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Effects of the Aromatase Inhibitor 7α-(4′-Amino)phenylthio-4-androstene-3,17-dione on 7,12-Dimethylbenz(a)anthracene-induced Mammary Carcinoma in Rats[1]

Robert W. Brueggemeier[2] and Pui-Kai Li

The College of Pharmacy and The OSU Comprehensive Cancer Center, The Ohio State University, Columbus, Ohio 43210

ABSTRACT

Inhibitors of aromatase, the cytochrome P-450 enzyme complex responsible for the biosynthesis of estrogens, may be useful therapeutic agents for the treatment of estrogen-dependent disease states such as breast and endometrial cancer. 7α-Substitution of androstenedione results in inhibitors of enhanced affinity for aromatase, with 7α-(4′-amino)phenylthio-4-androstene-3,17-dione (7α-APTA) exhibiting an apparent K_i of 18 nM and being among the most potent competitive inhibitors produced. The effects of this potent competitive 7α-substituted C_{19} aromatase inhibitor on reduction of the number and size of the 7,12-dimethylbenz(a)anthracene-induced mammary tumors in rats was investigated. Tumor-bearing rats receiving 25 or 50 mg 7α-APTA/kg/day demonstrated reductions in tumor volumes during the first week. Tumor volumes continued to decrease during the studies, resulting in tumor volume reductions of approximately 40 and 80%, respectively. Tumors in rats of the control group receiving only vehicle steadily increased in size during the studies. The tumor reductions in a 50-mg/kg/day-treated group were reversed by coadministration of 7α-APTA at 50 mg/kg/day and estradiol at 0.3 μg/kg/day for the last 3 weeks, indicating that the tumors were still responsive to estrogen. Plasma levels of estradiol were lower in the animals treated with 7α-APTA at the end of the treatments. Thus, 7α-APTA is effective in reducing tumor volumes in the estrogen-dependent 7,12-dimethylbenz(a)anthracene-induced mammary carcinoma rat model. These results encourage further development of these steroids as potential medicinal agents for the treatment of estrogen-dependent disease states such as breast and endometrial cancer.

INTRODUCTION

Aromatase is the cytochrome P-450 enzyme complex responsible for estrogen biosynthesis *in vivo*. Inhibitors of this enzyme complex may be useful in controlling reproductive processes and in treating estrogen-dependent disease states such as breast and endometrial cancer. These agents may be particularly effective in treating hormone-dependent breast cancer in postmenopausal patients since estrogen production would be suppressed by these agents in all tissues including peripheral sites. The therapeutic efficacies of aromatase inhibitors such as 4-hydroxyandrostenedione and aminoglutethimide are being investigated and these agents have been shown to cause regression of hormone-dependent breast tumors in both rats (1–3) and humans (4–6).

7α-Substitution of androstenedione results in inhibitors of enhanced affinity for aromatase (7–11), with 7α-APTA[3] being among the most potent competitive inhibitors produced to date (7). In microsomal enzyme preparations from human term placenta, these compounds exhibit apparent K_is ranging from 1 to 20 nM, with an apparent K_m for the substrate androstenedione of approximately 50 nM. 7α-APTA is also effective in inhibiting aromatase activity in MCF-7 human mammary cell culture, with 50% effective dose of 25 nM (12). These 7α-substituted steroids must be evaluated *in vivo* if a potential therapeutic agent is to be developed.

The administration of the carcinogen, DMBA, to young female rats results in the formation of estrogen-dependent mammary tumors (13). This has been one of the primary animal tumor models for studies of the biology and therapy of hormone-dependent tumors. Steroidal aromatase inhibitors have demonstrated effectiveness in reducing tumor volumes in this model (2, 3). The effects of the potent competitive 7α-substituted C_{19} aromatase inhibitor, 7α-APTA, on reducing the number and size of the DMBA-induced mammary tumors in rats are described here.

MATERIALS AND METHODS

Chemicals. Steroids were obtained from Steraloids (Wilton, NH) and checked for purity by melting point and thin layer chromatography. 7α-APTA and 4,6-ADD were prepared following the procedures of Brueggemeier *et al.* (7). 7,12-Dimethylbenz(a)anthracene was purchased from Aldrich Chemical Co., Milwaukee, WI. Estradiol radioimmunoassays were performed by the Department of Obstetrics and Gynecology, College of Medicine, Ohio State University.

