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BIOLOGICAL ASPECTS OF ANTIANDROGENS

RUDOLPH O. NERI and EDWIN A. PEETS

Departments of Physiology and Molecular Biology,
Division of Biological Research, Schering Corporation, Bloomfield,
New Jersey 07003, U.S.A.

SUMMARY

Flutamide (α,α,α-trifluoro-2-methyl-4′-nitro-m-propionotoluidide) at daily doses from 1–50 mg/kg reduced seminal vesicle and ventral prostate weights of intact male rats. A comparative study in castrated rats revealed that flutamide was equipotent to cyproterone acetate (CPA) as an antiandrogen. These potencies were substantiated by a newly developed assay measuring DNA synthesis rate (Sufrin and Coffey). No androgenic, estrogenic, anti-estrogenic, corticoid, progestational, anti-progestational, or antigonadotrophic activities were observed. Flutamide, given per os daily for either 6 weeks or 1 year to aged dogs with benign prostatic hyperplasia, reduced prostatic size in 6 weeks and the prostate remained reduced at 3, 6 and 12 months after the initiation of drug therapy. Flutamide, given im three times weekly for 4 weeks reduced the size of the baboon prostate (Müntzing *et al.*). In testosterone propionate-treated castrated rats CPA and flutamide inhibited ^{3}H-testosterone uptake and retention by prostate and prostate nuclei. In a study in 18 patients with far-advanced carcinoma of the prostate, there were seven positive responders, 10 failures and one inconclusive report, following the daily administration of flutamide (Stoliar and Albert). In a similar study, 9 of 12 patients in stage D prostatic carcinoma responded favorably (Prout and Irwin).

INTRODUCTION

The potential clinical applications of antiandrogens in prostatic hyperplasias, acne and hirsutism have prompted an intensive search for such agents by various laboratories. A number of antiandrogenic agents have been reported, most of which have associated with them other hormonal properties, particularly progestational activity. One of the more potent antiandrogenic agents described thus far is cyproterone acetate (CPA), a steroidal agent synthesized by Schering A.G., West Berlin, Germany [1]. Earlier reports suggesting beneficial effects of cyproterone acetate on benign prostatic hyperplasia (BPH) and prostatic carcinoma, were made by Scott and Wade[2] and Scott and Schirmer[3], respectively. Other antiandrogenic progestagens have been reported to show efficacy in BPH. Thus, Geller and co-workers[4] observed a beneficial effect with hydroxyprogesterone caproate while Rangno *et al.*[5] observed a significant reduction in the severity of BPH with medrogestone.

Since these antiandrogenic agents possess other hormonal properties which may negate their usefulness in prostatic hyperplasias, our efforts were directed toward finding an antiandrogen devoid of other hormonal activity.

This paper will extend the findings previously described [6] for the potent antiandrogenic agent, flutamide (α,α,α-trifluoro-2-methyl-4′-nitro-m-propionotoluidide) (Sch *13521*).

EXPERIMENTAL

1. *Studies in intact rats*

To assess the ability of flutamide to inibit endogenous androgens, 21–28 day old male rats weighing 55–60 g were given the compound daily for 3 weeks. Flutamide was suspended in an aqueous vehicle composed of 0·9% NaCl, 0·5% carboxymethyl cellulose, 0·4% polysorbate 80, and 0·9% benzyl alcohol and was always administered by gavage unless specified otherwise.

Twenty-four h after the last drug treatment, the rats were sacrificed and the seminal vesicles, ventral prostate, adrenals, thymus and testes were removed and weighed.

2. *Studies in castrate rats*

To determine the comparative potencies of flutamide and cyproterone acetate (CPA), male rats weighing 55–65 g were orchiectomized and drug treatment was begun the following day. Flutamide or CPA was administered orally at 10 mg/kg simultaneously with varying doses (mg/kg) of testosterone propionate injected sc in sesame oil daily for 7 days. Twenty-four h after the last treatment seminal vesicles and ventral prostates were removed and weighed.

3. *Studies in dogs*

Aged dogs (6 to 12 years) with benign prostatic hyperplasia (PH), as determined initially by rectal palpation were selected for this study. The prostate glands were exposed by an abdominal midline incision; caliper measurements of length, width and dorsoventral depth were made and wedge biopsies were taken before and after treatment. Histological and histochemical procedures and evaluations have been described previously [7]. In addition, blood studies including methemoglobin and plasma testosterone determinations were made as previously described[8].

