both subcutaneously dose levels tested, whereas MDL 18962 significantly reduced OAA only at 50 mg/kg per day (59% inhibition). Exemestane was also effective in inhibiting OAA when given orally, since at 100 and 200 mg/kg per day OAA was reduced by 72% and 74%, respectively. Serum LH and PRL levels are reported in Table 3. Since animals were killed independently of the oestrous cycle phase, a wide variability for both hormones was observed. A decrease in serum LH levels was evident in animals treated subcutaneously with exemestane, at doses of 10 (37% inhibition) and 50 mg/kg per day (45% inhibition), and with 50 mg/kg per day MDL 18962 (41% inhibition). No significant changes in serum LH levels were observed with oral exemestane, whereas a significant increase was caused by atamestane at 50 mg/kg per day subcutaneously.

Serum prolactin levels were very variable in all groups, and there was no significant difference between treated and control groups.

Effect on endocrine organ weights

The relative weights of pituitary, ovaries, adrenals and uterus of tumour-bearing rats measured at the end of the 4-week treatment are presented in Fig. 1. Exemestane given subcutaneously at 10 mg/kg per day significantly reduced pituitary, ovary and uterus weights; at 50 mg/kg per day this compound also reduced adrenal weight, whereas the effect on uterine weight was no longer evident. Atamestane had no detectable effects on organ weights, and MDL 18962 significantly reduced uterus weight at both subcutaneous dose levels and pituitary weight only at the highest dose employed. Oral exemestane, at 100 and 200 mg/kg, reduced pituitary and uterus weights.

Effect on serum LH levels in ovariectomised rats

Because of the variability in the concentrations of serum LH in cycling, tumour-bearing rats, the possible LH-lowering effect of exemestane and MDL 18962 was further investigated in rats with LH hypersecretion induced by ovariectomy. In the first experiment, the subcutaneous administration of exemestane at the dose of 10 mg/kg per day caused a significant decrease of LH levels (46% inhibition), while no effect was observed with oral exemestane at doses up to 100 mg/kg per day (Table 4). In a further experiment, 50 mg/kg exemestane per day subcutaneously reduced serum LH levels by 75%, whereas the same dose of MDL 18962 or 4-OHA caused a reduction of serum LH by 31% and 65%, respectively. As a comparison, testosterone propionate reduced serum LH levels by 92% at the subcutaneous dose of 1 mg/kg per day.

DISCUSSION

Results here presented show that exemestane, MDL 18962 and atamestane significantly reduced plasma oestradiol levels 6 h after subcutaneous dosing at 10 mg/kg in PMSG-primed prepuberal rats. Exemestane and MDL 18962 were also effective

Table 2. Effect of exemestane, atamestane and MDL 18962 on DMBA-induced mammary tumours in rats

Treatment	Dose* (mg/kg per day)	Rats	Tumours† evaluated	Effect at end of treatment CR	PR	NC	P	New tumours/ rat	Body weight gain (g)
Vehicle									
Subcutaneously/ orally	–	10	13	0(0)†	1(8)†	2(15)†	10(77)†	1.5	24(1)‡
Exemestane									
Subcutaneously	10	9	10	2(20)	1(10)	2(20)	5(50)	0.2	53(4)
	50	9	11	6(55)	2(18)	1(9)	2(18)	0.1	62(4)
	100	10	14	0(0)	1(7)	6(43)	7(50)	1.4	30(5)
Orally	200	10	15	0(0)	0(0)	1(7)	14(93)	0.6	30(4)
Atamestane									
Subcutaneously	10	9	16	1(6)	2(12)	4(25)	9(57)	1.8	22(8)
	50	9	14	0(0)	0(0)	4(28)	10(72)	3.0	38(2)
MDL 18962									
Subcutaneously	10	9	13	0(0)	0(0)	1(8)	12(92)	3.2	25(4)
	50	9	12	0(0)	1(8)	1(8)	10(84)	1.4	36(4)

*The compounds were administered twice a day, 6 days a week for 4 weeks.
CR = complete remission, PR = partial remission, NC = no change, P = progression.
†No. (%), ‡mean (S.E.).