Animals. Female Sprague-Dawley rats (50–60 days old) were purchased from Harlan Industries, Inc., Cumberland, IN. Animals were housed in metal cages containing ground corncob (Anderson's, Maumee, OH), provided Purina laboratory chow and water *ad libitum*, and maintained in an American Association for Accreditation of Laboratory Animal Care-accredited animal facility with a 12-h alternating light/dark cycle.

Induction of Tumors. Female rats (Sprague-Dawley, 50 days old) were gavaged with 20 mg of DMBA in 2 ml of sesame oil per rat (13). Each week the animals were examined for the appearance of tumors and any tumors present were measured with calipers. Rats were selected for the study when at least one tumor had a diameter of 2 cm, which was about 4 months after the administration of DMBA. The tumor volume was calculated using the equation

$$v = (4/3)\pi r_1^2 r_2$$

where r_1 is the minor radius. Prior to treatment, rats were divided into groups consisting of animals (6 or 7) with the same number of tumors per rat and the same average tumor volume. Average tumor volumes per group at the beginning of treatments ranged from 2.44 ± 0.99 (SD) cm^3 to 4.48 ± 1.94 cm^3.

Treatment with Aromatase Inhibitor. 7α-APTA was examined at doses of 25 and 50 mg/kg rat/day. The compound was dissolved in sesame oil (0.5 ml/injection) and each rat was given a s.c. injection daily. The rats were weighed twice a week and the number and volume of the tumors present were determined over a 6-week period. Another group of tumor-bearing rats received 4,6-androstadiene-3,17-dione (4,6-ADD) at a dose of 50 mg/kg rat/day. Rats in the control group received only sesame oil (0.5 ml daily).

Cotreatment with Aromatase Inhibitor and Estradiol. 7α-APTA (50 mg/kg) was dissolved in sesame oil (0.5 ml/injection) and each rat was

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[2] To whom requests for reprints should be addressed, at College of Pharmacy, The Ohio State University, 500 West 12th Avenue, Columbus, OH 43210.

[3] The abbreviations used are: 7α-APTA, 7α-(4′-amino)phenylthio-4-androstene-3,17-dione; DMBA, 7,12-dimethylbenz(a)anthracene; 4,6-ADD, 4,6-androstadiene-3,17-dione.

given a s.c. injection daily for 3 weeks. Beginning week 4, 7α-APTA (50 mg/kg) and estradiol (0.3 μg/kg) were dissolved in sesame oil (0.5 ml/injection) and each rat was given a s.c. injection daily for 3 more weeks. The rats were weighed twice a week and the number and volume of the tumors present were determined over a 6-week period.

RESULTS AND DISCUSSION

The initial 7α-substituted C_{19} steroidal aromatase inhibitor selected for this study was 7α-APTA. This competitive inhibitor was injected daily for 6 weeks at dosages of 25 or 50 mg/kg/day in vehicle, and rats in the control group received only vehicle. The tumors of the control group grew steadily during the study, reaching an increase in total tumor volumes of approximately 550% of the original volumes (Fig. 1). On the other hand, the 7α-APTA-treated groups demonstrated a reduction in tumor volumes during the first week (Fig. 1). Furthermore, tumor volumes continued to decrease to less than 20% of the original volumes (80% reduction) during the last 2 weeks of the treatment with 50 mg/kg/day. The group receiving 25 mg/kg/day responded with approximately a 50% reduction in tumor volume by the second week of treatment and maintained a 30 to 40% reduction of total tumor volumes throughout the rest of the 6-week study. Thus, effective reduction of tumor volumes was observed with 7α-APTA at doses of 25 and 50 mg/kg/day (Fig. 1; Table 1). Approximately 80% of tumors responded either completely or partially to 7α-APTA at the two doses examined.