Flutamide was placed in gelatin capsules and administered orally daily for 6 weeks or one year.

Table 1. Inhibition of endogenous androgens in immature male rats by the oral administration of flutamide for three weeks

DOSE (mg/kg)	SEMINAL VESICLES	VENTRAL PROSTATE	ADRENALS	THYMUS	TESTES
-	118.6 ± 15.1	205.1 ± 14.2	35.6 ± 1.6	630 ± 99	2250 ± 56
1	91.4 ± 12.6	138.2 ± 8.4*	41.1 ± 0.9	598 ± 57	2196 ± 66
5	78.2 ± 3.8*	133.4 ± 17.5*	40.3 ± 2.3	725 ± 64	2145 ± 31
10	57.8 ± 5.5[a]	129.7 ± 11.3*	38.4 ± 1.3	640 ± 90	2290 ± 11
25	30.5 ± 3.3[a]	93.1 ± 5.7[a]	38.7 ± 2.5	640 ± 107	2210 ± 92

*Each weight (mg ± SE) is the mean of 5 rats.
*Significantly different from mean of control group, $p<0.05$.
[a]Significantly different from mean of control group, $p<0.01$.

RESULTS

1. *Studies in intact rats*

Ventral prostate weights from rats given flutamide at 1–25 mg/kg were significantly less than those of the control group (Table 1). Seminal vesicle weights were significantly less than controls in rats given 5–25 mg/kg.

2. *Studies in castrated rats*

The data shown in Figs. 1 and 2 indicate that CPA and flutamide, each administered orally at 10 mg/kg, significantly reduced seminal vesicle and ventral prostate wt. when given simultaneously with varying doses of testosterone propionate. In each instance except the seminal vesicle response at 0·125 mg/kg in which CPA and flutamide gave an equal inhibitory response (Fig. 1, 2), flutamide was more effective than CPA in inhibiting testosterone propionate-induced seminal vesicle and ventral prostate weight increases. Statistical analysis of the data revealed that on the basis of seminal vesicle response, CPA was 0·8 times as active as flutamide whereas on the basis of ventral prostate response, CPA was 0·7 times as active as flutamide. However, because of the differences in molecular weights, analysis on an equimolar basis indicated that CPA was 1·2 and 1·4 times as active as flutamide based on the ventral prostate and seminal vesicle response.

3. *Studies in dogs*

Prostatic volumes and epithelial cell heights were significantly reduced after daily administration of flutamide for 6 weeks at dose levels from 5–25 mg/kg (Fig. 3). In addition, acid phosphatase was essentially obliterated. Before drug treatment the acini were

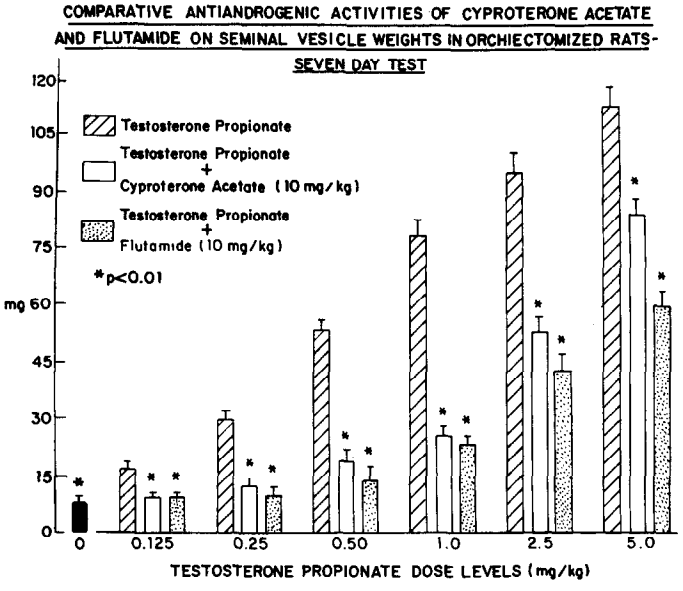

Fig. 1. Comparative antiandrogenic activities of cyproterone acetate and flutamide on testosterone propionate-induced increases of seminal vesicle weights in orchiectomized rats. The testosterone propionate (sc) dose was varied and injected simultaneously with either cyproterone acetate or flutamide, both given orally at a constant dose of 10 mg/kg for 7 days. Vertical lines represent the standard errors of the means.

