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Effect of the irreversible aromatase inhibitor FCE 24304 on DMBA-induced mammary tumors in ovariectomized rats treated with testosterone

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Summary. The antitumor activity of the irreversible aromatase inhibitor FCE 24304 (6-methylenandrosta-1,4-diene-3,17-dione) was studied in ovariectomized, testosterone propionate (TP)-treated rats with 7,12-dimethylbenzanthracene (DMBA)-induced mammary tumors that were used as a postmenopausal tumor model. When given s.c. at 20 mg/kg per day, 3 days a week for 4 weeks, TP was effective in maintaining tumor growth in ovariectomized rats (51% tumor regression in control animals vs 94% in ovariectomized rats). FCE 24304 given s.c. twice daily at 100 mg/kg per day, 6 days a week for 4 weeks, induced 96% regression, thus inhibiting the growth-promoting effect of TP. When the effect of various doses of FCE 24304 was evaluated, in comparison with a 52% tumor regression rate in control (ovariectomized, TP-treated) rats, tumor regressions amounted to 88% and 96% at s.c. FCE 24304 doses of 10 and 50 mg/kg per day, respectively, and to 76%, 88%, and 81% at oral doses of 10, 50, and 100 mg/kg per day, respectively. When FCE 24304 was given alone to ovariectomized rats, it did not affect ovariectomy-induced tumor regression (87% vs 94%). In conclusion, FCE 24304 was effective by both the s.c. and oral routes against DMBA-induced mammary tumors in ovariectomized TP-treated rats, a postmenopausal mammary tumor model.

Introduction

FCE 24304 (6-methylenandrosta-1,4-diene-3,17-dione) is a novel irreversible aromatase inhibitor [3, 7]. When given s.c., the compound was shown to be highly effective against 7,12-dimethylbenzanthracene (DMBA)-induced mammary tumors in intact cycling rats, with a positive correlation between its antitumor activity and the decrease in ovarian aromatase activity [17]. In this tumor model, which simulates the endocrine characteristics of premenopausal women, ovarian aromatization is the main source of circulating estrogens. However, aromatization occurs not only in the ovaries but also in peripheral tissues [10]; this extraovarian synthesis increases after menopause and becomes the main source of estrogens [9].

In the present study, we further investigated the antitumor effect of FCE 24304 given either s.c. or orally in a postmenopausal tumor model [8]. The model is based on the observation that testosterone administration can prevent the regression of DMBA-induced tumors in ovariectomized rats. This effect has been ascribed to the peripheral conversion of androgens to estrogens; in support of this contention, it has been shown that this testosterone-induced tumor growth can be fully counteracted by aromatase inhibitors [8]. The possible influence of FCE 24304 alone on ovariectomy-induced tumor regression was also evaluated.

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Materials and methods

Animals. Female Sprague-Dawley (IOPS-OFA) rats were supplied by Iffa-Credo, France. The animals were housed 4–5 per cage and maintained in an air-conditioned room with controlled temperature (23° C) and light (12 h light from 7:00 a.m. to 7:00 p.m.).

Mammary tumor model. At 50–54 days of age, rats were dosed intragastrically with 20 mg DMBA (Sigma Chemical Co.) dissolved in sesame oil (1 ml/rat). Starting 40 days after DMBA treatment, animals were examined weekly by palpation; when at least one tumor measuring 1 cm in diameter was found, the rats were ovariectomized while under ether anesthesia and placed sequentially into experimental groups (10–19 rats/test group). Animals with no tumors by day 150 were discarded. The two perpendicular tumor axes were measured with calipers twice weekly during treatment. Tumor weight was calculated according to the formula $d^2 \times D/2$, where d is the minimal and D, the maximal diameter [6]. At the end of the treatment period, tumor response to the drug was designated as CR (complete remission, disappearance of the tumor), PR (partial remission, >50% reduction in tumor weight), NC (no change, <50% increase or decrease) or P (progression, >50% increase).

Maintenance of tumor growth. Testosterone propionate (TP) (Sigma Chemical Co.) was given at 20 mg/kg per day, 3 days/week for 4 weeks, starting 2 days after ovariectomy. The compound, dissolved in benzyl alcohol and diluted in sesame oil, was given s.c. in a volume of 2 ml/kg body wt.

Drug and treatment schedule. FCE 24304 (Farmitalia Carlo Erba, Milano, Italy) was dissolved in benzyl alcohol and diluted in sesame oil when given s.c. or suspended in 0.5%

Table 1. Effect of testosterone propionate (TP) alone or combined with FCE 24304 in ovariectomized rats bearing DMBA-induced mammary tumors

Treatment:		Rats (*n*)	Tumors[c] (*n*)	Effect at the end of treatment:					BWC (g)
TP[a]	FCE 24304[b]			Number (%) of				New tumors/ rat	
				CR	PR	NC	P		
–	–	19	39	26 (66)	11 (28)	1 (3)	1 (3)	0	+56±3
+	–	19	41	14 (34)	7 (17)	9 (22)	11 (27)	0.5	+36±2
+	+	11	23	18 (78)	4 (18)	1 (4)	0 (0)	0.1	+60±2