Tumor-bearing animals receiving 4,6-ADD at 50 mg/kg/day demonstrated only a weak response of 10% reduction of tumor volume during the first 2 weeks of treatment (Fig. 1). The tumors slowly began to increase in tumor volume at 3 weeks of treatment and were approximately 50% larger at the end of the 6-week study. Thus, 4,6-ADD is much less effective in reducing tumor volumes. 4,6-ADD was selected in this study for two reasons: (*a*) 4,6-ADD is a weaker aromatase inhibitor than 7α-APTA and *in vivo* comparisons of these two steroids would be useful; (*b*) possible metabolism of 7α-APTA could occur via oxidation of the thioether and result in cleavage of the side chain of 7α-APTA to produce 4,6-ADD. Since 7α-APTA is significantly more effective in reducing tumor volumes in tumor-bearing rats than 4,6-ADD, the data suggest that the effects of 7α-APTA are due to the intrinsic activity of the 7α-substituted inhibitor and not to its degradation to 4,6-ADD.

Since 7α-APTA was effective at a dose of 50 mg/kg rat/day, experiments were performed to determine if this tumor reduction is due to inhibition of estrogen biosynthesis. Again, the tumors responded to 7α-APTA treatment with tumor reduction of 70% of the original volume during the first 3 weeks. Beginning at week 3, the coadministration of estradiol resulted in tumor growth (Fig. 2). Thus, the reversal of tumor reduction was observed in 10 of 12 tumors, resulting in 6 tumors having a greater volume than their original volumes at the end of the treatment period. Interestingly, 2 tumors that completely regressed during the first 3 weeks did not reappear upon estradiol coadministration.

A summary of the results of changes in tumor volume during the 6-week treatment period are presented in Table 1. The overall percentage of tumors responding completely or partially to 7α-APTA at both the 25 and 50 mg/kg/day doses was 75 to 80% (9 of 12 and 8 of 10, respectively). Coadministration of estradiol and 7α-APTA resulted in 50% of the tumors (6 of 12) having larger volumes at the end of the 6-week study than at the start of treatment. In the control group, 9 of 10 tumors (90%) grew.

Finally, the plasma estradiol concentrations were determined at the end of the six-week study in the treated animals. The results are shown in Fig. 3. 7α-APTA dramatically lowered plasma estradiol concentrations at both the 25 and 50 mg/kg/day doses to concentrations of 981 and 777 pg/ml, respectively.

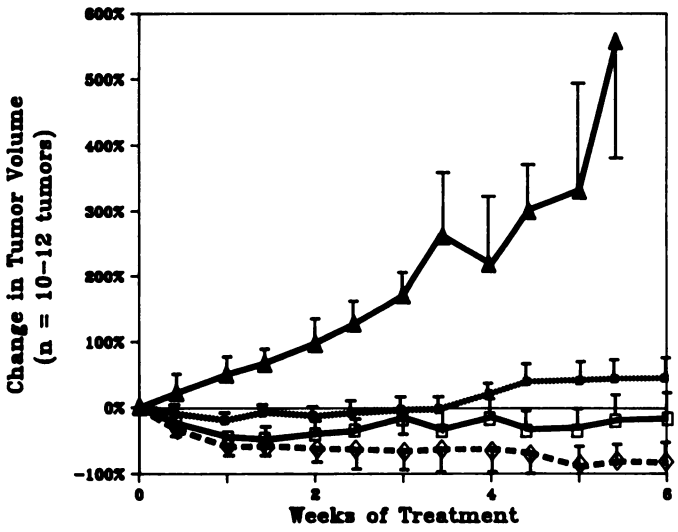

Fig. 1. Effects of 7α-APTA on tumor regression. Tumor-bearing rats were treated s.c. with 7α-APTA at 25 mg/kg/day (□), 7α-APTA at 50 mg/kg/day (◇), or 4,6-ADD at 50 mg/kg/day (■) in sesame oil suspensions. Control group of rats (▲) received only vehicle. Average tumor volumes per group at the beginning of treatments ranged from 2.44 ± 0.99 to 4.48 ± 1.94 cm^3.

Table 1 *Effects of 7α-APTA on rat mammary tumors*

Tumors	25 mg/kg/day	50 mg/kg/day	50 mg/kg/day + estradiol[a] (0.3 μg/kg/day)	Controls
Completely regressed	5	3	2	0
Regressed to less than 0.02 cc^3	0	1	0	0
Regressed greater than half-volume	2	2	2	0
Regressed less than half-volume	2	2	2	1
Have grown	3	2	6	9

[a] Estradiol (0.3 μg/kg/day) was coinjected with 7α-APTA beginning week 4.

