Effect of the Preestrogen 4-Androstene-3,17-dion-19-al on the Dunning R3327 Prostatic Adenocarcinoma

Thomas A. Marks1 and Vladimir Petrow2

Research Triangle Institute, Research Triangle Park, North Carolina 27709

ABSTRACT

The purpose of this pilot study was to determine if biogenetic precursors of estrone such as 4-androstene-3,17-dion-19-al, which is virtually devoid of thrombotic potential as well as androgenic and uterotrophic activity, could replace estrogen in the treatment of the hormone-sensitive Dunning R3327 prostatic adenocarcinoma in the male Copenhagen rat. If such were the case, the way would be open to an improved form of palliative therapy of prostatic cancer with the potential for decreased estrogenic side effects and cardiovascular complications. To this end, the R3327 tumor was transplanted (Day 0) into the flank of 10-week-old male Copenhagen rats, and treatment was begun 20 weeks later at which time the tumors reached a mean volume of 2160 cu cm. In addition to 4-androstene-3,17-dion-19-al (1 and 10 mg/day), diethylstilbestrol (33 µg/day) and 17β-estradiol (3.3 and 33 µg/day) were studied (daily for 60 days). At 1 mg/day, 4-androstene-3,17-dion-19-al produced a 43% inhibition of tumor growth (Day 203) while, in the 10 mg/day group, a 72% inhibition of tumor growth was measured on Day 196 (roughly equivalent to that produced by estradiol at 3.3 µg/day), with a 50% inhibition on Day 231. It is concluded that the tumor-inhibiting activity of 4-androstene-3,17-dion-19-al, coupled with its very low thrombotic potential, indicated that orally active analogues of this steroid may offer advantages over estrogens in the palliative treatment of prostatic cancer.

INTRODUCTION

Although cancer of the prostate is the second most frequent cancer and the third most frequent cause of death in males, the appropriate treatment for each stage of the disease is still controversial (33). Survival rates for Stage 0 carcinoma of the prostate have remained virtually unchanged for over 35 years (10, 22). The main standby for patients with advanced disease is still orchiectomy with supplemental estrogen therapy (1). New therapeutic approaches under the aegis of the National Prostatic Cancer Project are now under detailed study (8, 29), however, the beneficial effects of estrogens in prostatic cancer stem from: (a) the inhibition of LH release with a resulting fall in circulating testosterone levels; (b) the blocking of androgen synthesis directly within the Leydig cells (3); and (c) direct inhibition of the enzyme 5α-reductase within the prostate (17). In addition, since estrogen receptors are present in the prostate (23), they presumably play a functional, but presently unknown, role in the effect of the hormone on this organ. Finally, estrogens may exert a direct cytotoxic effect on the tumor.

In seeking to bypass the cardiovascular complications of estrogen therapy, we turned to the biogenetic precursors of the hormone within the cell. 4-Androstene-3,17-dione is converted into estrone in the body (Chart 1) by the NADPH-dependent enzyme aromatase via 19-oxygenated intermediates (Steroids II and III) (21, 32). These 2 little-studied derivatives of 4-androstene-3,17-dione are characterized by a very low thrombotic potential (20) and by very low androgenic and peripheral estrogenic activities (2, 13, 18, 24). We believe that the lack of systemic estrogenic activity and, hence, thrombotic potential stems from the low level of aromatase in blood and, in the female, in such organs as the uterus and mammary glands (5, 15) which, therefore, are incapable of converting Structures II and III into the hormonally active estrone. In tissues rich in both aromatase and RE, in contrast, Steroids II and III undergo aromatization into estrone, which is then taken up by copresent RE, inducing an estrogenic response. Estrogen overspill, if present, leads to release of estrone into the general circulation. In support of this concept, Structure II has been shown to replace both testosterone and estradiol in maintaining mating behavior in the castrated, adrenalectomized rat, whose behavior is generally believed to be associated with testosterone aromatization in rat brain areas rich in both aromatase and RE (7, 13, 24).