Table 3. Effect of exemestane, atamestane and MDL 18962 on ovarian microsomal aromatase and serum LH and prolactin levels

Treatment	Dose† (mg/kg per day)	Rats	Ovarian aromatase‡ (pmol E/ovaries/h)	Serum LH (ng/ml)	Serum prolactin (ng/ml)
Vehicle					
Subcutaneously	–	10	2.69(0.24–18.79)*	0.49(0.23–0.94)	18.3(3.2–295)
Exemestane					
Subcutaneously	10	9	0.39(<0.05–1.49)‡	0.31(0.24–0.50)	9.1(4.0–53.6)
	50	9	0.18(<0.05–0.75)‡	0.27(0.21–0.32)‡	18.7(5.5–56.5)
Orally	100	10	0.75(<0.05–2.90)†	0.38(0.24–1.24)	9.3(2.8–247)
	200	10	0.70(0.07–4.67)‡	0.38(0.23–0.63)	8.9(1.4–205)
Atamestane					
Subcutaneously	10	9	3.78(<0.05–10.81)	0.46(0.27–0.94)	65.4(4.4–>320)
	50	9	4.51(1.72–7.42)	0.78(0.65–16.66)†	15.0(6.7–>320)
MDL 18962					
Subcutaneously	10	9	2.03(<0.05–4.92)	0.51(0.31–10.69)	28.0(4.3–>320)
	50	9	1.10(<0.05–2.45)‡	0.29(0.21–0.71)	20.7(2.3–>320)

*Median (range).
†$P < 0.05$, ‡$P < 0.01$ vs. vehicle-treated group (Dunn's test).

in reducing ovarian aromatase activity in these animals, whereas atamestane was ineffective in these conditions. Atamestane has previously been shown by Henderson *et al.* [12] to reduce serum oestradiol levels in a similar model in juvenile rats, at subcutaneous doses of 1–3 mg/animal; however, its effect on ovarian aromatase has not been reported. Our results on the ineffectiveness of atamestane to influence ovarian aromatase activity could be explained by the slow enzyme inactivation rate ($t_{1/2}$ 64 min) of this compound, as described by Henderson *et al.* [7], and by the possibility that *in vivo* this compound acts mainly as a competitive, reversible inhibitor of aromatase. *In vivo* ovarian aromatase inactivation by exemestane and MDL 18962 has already been reported [8, 16].

As regards the antitumour activity studies, we tested the compounds in the DMBA-induced mammary tumour model at subcutaneous doses equal (10 mg/kg) and five times higher (50 mg/kg) than those effective in lowering plasma oestradiol levels in PMSG-primed rats. In addition, exemestane was also administered orally, at doses of 100 and 200 mg/kg since the compound has previously been reported to be slightly effective at 100 mg/kg in this tumour model [14], although its aromatase inhibitory potency by the oral route was only two times lower