* Represent significant differences ($P < 0·01$) between the combination of testosterone propionate and drug-treated rats vs testosterone propionate-treated rats alone: N = 6. (Permission to duplicate granted by Editor and publisher of *Endocrinology*).

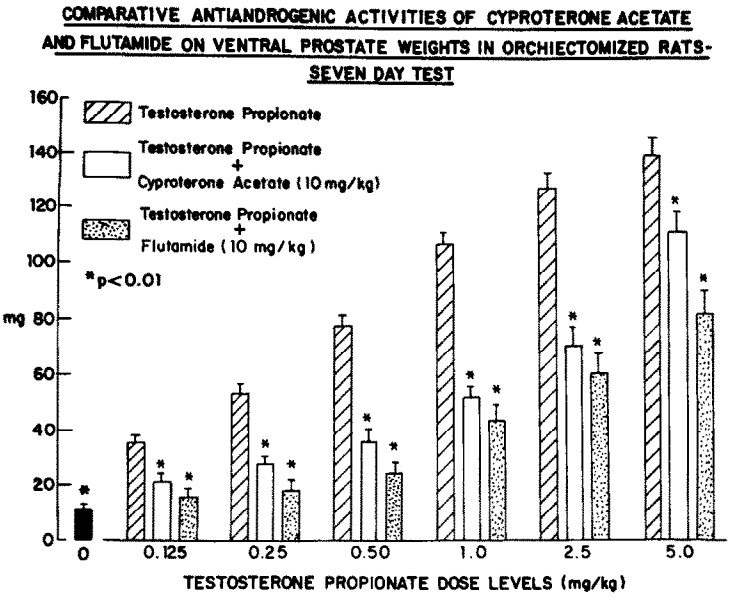

Fig. 2. Comparative antiandrogenic activities of cyproterone acetate and flutamide on testosterone propionate-induced increases of ventral prostate weights in orchiectomized rats. The testosterone propionate (sc) dose was varied and injected simultaneously with either cyproterone acetate or flutamide, both given orally at a constant dose of 10 mg/kg for 7 days. Vertical lines represent the standard errors of the means.

* Represent significant differences ($P < 0{\cdot}01$) between the combination of testosterone propionate and drug-induced rats vs testosterone propionate-treated rats alone: N = 6. (Permission to duplicate granted by Editor and publisher of *Endocrinology*).

large, irregularly shaped structures lined with either cuboidal or long columnar cells. Small amounts of stroma were present. After treatment with flutamide (5–50 mg/kg) a pronounced regression of the glandular epithelia occurred. Testosterone plasma levels were measured in some dogs. In two dogs that received 5 mg/kg the average plasma levels were essentially unchanged (0·15 0·12 μg/100 ml). However, in 2 dogs that received 10 mg/kg, the average plasma testosterone levels increased from 0·126 to 0·284 μg/100 ml, a per cent change of + 130. Similarly, in another dog treated with 15 mg/kg, plasma testosterone increased + 107% (0·161 to 0·334 μg/100 ml).

The examination of peripheral blood and bone marrow elements in all dogs revealed no significant alteration after 6 weeks of treatment with flutamide.

