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# Antitumor activity of the aromatase inhibitor FCE 24928 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 24928 (4-aminoandrosta-1,4,6-triene-3,17-dione) was studied in ovariectomized and testosterone propionate (TP)-treated rats bearing 7,12-dimethylbenzanthracene (DMBA)-induced mammary tumors. This experimental condition was used as a model of postmenopausal breast cancer. TP was given s.c. three times per week for 4 weeks at a dose of 20 mg/kg per day, a treatment that is effective in maintaining tumor growth in ovariectomized rats (89% growing tumors). FCE 24928 given s.c. twice daily 6 days/week for 4 weeks at doses of 10 and 30 mg/kg per day inhibited the tumor growth-promoting effect of TP as shown by 81% and 80% tumor-regression rates. When FCE 24928 was given at various dose levels either s.c. (3, 10, and 30 mg/kg daily) or orally (10, 30, and 100 mg/kg per day), tumor regressions were observed at all doses, amounting to 63%–93% and 63%–72%, respectively. In addition, FCE 24928 given alone (30 mg/kg daily s.c.) to ovariectomized rats did not affect ovariectomy-induced tumor regression. In conclusion, following both s.c. and oral administration, FCE 24928 was effective against DMBA-induced mammary tumors in ovariectomized TP-treated rats, a postmenopausal mammary tumor model.

## Introduction

FCE 24928 (4-aminoandrosta-1,4,6-triene-3,17 dione) is a novel irreversible aromatase inhibitor [12]. This compound induces a time-dependent inhibition of human placental aromatase ($t_{1/2}$, 4 min; $K_i$, 59 nM). In pregnant mare's serum gonadotropin-treated adult rats, microsomal ovarian aromatase activity was reduced at 24 h after FCE 24928 dosing by both the s.c. [effective dose for 50% reduction of aromatase ($ED_{50}$), 1.2 mg/kg] and the oral ($ED_{50}$, 14.1 mg/kg) routes. It has also been shown that this product is devoid of intrinsic androgenic activity, lacks significant binding affinity for androgen and estrogen receptors, and does not inhibit 5α-reductase or desmolase [12]. In recent years, the use of inhibitors of estrogen biosynthesis has become the focus of much attention as an alternative strategy for the treatment of postmenopausal breast cancer [8, 13]. In this connection it is relevant that in premenopausal women, ovarian aromatization is the main source of circulating estrogens, whereas under postmenopausal conditions, extraovarian tissues (e.g., adipose tissue, skeletal muscle, mammary tissue) are the main sites of aromatase activity [3].

In the present study we investigated the antitumor effect of FCE 24928 using a postmenopausal mammary tumor model in rats. The model is based on the observation that administration of testosterone can prevent the regression of 7,12-dimethylbenzanthracene (DMBA)-induced tumors seen after ovariectomy in rats. This effect has been ascribed to the peripheral conversion of androgens to estrogens; in fact, this testosterone-induced effect can be fully inhibited by aromatase inhibitors [6, 16]. We evaluated the antitumor activity of FCE 24928 given s.c. and p.o. in this tumor model and investigated the possible influence of this compound alone on the regression of tumors induced by ovariectomy in rats.

## Materials and methods

*Animals.* Female Sprague-Dawley (IOPS-OFA) rats were supplied by Iffa-Credo, France. The animals were housed at 4–5 per cage and were 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 an age of 50–54 days, rats were dosed intragastrically with 20 mg DMBA (Sigma Chemical Co.) dissolved in sesame oil (1 ml/rat). Starting at 40 days after DMBA treatment, animals were examined weekly by palpation; when at least one tumor measuring

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**Table 1.** Effect of FCE 24928 on DMBA-induced mammary tumors in ovariectomized rats treated with TP

| Group number | Treatment | | Rats (*n*) | Tumors[c] (*n*) | Effect at the end of treatment | | | | | BWG (g) |
|---|---|---|---|---|---|---|---|---|---|---|
| | TP[a] | FCE 24928[b] (mg/kg daily) | | | Number of responses | | | | New tumors/ rat | |
| | | | | | CR | PR | NC | PD | | |
| 1 | + | – | 10 | 18 | 0 ( 0) | 2 (11%) | 6 (33%) | 10 (56%) | 0.8 | 56 ± 3 |
| 2 | + | 10 s.c. | 9 | 16 | 8 (50%) | 5 (31%) | 3 (19%) | 0 ( 0) | 0.2 | 78 ± 4** |
| 3 | + | 30 s.c. | 10 | 20 | 12 (60%) | 4 (20%) | 1 ( 5%) | 3 (15%) | 0.1 | 80 ± 4** |
| 4 | – | – | 10 | 21 | 15 (71%) | 6 (29%) | 0 ( 0) | 0 ( 0) | 0 | 75 ± 4** |
| 5 | Intact rats | | 9 | 19 | 0 ( 0) | 2 (10%) | 3 (16%) | 14 (74%) | 1.1 | 6 ± 5[d] |