[a] TP was given s.c. at a dose of 20 mg/kg per day, 3 days/week for 4 weeks, starting 2 days after ovariectomy
[b] FCE 24304 was given s.c. twice daily at a dose of 100 mg/kg per day, 6 days/week for 4 weeks, starting 2 days after ovariectomy
[c] Tumors were induced by a single gastric intubation of 20 mg DMBA in 50- to 54-day-old female Sprague-Dawley rats
CR, complete remission; PR, partial remission (reduction in tumor weight of >50% of the initial tumor weight); NC, no change (increase or decrease in tumor weight, <50%); P, progression (increase in tumor weight, >50%); BWC, mean body wt. change ± SE (difference between intitial and final body wt.)

methocel containing 0.4% Tween-80 when given orally. Treatments were given twice daily (10:00 a.m. and 4:00 p.m.), 6 days/week for 4 weeks. The compound was given s.c. in a volume of 2 ml/kg body wt. and orally in a volume of 5 ml/kg. Treatments started 2 days after ovariectomy.

Results

Effect of FCE 24304 in ovariectomized, tumor-bearing rats treated with TP

In the first experiment, the ability of TP to maintain tumor growth in ovariectomized rats bearing DMBA-induced tumors was investigated and the effect of FCE 24304 was preliminarily studied under these experimental conditions. Ovariectomy alone resulted in 94% (66% CR+28% PR) tumor regression (Table 1). TP partially prevented ovariectomy-induced tumor regression; in fact, in this group 51% (34% CR+17% PR) tumor regression was observed. This effect of TP was completely inhibited by FCE 24304 given s.c. at a high dose (100 mg/kg daily), as shown by the finding that tumor regression in this group amounted to 96% (78% CR+18% PR). Table 1 also shows that the number of new tumors (i.e., tumors that appeared during the treatment period) was higher in the ovariectomized TP-treated group than in animals treated by ovariectomy alone, and this effect was also antagonized by FCE 24304. TP treatment slightly reduced the gain in body weight observed in ovariectomized rats; combined treatment with TP and FCE 24304 did not affect body weight.

The effect of FCE 24304 given s.c. or orally at different dose levels in ovariectomized TP-treated rats with DMBA-induced tumors is shown in Table 2. In confirmation of the results of the previous experiment, TP maintained tumor growth in ovariectomized rats, as shown by 52% (14% CR+38% PR) tumor regression compared with 86% (60% CR+26% PR) in the ovariectomized control group. When given s.c., FCE 24304 was effective at both doses tested, with tumor regression amounting to 88% (48% CR+40% PR) at a dose of 10 mg/kg per day and 96% (81% CR+15% PR) at 50 mg/kg per day. The compound was also effective orally, inducing 76% (60% CR+16% PR) tumor regression at 10 mg/kg per day, 88% (46%+42% PR) at 50 mg/kg daily, and 81% (27% CR+54% PR) at 100 mg/kg per day. The number of new tumors was reduced in all FCE 24304-treated groups. The body wt. increase seen in the 4-week treatment period indicated a slight anabolizing effect for FCE 24304 only after s.c. dosing.

Table 2. Effect of FCE 24304 in ovariectomized rats bearing DMBA-induced mammary tumors that were treated with testosterone propionate (TP)

Treatment:		Rats (*n*)	Tumors[c] (*n*)	Effect at the end of treatment:					BWC (g)
TP[a]	FCE 24304[b] (mg/kg per day)			Number (%) of				New tumors/ rat	
				CR	PR	NC	P		
–	–	10	15	9 (60)	4 (26)	1 (7)	1 (7)	0	+70±5
+	–	13	21	3 (14)	8 (38)	0 (0)	10 (48)	0.6	+50±3
+	10 s.c.	14	25	12 (48)	10 (40)	0 (0)	3 (12)	0	+86±4
+	50 s.c.	17	26	21 (81)	4 (15)	0 (0)	1 (4)	0.1	+80±4
+	10 p.o.	14	25	15 (60)	4 (16)	5 (20)	1 (4)	0.2	+53±3
+	50 p.o.	15	26	12 (46)	11 (42)	2 (8)	1 (4)	0.3	+55±4
+	100 p.o.	15	26	7 (27)	14 (54)	1 (4)	4 (15)	0.1	+61±3

[a] TP administration as shown in footnote to Table 1
[b] FCE 24304 was given twice a day, 6 days/week for 4 weeks, starting 2 days after ovariectomy
[c] Tumors were induced as shown in footnote to Table 1

Table 3. Effect of FCE 24304 alone in ovariectomized rats bearing DMBA-induced mammary tumors

FCE 24304 (mg/kg per day)	Rats (*n*)	Tumors[c] (*n*)	Effect at the end of treatment:					BWC (g)
			Number (%) of				New tumors/ rat	
			CR	PR	NC	P		
0	11	17	13 (76)	3 (18)	0 (0)	1 (6)	0	+ 49 ± 5
50 s.c.	11	16	13 (82)	1 (6)	1 (6)	1 (6)	0	+ 56 ± 4