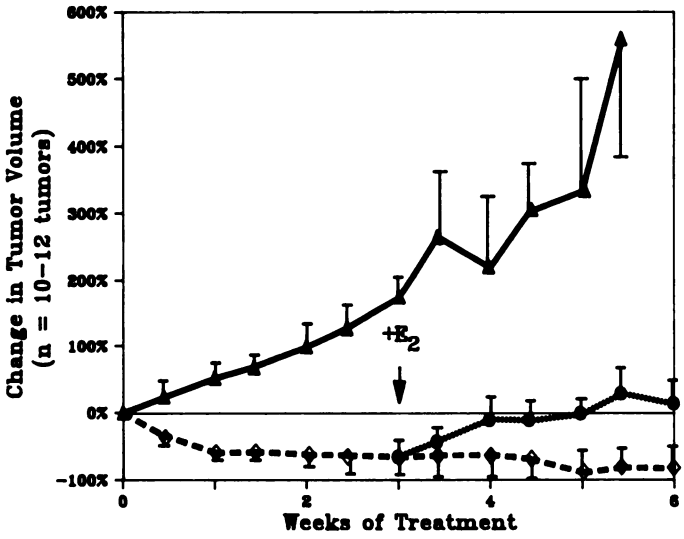

Fig. 2. Effects of coadministration of estradiol (E_2) and 7α-APTA on tumor regression. Tumor-bearing rats were treated s.c. with 7α-APTA at 50 mg/kg/day (◇) in sesame oil suspension for 3 weeks. The rats then received both 7α-APTA at 50 mg/kg/day and estradiol at 0.3 μg/kg/day (●) for weeks 4 through 6. Control group of rats (▲) received only vehicle. Average tumor volumes per group at the beginning of treatments ranged from 2.44 ± 0.99 to 4.48 ± 1.94 cm^3.

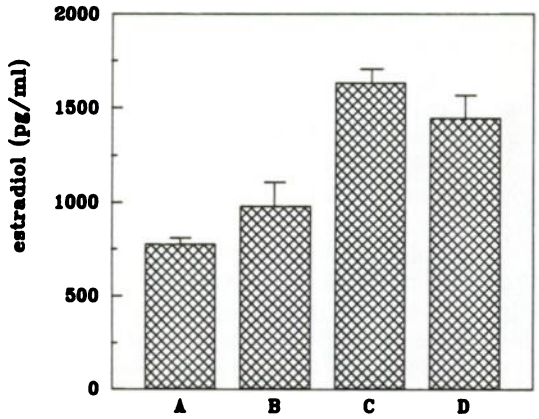

Fig. 3. Plasma estradiol concentrations in tumor-bearing rats. At the end of the 6-week treatment, plasma samples were obtained from the tumor-bearing rats (n = 5–7) and estradiol concentrations were determined by radioimmunoassays. Plasma estradiol levels were determined in rats treated s.c. with 7α-APTA at 50 mg/kg/day (*A*), 7α-APTA at 25 mg/kg/day (*B*), 4,6-ADD at 50 mg/kg/day (*C*), or 7α-APTA at 50 mg/kg/day for the first 3 weeks, followed by both 7α-APTA at 50 mg/kg/day and estradiol at 0.3 μg/kg/day for the last 3 weeks (*D*).

Plasma estradiol concentrations in the animals receiving the coadministration of estradiol with 7α-APTA were 1446 pg/ml and in animals receiving 4,6-ADD they were 1634 pg/ml. After 5 weeks, the surviving control animals that received only vehicle had large tumors, diminished weight, and widely varied plasma estradiol levels ranging from 640 to 2800 pg/ml. Normal female adult rats have plasma estradiol levels of approximately 1400 pg/ml (1).

Thus, 7α-APTA is effective in reducing tumor volumes in the estrogen-dependent DMBA-induced mammary carcinoma rat model. Administration of 7α-APTA to tumor-bearing animals at a dose of 50 mg/kg/day reduced tumor volumes by 80% during a 6-week treatment schedule. In addition, plasma estradiol levels in these animals were decreased by approximately 50%. These results indicate that 7α-APTA is effective *in vivo* and encourage further development of these steroids as potential medicinal agents for the treatment of estrogen-dependent disease states such as breast and endometrial cancer.

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