Estrogens exert their main palliative effects in prostatic cancer through the pituitary-hypothalamic axis by inhibiting the release of LH. The human hypothalamus is rich in both aromatase and RE (6, 27). In addition, in the castrate rhesus male monkey, Structure II has been shown to lower LH levels (25). Therefore, we deemed it likely that II and III might be able to selectively inhibit LH and, hence, testosterone levels after aromatization to estrone in the hypothalamus at dose levels that did not activate the blood coagulation mechanisms. If this could be achieved, then antiprostatic effects might be obtained without concomitant cardiovascular complications. Since we have previously found some aromatase activity in the homogenate of the Dunning R3327 prostatic adenocarcinoma, using the assay of Thompson and Slieter (32), we hoped that administration of Structures II or III to the tumor-bearing animal would lead to selective in situ
conversion of the steroid to estrone within the prostate tumor, thereby increasing the therapeutic benefits resulting from LH inhibition. As Structure II undergoes significant conjugation in vivo (14, 16), Structure III was selected for the pilot study. The Dunning R3327 prostatic adenocarcinoma implanted into the male Copenhagen rat (4, 9, 26) was used throughout, since it is a well-differentiated transplantable tumor containing both hormone-sensitive and hormone-insensitive cells.

The first experiment compares the effect of 4-androstene-3,17-dion-19-al and 17β-estradiol on the prostate of the Copenhagen rat with and without the R3327 tumor. The effects of these 2 steroids, as well as of DES, on this tumor model were also compared. Part of these data were presented in a preliminary communication (20).

MATERIALS AND METHODS

**Animals.** Male Copenhagen rats were obtained from Mammalian Genetics and Animal Production Section, Drug Research and Development Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, Md.

**Tumor.** Male Copenhagen × Fischer F₁ rats containing R3327 prostatic adenocarcinoma (implanted 5 months earlier) were obtained from Dr. Norman H. Altman of the Papanicolaou Cancer Research Institute at Miami, Inc., Miami, Fla. Tumors from donor rats were excised and cleared of membrane (capsule) and necrotic tissue. The tumors were then cut into pieces of about 2 cu mm with a scalpel blade. A tumor piece was then implanted s.c. into the right flank of 138 male Copenhagen rats using a trocar (Cancer Implant Needle, 13-gauge, three-fourth-inch long; Becton, Dickinson & Co., Rutherford, N. J.) attached to a 5-ml syringe. The tumor piece was loaded into the syringe barrel through the breech and was positioned at the rear entrance of the trocar. By postimplantation on Day 140, 132 rats had palpable tumors of various sizes. The tumors were measured with calipers and, based on their tumor volume (11), were systematically distributed into 5 treatment groups and a vehicle control group (16 rats each), plus an untreated control group (20 rats). The mean body weight of the rats on the day before the day treatment was begun was 306.4 g, and the mean tumor volume was 3454.6 cu mm at the start. There was an untreated control group in both experiments as well as a vehicle (0.5% methylcellulose) control group.

**Test Agents.** DES and 17β-estradiol were purchased from Sigma Chemical Co., St. Louis, Mo. The 4-androstene-3,17-dion-19-al (RMI-11985-07; Batch R-25640; 98% pure) used in the antitumor study was a gift from Merrell-National Laboratories (now Merrell-Dow), Cincinnati, Ohio. The 3 compounds were suspended in a 0.5% (w/v) methylcellulose (Matheson, Coleman & Bell, Norwood, Ohio) water vehicle. The androstene-3,17-dion-19-al used in the prostate study was prepared by Dr. C. E. Cook at the Research Triangle Institute. In this study, the steroids were also suspended in 0.5% methylcellulose (water).

**Drug Administration in the Antitumor Study.** The steroids were injected s.c. (0.5 ml) for 60 days (141 to 200 days after tumor implantation); 4-androstene-3,17-dion-19-al was administered at 10 or 1 mg/day, and 17β-estradiol was given at 33 and 3.3 μg/day. The vehicle control group received 0.5% methylcellulose.

**Prostate Study.** The steroids were injected s.c. on a 0.1% (1.0 ml/kg) body weight basis for 14 days. The 4-androstene-3,17-dion-19-al dose levels were 30 and 3 mg/kg/day, while 17β-estradiol was given at 100 and 10 μg/kg/day. Vehicle and untreated control groups also were included in this study.

**Statistical Methods in the Prostate Study.** A log transformation of the data was used in order to stabilize group variances (30). A 2-way analysis of variance on logs of prostate weights was used to test treatment, tumor status, and treatment multiplied by tumor-status interaction effects for normal and tumor-bearing data combined. Pairwise comparisons of treatment groups to the vehicle control group were made using the least significant difference method. Tests of treatment group differences and pairwise comparisons of treatment groups to the vehicle control group were also made within normal and tumor-bearing groups separately, using the error term from the 2-way analysis of variance. All tests of statistical significance were performed on log-transformed data.