FCE 24304 administration and tumor induction were carried out as shown in footnote to Table 1

Effect of FCE 24304 alone in ovariectomized, tumor-bearing rats

To investigate the possible influence of FCE 24304 on ovariectomy-induced tumor regression, the compound was given alone to ovariectomized rats at the s.c. dose of 50 mg/kg per day, 6 days/week for 4 weeks. The compound was ineffective in stimulating tumor growth. In fact, the percentage of tumor regression in the ovariectomized, FCE 24304-treated group was 87% (81% CR + 6% PR), a value comparable with the 94% (76% CR + 18% PR) incidence seen in the ovariectomized control group (Table 3). The compound had no effect on body weight under these experimental conditions.

Discussion

The present results show that the irreversible aromatase inhibitor FCE 24304 was highly effective by both the s.c. and oral routes against DMBA-induced mammary tumors in ovariectomized rats treated with TP, a postmenopausal tumor model.

In previous studies [18] using the DMBA-induced cycling tumor model in intact rats, FCE 24304 was highly effective when given s.c. but had little activity when given orally. The intact, tumor-bearing rat model can be considered to be a premenopausal model in which estrogens are mainly produced by the ovaries. The low antitumor activity of oral FCE 24304 in this model can reasonably be ascribed to a feedback compensatory increase in gonadotropins as a consequence of a decrease in estrogen synthesis, which can overcome aromatase inhibition through the stimulation of ovarian aromatase synthesis. FCE 24304 given s.c. showed weak androgenic activity [3] that could prevent feedback gonadotropin increase; however, such androgenic activity was several times lower after oral dosing (D. Giudici et al., unpublished results). Other aromatase inhibitors, such as aminoglutethimide [16] and SH 489 [13], have been reported to be only marginally effective in DMBA-induced tumors in intact rats, whereas 4-OH-androstenedione, a weakly androgenic aromatase inhibitor, had strong antitumor activity in this model [1].

Since estrogen synthesis in the postmenopausal condition is mainly due to peripheral extraovarian androgen aromatization (i.e., adipose tissue, skeletal muscle, mammary tissue) and peripheral aromatase does not appear to be regulated by gonadotropins [11], we decided to investigate the effectiveness of FCE 24304 in a postmenopausal tumor model.

To reproduce the condition of breast cancer in postmenopausal women, rats that had developed tumors were ovariectomized and TP was given to prevent ovariectomy-induced tumor regression. The ability of TP to maintain tumor growth in ovariectomized rats has been attributed to its conversion to estrogens through peripheral aromatization. An important site of aromatization is the hypothalamus [12], and in rats testosterone was shown to stimulate prolactin secretion after its hypothalamic conversion to estrogens [5, 14]. Since DMBA-induced tumors are estrogen- and prolactin-dependent, this prolactin increase could contribute to the restoration of tumor growth in ovariectomized, TP-treated rats. Peripheral aromatization might also occur in adipose tissue of rats, since there is some evidence of increased aromatization of testosterone in obese rats [4]. In addition, aromatase activity has been detected in human breast-cancer tissue [15], and local estrogen production might also be important for tumor growth in this tumor model. The TP-stimulated tumor growth in ovariectomized rats can be fully inhibited by aromatase inhibitors [8]. A similar tumor-growth-promoting effect is described for 19-hydroxy-testosterone, and aromatase inhibitors have been reported to counteract this effect [13].

The present findings confirm that TP can partially prevent ovariectomy-induced tumor regressions in the DMBA-induced mammary tumor model. In the two experiments reported, ovariectomy caused 94% and 86% tumor regression, which TP reduced to 51% and 52%. In this model FCE 24304 proved to be highly effective by both the s.c. and oral routes. In fact, at doses ranging from 10 to 100 mg/kg per day, FCE 24304 induced 88%–96% and 76%–88% tumor regression by the s.c. and oral routes, respectively, compared with 52% tumor regression in control (ovariectomized, TP-treated) rats. These results are in accordance with data obtained in the aromatase inactivation test in pregnant mare's serum gonadotropin (PMSG)-pretreated rats; under these conditions, a single dose of FCE 24304 was effective by both the oral (ED_{50}, 3.7 mg/kg) and the s.c. route (ED_{50}, 1.8 mg/kg) [3].

FCE 24304 given alone did not affect ovariectomy-induced tumor regression. These data confirm that FCE 24304 has no intrinsic estrogenic effect [3] that could result in the stimulation of tumor growth in ovariectomized rats previously described for tamoxifen [2]. In addition, this novel, steroidal, irreversible aromatase inhibitor does not inhibit desmolase (cholesterol side-chain cleavage enzyme), even at very high concentrations (E. Di Salle et al., unpublished results), in contrast to aminoglutethimide. The selectivity and potency of FCE 24304 may therefore offer a therapeutic advantage in the management of postmenopausal breast-cancer patients.

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