[a] TP was given s.c. 3 days/week for 4 weeks at a dose of 20 mg/kg per day starting 2 days after ovariectomy

[b] FCE 24928 was given twice daily 6 days/week for 4 weeks starting 2 days after ovariectomy

[c] Tumors were induced by a single gastric intubation of 20 mg DMBA into 50- to 54-day-old female Sprague-Dawley rats

[d] Not included in the statistical analysis

CR, Complete remission; PR, partial remission (reduction of >50% in the initial tumor weight); NC, no change (increase or decrease of <50% in the initial tumor weight); PD, progressive disease (increase of >50% in tumor weight); BWG, body weight gain (mean ± SE)

** $P < 0.01$ vs group 1 (Dunnett's test)

1 cm in diameter was found, the rats were ovariectomized using ether anesthesia and placed sequentially into experimental groups (9–10 rats/test group). Animals that failed to develop tumors by day 150 were discarded. The two perpendicular tumor axes were measured with calipers once weekly during treatment. Tumor weight was calculated according to the formula $d^2 \times D/2$, where $d$ is the minimal diameter and $D$, the maximal diameter [2]. Tumor growth in the control and treated groups was expressed as a percentage of the initial tumor weight, measured on the 1st day of treatment and taken as 100%. At the end of the treatment period, the response of each tumor 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 in tumor weight), or PD (progressive disease, >50% increase in tumor weight).

*Maintenance of tumor growth.* Testosterone propionate (TP; Sigma Chemical Co.) was given at 20 mg/kg three times per week (Monday, Wednesday, and Friday) for 4 weeks starting at 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 24928 (Farmitalia Carlo Erba, Milano, Italy) was dissolved in benzyl alcohol and diluted in sesame oil for s.c. injection or suspended in 0.5% Methocel (methylcellulose 400) containing 0.4% Tween 80 for oral administration. 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 were started at 2 days after ovariectomy.

*Experimental design.* For investigation of the antitumor activity of FCE 24928, two experiments were performed in ovariectomized and TP-supplemented rats. At 2 days after ovariectomy, animals were randomly allocated to receive TP plus vehicle (sesame oil containing 5% benzyl alcohol or 0.5% Methocel containing 0.4% Tween 80) or TP plus FCE 24928. In the first experiment, FCE 24928 was given s.c. at two dose levels; in the second experiment it was given s.c. or orally at three dose levels. Both of the experiments also included a group of ovariectomized rats that were not treated with TP. In addition, in the first experiment a group of intact, untreated animals was also included.

We also determined the effect of FCE 24928 on the regression of tumors induced by ovariectomy. At 2 days after ovariectomy, animals were randomly allocated into two groups receiving either the vehicle (sesame oil containing 5% benzyl alcohol) or FCE 24928 at a daily s.c. dose of 30 mg/kg.

## Results

### *Effect of FCE 24928 in ovariectomized tumor-bearing rats treated with TP*

Table 1 shows the effects of ovariectomy, TP treatment of ovariectomized rats, and combined treatment with TP and FCE 24928 of ovariectomized rats. Ovariectomy alone (group 4) resulted in a 100% (71% CR+29% PR) tumor-regression rate as compared with the 10% (0 CR+10% PR) value obtained in intact rats (group 5). In this experiment, TP treatment (group 1) almost completely prevented the regression of tumors induced by ovariectomy; in fact, only 11% (0 CR+11% PR) of the tumors regressed, a percentage similar to that observed in intact rats. The effect of TP was inhibited by FCE 24928 (groups 2 and 3) at the two doses tested; indeed, at a daily s.c. dose of 10 mg/kg, the compound caused 81% (50% CR+31% PR) of the tumors to regress, and 80% (60% CR+20% PR) regression rates were recorded following s.c. injections of 30 mg/kg per day. Table 1 also shows that the numbers of new tumors (i.e., tumors that appeared during the treatment period) were higher in the ovariectomized TP-treated group than in animals that had undergone ovariectomy alone and that the effect of TP on this parameter was also clearly inhibited by FCE 24928. The body weight gain induced by castration (group 4 vs group 5) was slightly reduced by TP treatment (group 1 vs group 4). Combined treatment with TP and FCE 24928 increased the body weight gain in comparison with TP alone.