RESULTS

Table 1 shows the effect (14-day treatment) of 4-androstene-3,17-dion-19-al (Structure III) (3 and 30 mg/kg/day) and 17β-estradiol (0.01 and 0.10 mg/kg/day) on the prostate weight of Copenhagen rats, both with and without the implanted Dunning R3327 adenocarcinoma. Overall treatment group differences were statistically significant (p < 0.01) for normal rats, tumor-bearing rats, and normal and tumor-bearing rats combined. Pairwise comparisons of treatment groups to the untreated control group showed all but the 0.5% methylcellulose group to be significantly different from the untreated control group. For normal rats and tumor-bearing rats tested separately, the methylcellulose group was not significantly different from the untreated control group. For normal rats and tumor-bearing rats tested separately, the methylcellulose group was not significantly different from the untreated control group at the 0.05 level. However, for all rats combined, the methylcellulose group was significantly (p < 0.05) different from the untreated control group.

The apparent effect of 0.5% methylcellulose on prostate weight was a surprise. However, it was unlikely that such an effect influenced the results of the antitumor study. In this latter
study, there were no apparent differences between the mean tumor volumes of the untreated controls and the vehicle controls. Thus, the 2 control groups were combined.

The relative change in tumor volume of the Dunning R3327 adenocarcinoma over time is plotted in Chart 2. During the 91 days that tumor growth was measured, there was an almost 7-fold increase in tumor volume (from approximately 2.2 to 14.9 ml) in the control group (i.e., combined untreated and vehicle control groups). During the 60-day injection period, the tumor volume of the control group increased almost 4-fold. In contrast, the administration of 17β-estradiol or DES at 33 μg/day reduced tumor growth to about 2- to 3-fold for the 91 days. In fact, there was little or no tumor growth during the 60 days in which these agents were injected at this concentration.

4-Androstene-3,17-dion-19-al (Steroid III) produced an antitumor effect at 10 mg/day, comparable to that produced by 17β-estradiol at 3.3 μg/day. The tumor volume in these two test groups increased about 2-fold during the 60-day injection period as compared to the 4-fold increase for the controls. When the study was terminated (31 days later), the tumor volumes of these 2 groups had increased 4.2-fold as compared to 6.9-fold for the controls (T/C = 50.4% for Steroid III). The 1-mg/day dose level of Steroid III also had an antitumor effect with a tumor volume about 2-fold for the 91 days. On Day 231, this dose group (4.8-fold increase in tumor volume) was 68.5% (T/C) of the controls. Thus, all treatments led to at least a 30% reduction in tumor volume at 31 days after drug administration, and at least a 49% reduction after the treatment period.

DISCUSSION

It has been stated (28) that the failure to uncover a drug for prostatic cancer with an improved therapeutic index has been due to failure to design and synthesize new agents with a selective action upon the prostatic cell and not upon the short-comings of the test systems. While this is undoubtedly the case, no information was available, when the work was started, on how such prostatic selectivity could be achieved. It seemed possible, however, for reasons outlined above, that some degree of therapeutic selectivity, relative to the thrombotic potential of 17β-estradiol, would result by using the preestrogen Steroid II or III. These compounds appear to be converted to estrone in the hypothalamus, where it was hoped that the resulting inhibition of LH release could be achieved at dose levels which would not activate blood coagulation mechanisms.

4-Androstene-3,17-dion-19-al (Steroid III) produced significant inhibition of tumor growth at 1 and 10 mg/day. At the 1 mg/day dose level, a 43% inhibition of tumor growth resulted on Day 203. AT the 10 mg/day dose level, a 43% inhibition of tumor growth resulted on Day 203. At this dose level, however, Steroid III had effect neither upon the activity of antithrombin III, nor the levels of fibrin monomer or fibrin degradation products in the ovariectomized rat model of MacKenzie et al. (19), nor upon ADP and collagen-induced platelet aggregation (20). In contrast, an equivalent uterotrophic dose of ethyl estradiol produced significant effects upon all of these coagulation parameters (20). That the biological effects of Steroid III were the result of aromatization to estrone from the comparable antiprostatic effects and tumor-inhibiting effects shown by equiuterotrophic doses of the 2 compounds. Thus, the preestrogen Steroid III fulfills the objectives of this pilot study in showing lowered thrombosis-inducing effects relative to 17β-estradiol at equivalent tumor-inhibiting dose levels. Solubility problems with Steroid III prevented us from matching the antitumor effects seen in this experiment with 17β-estradiol and DES at 33 μg/day. However, an analogue of 4-androstene-3,17-dion-19-al and preferably one which can be made available is needed for clinical studies on frank estrogens in the palliative therapy of prostatic tumors.

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