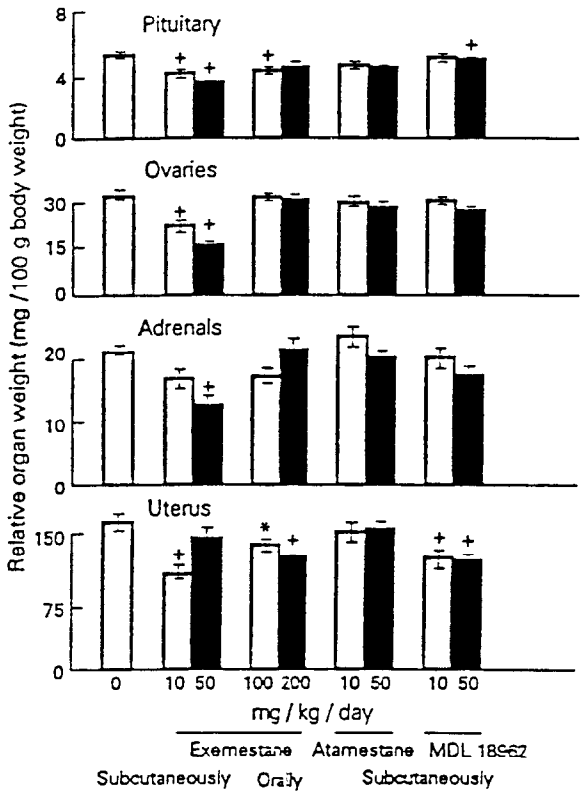

Fig. 1. Effect of exemestane, atamestane and MDL 18962 on relative organ weights of rats with DMBA-induced mammary tumours. Bars represent mean (S.E.) (9–10 rats per group).
***P* < 0.05 and +*P* < 0.01 vs. controls (Dunn's test).**

than after subcutaneous dosing in PMSG-primed rats [8]. Results here presented show that the subcutaneous administration of exemestane to DMBA-induced tumour-bearing rats was associated not only with potent inhibition of ovarian aromatase but also with marked inhibitory effects on the growth of established tumours and with a clear preventive effect on the appearance of new tumours during the treatment period.

Conversely, neither subcutaneous MDL 18962 and atamestane, nor oral exemestane were found to affect DMBA-induced tumour growth in intact rats. Ovarian aromatase activity of tumour-bearing rats was reduced by the higher subcutaneous dose of MDL 18962, whereas atamestane was ineffective at both doses, thus confirming the results previously obtained in PMSG-primed rats. Oral exemestane reduced ovarian aromatase activity by 72–74%, as previously shown in the same tumour model and in PMSG-primed adult rats [8, 14]. Serum LH levels in tumour-bearing rats were reduced at both subcutaneous exemestane doses and at the higher dose of MDL 18962, whereas oral exemestane was completely ineffective in this regard. Atamestane slightly increased serum LH levels at 50 mg/kg per day subcutaneously, while it was ineffective at the lower dose. The relative ovarian weight was significantly reduced only after subcutaneous exemestane, in agreement with the more marked reduction of LH.

To further characterise the possible direct effect of exemestane and MDL 18962 on LH secretion, and to avoid the influence of circulating oestrogens on the feedback mechanism as in intact DMBA-treated rats, the compounds were given to ovariectomised rats. A marked reduction of ovariectomy-induced LH hypersecretion was observed with subcutaneous exemestane treatment, and a comparable inhibition was shown with MDL 18962 but only at approximately 5-fold higher doses. No effect was conversely observed with oral exemestane, thus confirming the results observed in intact rats. As a comparison, the effect of the subcutaneous 50 mg/kg per day dose of exemestane (75% inhibition) was similar to that caused by the same dose of 4-OHA (65% inhibition), but less marked than that observed with 1 mg/kg per day testosterone propionate (92% inhibition). The direct inhibitory effect on LH secretion by subcutaneous exemestane, which is likely due to its slight androgenic effect [11], may contribute to its efficacy in causing regression of DMBA-induced mammary tumours in rats, as has already been shown for 4-OHA [3, 4]. The ineffectiveness of oral exemestane in lowering LH levels is in agreement with the very low androgenic activity by this administration route (D.G. *et al.*), whereas its aromatase inhibitory effect was previously observed in PMSG-primed rats after both subcutaneous and oral dosage [8]. Thus, in intact DMBA tumour-bearing rats, aromatase inactivation by oral exemestane could be partly overcome by a compensatory feedback mechanism on gonadotropin secretion. However, the residual ovarian aromatase activity in these orally treated rats could possibly maintain enough oestrogen synthesis to sustain tumour growth, though it would be inadequate to maintain uterus weight which was in fact significantly reduced. Uterus weight was also reduced at the lower subcutaneous dose of exemestane, whereas no effect was observed at the higher dose, possibly because of a direct "androgenic" stimulation of the uterus after this administration route, as already described also for 4-OHA [4].