In a second part of the study, the effect of FCE 24928 given either s.c. or orally over a broader dose range was explored. The results are shown in Table 2. Although the effect was not as marked as that observed in the previous experiment, TP maintained tumor growth in ovariectomized rats as demonstrated by a tumor-regression rate of 42% (32% CR+10% PR, group 1) as compared with the 79% (74% CR+5% PR) value obtained in the animals that had undergone ovariectomy alone (group 8). When given s.c., FCE 24928 was effective at the lowest dose tested; in fact, it resulted in tumor-regression rates of 78% (55% CR+23% PR) at 3 mg/kg per day, 63%

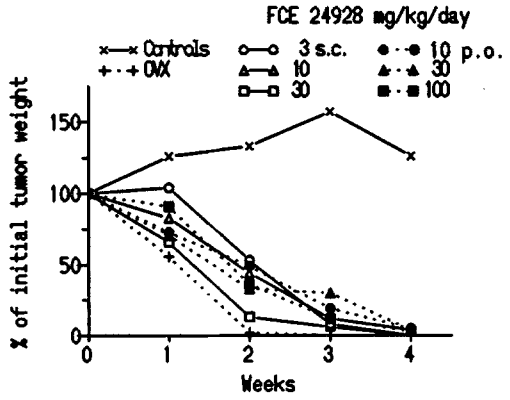


**Fig. 1.** Effect of various doses of FCE 24928 on the median weight (expressed as a percentage of the initial value) of DMBA-induced mammary tumors in ovariectomized rats treated with TP (17–40 tumors/group). *OVX*, ovariectomized rats that were not treated with TP. Weights significantly lower ($P < 0.01$, Kruskall-Wallis test) than the control values were observed beginning at week 1 in the OVX group, beginning at week 2 in rats receiving s.c. FCE 24928 at doses of 10 and 30 mg/kg, and beginning at week 3 in all other groups

(44% CR+19% PR) at 10 mg/kg per day, and 93% (60% CR+33% PR) at 30 mg/kg per day. This compound was also effective when given orally, producing tumor-regression rates of 63% (21% CR+42% PR) at a dose of 10 mg/kg per day, 72% (50% CR+22% PR) at 30 mg/kg per day, and 65% (47% CR+18% PR) at 100 mg/kg per day. TP treatment was not associated with the appearance of new tumors in this experiment. Rats receiving s.c. injections of FCE 24928 showed a slight increase in body weight gain in comparison with the ovariectomized TP-treated controls, whereas no change was observed following oral drug treatment.

Figure 1 illustrates the tumor growth (expressed as a percentage of the initial tumor weight) recorded during the 4-week treatment period in controls and treated groups. The results of the two experiments were combined. Subcutaneous treatment with FCE 24928 resulted in a significant inhibition of tumor growth starting at the 2nd (10 and 30 mg/kg daily) or 3rd (3 mg/kg daily) week of treatment. Oral FCE 24928 was also effective in suppressing tumor growth with a significant inhibition being observed beginning at week 3 in all of the treated groups.

### *Effect of FCE 24928 alone in ovariectomized tumor-bearing rats*

To exclude that treatment with FCE 24928 might be associated with hormonal activities (e.g., estrogenic) that may influence tumor growth, s.c. injections of the compound were given 6 days a week for 4 weeks at a daily dose of 30 mg/kg to rats that had been subjected to ovariectomy alone. It can be seen from Table 3 that the compound was ineffective in stimulating tumor growth. In fact, the percentage of tumor regression recorded in the ovariec-

**Table 2.** Effect of FCE 24928 on DMBA-induced mammary tumors in ovariectomized rats treated with TP

| Group number | Treatment | | Rats (*n*) | Tumors (*n*) | Effect at the end of treatment | | | | | BWG (g) |
|---|---|---|---|---|---|---|---|---|---|---|
| | TP | FCE 24928 (mg/kg daily) | | | Number of responses | | | | New tumors/ rat | |
| | | | | | CR | PR | NC | PD | | |
| 1 | + | – | 10 | 19 | 6 (32%) | 2 (10%) | 6 (32%) | 5 (26%) | 0 | 46±3 |
| 2 | + | 3 s.c. | 10 | 22 | 12 (55%) | 5 (23%) | 1 ( 4%) | 4 (18%) | 0 | 70±5** |
| 3 | + | 10 s.c. | 10 | 16 | 7 (44%) | 3 (19%) | 2 (12%) | 4 (25%) | 0.2 | 72±3** |
| 4 | + | 30 s.c. | 9 | 15 | 9 (60%) | 5 (33%) | 1 ( 7%) | 0 ( 0) | 0 | 61±6* |
| 5 | + | 10 p.o. | 10 | 19 | 4 (21%) | 8 (42%) | 4 (21%) | 3 (16%) | 0.2 | 45±2 |
| 6 | + | 30 p.o. | 10 | 18 | 9 (50%) | 4 (22%) | 3 (17%) | 2 (11%) | 0.1 | 42±4 |
| 7 | + | 100 p.o. | 10 | 17 | 8 (47%) | 3 (18%) | 4 (23%) | 2 (12%) | 0.2 | 44±2 |
| 8 | – | – | 10 | 19 | 14 (74%) | 1 ( 5%) | 3 (16%) | 1 ( 5%) | 0 | 56±3 |

TP and FCE 24928 administration and tumor induction were carried out as shown in the footnote to Table 1
* $P < 0.05$, ** $P < 0.01$ vs group 1 (Dunnett's test)

**Table 3.** Effect of FCE 24928 alone on DMBA-induced mammary tumors in ovariectomized rats

| FCE 24928 (mg/kg per day s.c.) | Rats (*n*) | Tumors (*n*) | Effect at the end of treatment | | | | | BWG (g) |
|---|---|---|---|---|---|---|---|---|
| | | | Number of responses | | | | New tumors/ rat | |
| | | | CR | PR | NC | PD | | |
| 0 | 10 | 19 | 14 (74%) | 1 (5%) | 3 (16%) | 1 (5%) | 0 | 56±3 |
| 30 | 10 | 18 | 14 (78%) | 3 (17%) | 0 (0) | 1 (5%) | 0 | 57±5 |

FCE 24928 administration and tumor induction were carried out as shown in the footnote to Table 1

tomized, FCE 24928-treated group was 95% (78% CR+17% PR) as compared with the 79% (74% CR+5% PR) value obtained in the animals that had undergone ovariectomy alone. Under these conditions, no effect on body weight gain was observed.

## Discussion

The results presented herein show that administration of the irreversible aromatase inhibitor FCE 24928 by the s.c. and oral routes was highly effective against DMBA-induced mammary tumors in ovariectomized rats treated with TP, a "postmenopausal" tumor model. This experimental model was employed in view of the observation that the DMBA-induced mammary tumor in intact rats, a "premenopausal" model, is not suitable for use in the evaluation of "pure" (e.g., devoid of androgenic activity) steroidal aromatase inhibitors [18]. In fact, the inhibition of ovarian estrogen synthesis in the intact rat may lead to increased serum gonadotropin levels via a feedback regulatory mechanism. This increase stimulates ovarian aromatase synthesis and therefore counteracts the effect of "pure" aromatase inhibitors. Following their s.c. administration, the irreversible aromatase inhibitors 4-hydroxyandrostenedione [14] and exemestane [17, 18] have been found to be highly effective in the "premenopausal" model, probably because their weak androgenic activity [11, 12, 15] prevents an increase in gonadotropins and therefore contributes to their antitumor activity. Conversely, compounds that lack intrinsic antigonadotropic activity, such as atamestane or oral exemestane, exhibit only slight, if any, antitumor activity in the "premenopausal" DMBA-induced tumor model [18].

Since the new irreversible aromatase inhibitor FCE 24928 is devoid of intrinsic androgenic and, consequently, antigonadotropic activity [12], we investigated its antitumor activity in the "postmenopausal" tumor model. In fact, estrogen synthesis under postmenopausal conditions is mainly due to peripheral, extraovarian (e.g., adipose tissue, skeletal muscle, mammary tissue) androgen aromatization, and peripheral aromatase does not appear to be regulated by gonadotropins [9]. To reproduce the conditions of postmenopausal breast cancer, we subjected tumor-bearing rats to ovariectomy and treated them with TP to prevent ovariectomy-induced tumor regression. The ability of TP to maintain tumor growth has been attributed to its conversion to estrogens through peripheral aromatization [6, 16]. The present findings confirm that TP can prevent ovariectomy-induced tumor regression in the DMBA-induced mammary tumor model; the very strong effect demonstrated for TP in the experiment presented in Table 1 (practically complete prevention of regression) is in our experience an unusual result, whereas its effect in the experiment shown in Table 2 (partial prevention of regressions) is more in accordance with previous findings [16].

In this study, FCE 24928 given by the s.c. and oral routes showed clear antitumor activity. Following its s.c. administration, the compound was effective starting at the lowest dose tested (3 mg/kg per day), causing 78% of the tumors to regress, and at the highest dose used (30 mg/kg per day) it produced a tumor-regression rate of 93% as compared with the control value of 42%. When given by the oral route, the compound was also effective at doses ranging from 10 to 100 mg/kg per day (63%–72% tumor-regression rates). In addition, to exclude that FCE 24928 might have intrinsic hormonal activity (e.g., estrogenic), we gave the compound alone to ovariectomized tumor-bearing rats. In this experiment, no stimulation of tumor growth was observed.

The selectivity and potency of FCE 24928 and its activity following oral administration render this compound an interesting candidate for clinical testing for the treatment of breast cancer in postmenopausal women. In addition, since increasing experimental and clinical data suggest that estrogens may be involved in the pathogenesis of benign prostatic hyperplasia [1, 4, 5, 7, 10], a compound that lacks intrinsic androgenic activity such as FCE 24928, could be a promising candidate for testing of the therapeutic potential of aromatase inhibitors in this disease.

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