Atamestane has been shown by Nishino *et al.* [17, 18] to cause, at 30–150 mg/kg per day subcutaneously, a slight inhibi-

Table 4. Effect of treatment with exemestane, MDL 18962, 4-OHA and testosterone propionate on serum LH levels in ovariectomised rats

Experiment	Treatment	Dose (mg/kg per day)	Serum LH (ng/ml)
1	Vehicle		
	Subcutaneously/orally	–	6.7(0.7)*
	Exemestane		
	Subcutaneously	3	5.5(0.5)
		10	3.6(0.5)†
	Orally	30	6.6(0.5)
		100	6.7(0.5)
2	Vehicle		
	Subcutaneously	–	8.0(0.9)
	Exemestane		
	Subcutaneously	10	5.3(0.6)†
		50	2.0(0.3)†
	MDL 18962		
	Subcutaneously	10	7.5(0.4)
		50	5.5(0.4)†
	4-OHA		
	Subcutaneously	50	2.8(0.6)†
	Testosterone propionate		
	Subcutaneously	1	0.6(0.1)†
		5	0.5(0.1)†

*Mean (S.E.) (6–7 rats per group).
†$P < 0.01$ vs. vehicle treated group (Dunn's test).

tory effect on tumour growth and an increase of serum LH levels in intact DMBA-treated rats. Data reported here show that atamestane up to 50 mg/kg per day subcutaneously was not effective on tumour growth and on ovarian aromatase activity, and did not change any endocrine organ weight. An increase in serum LH levels was observed at the higher dose, probably related to reduction of oestrogen biosynthesis, as also suggested by Nishino *et al.* [18]. In addition, and in contrast with subcutaneous exemestane, atamestane has been reported to have no androgenic or antigonadotrophic activity in rats [12]. A significant reduction of DMBA tumour growth in rats has been reported by Puett *et al.* [16] with MDL 18962 given subcutaneously at 50 mg/kg per day for 2 weeks. In contrast, we have not found any antitumoral activity for this compound given subcutaneously up to 50 mg/kg per day either at 2 weeks (data not shown) or at the end of the 4-week treatment regimen. The basis for this discrepancy remains at the moment unexplained. Though not effective on tumour growth, MDL 18962 caused a decrease in ovarian aromatase activity, uterine weight and serum LH levels at 50 mg/kg per day in the same animals. In addition, MDL 18962 was found to decrease LH hypersecretion in ovariectomised rats at 50 mg/kg per day, an effect possibly related to its slight androgenic activity [10]. However, the antigonadotrophic effect of MDL 18962 in both intact DMBA-treated and ovariectomised rats was less marked than that of subcutaneous exemestane.

In conclusion, taken collectively, results reported here suggest that steroidal compounds which are "pure" irreversible aromatase inhibitors, i.e. lacking any intrinsic antigonadotrophic activity, such as atamestane and oral exemestane, can have low or no antitumour effect in a "premenopausal" tumour model in intact rats, where feedback mechanisms can overcome drug-induced enzyme inhibition. As mentioned, the tumour model investigated here can be regarded as representative of the premenopausal status but not of the postmenopausal condition. In postmenopause, oestrogens are mainly produced by peripheral aromatisation (e.g. in adipose tissue and skeletal muscle), which is not regulated by gonadotropins [19]. An experimental "postmenopausal" breast cancer model in rats is represented by the DMBA-induced tumour in ovariectomised rats treated with testosterone propionate or 19-hydroxy-testosterone, compounds that being peripherally aromatised to oestrogens, sustain tumour growth. Tested in the latter experimental model, exemestane, by both the subcutaneous and the oral route [20], and subcutaneous atamestane [17] were shown to be very effective in reducing tumour growth. This finding indicates that in a tumour model where only peripheral aromatisation is involved, specific inhibition of this enzymatic complex can result in significant antitumour activity.

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