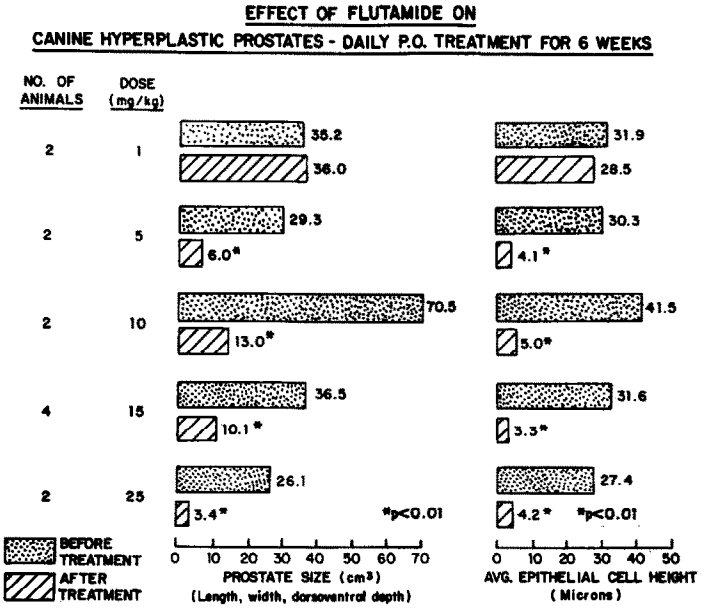

Fig. 3. Effect of flutamide on canine hyperplastic prostates. Dogs treated daily for 6 weeks. Compound placed in gelatin capsules and given orally.

In addition to these negative findings on the blood elements no significant elevation of methemoglobin occurred during treatment.

Administration of flutamide daily to three dogs for one year at 5 mg/kg suppressed prostatic growth throughout the entire treatment period. In the first dog, the initial pretreatment biopsy showed a prostate volume of 26·2 cm.^{3} and an epithelial cell height of 31·8 μm. After remeasuring the prostate *in situ* at 3 and 6 months a reduction in prostate volume occurred, 12·5 cm.^{3} and 10·6 cm.^{3} respectively. At autopsy after 1 year of treatment the prostate volume was 5·3 cm.^{3} and the epithelial cell height decreased to 1·6 cm.^{3} The second dog responded similarly to the first dog. The third dog exhibited the same pattern of response as the other two dogs resulting in prostatic atrophy. The pretreatment prostatic volume and epithelial cell heights measured 28·8 cm.^{3} and 33·4 μm, respectively. The 3 and 6 month prostate volume was 14·9 cm.^{3} and 15·6 cm.^{3}, respectively. In order to determine whether prostate size reverted to its hypertrophic condition as seen prior to treatment drug administration was suspended following 1 year treatment and the prostate was measured in situ after 1 year and a second biopsy was taken to determine epithelial cell height. Prostate volume was 12·5 cm.^{3} and epithelial cell height was 4·8 μm. Eight weeks later during which the dog received no drug, the animal was autopsied. The prostate volume increased to 38·2 cm.^{3} and the epithelial cell height increased to 29·2 μm, reverting to the pretreatment hyperplastic state. Thus, this study indicates that prostatic atrophy is maintained for one year at a minimally effective antiandrogenic dose of 5 mg/kg daily but that this effect is reversed following cessation of treatment.

4. *Other observations*

The antiandrogenic activity of flutamide and its comparable potency to cyproterone acetate was corroborated in a newly developed assay system measuring rates of DNA synthesis [9]. In this system, orchiectomized rats are treated with androgen simultaneously with the test compounds for three days. Twenty-four hours following the last treatment, the prostates and seminal vesicles are removed, incubated with labeled thymidine and the rate of DNA synthesis measured. The following antiandrogens were tested for their effectiveness in this system and the order of potency was observed to be Sch *13521* (flutamide) $\geq$ cyproterone acetate > Mk-316 [2′,3α′-tetrohydrofuran-2′-spiro-17-(6α,7α-difluoromethylene-1α,2α-methylene)-4-androstene-3-one] > R-2956 (17β-hydroxy-2α,2β,17α-trimethyl 8α-estra-4,9,11-triene-3-one) > medrogestone.

In a portion of a study reported by Müntzing and co-workers [10] flutamide was compared to estracyt (nitrogen mustard of 17β-estradiol) and stilbestrol for its ability to reduce the size of the prostate of the baboon. Estracyt and stilbestrol were injected intravenously 3 times weekly for 4 weeks and flutamide was injected intramuscularly also 3 times weekly for 4 weeks. All drugs were given at a dose of 5 mg/kg. The data indicated that a greater reduction in prostate size occurred with flutamide than with estracyt and stilbestrol although the latter two agents also significantly reduced prostate size. Histological examination revealed that the most pronounced changes were observed in the acinar nuclei which became flattened, and the glandular tissue which decreased in amount.

Several studies have indicated that CPA exerts its antiandrogenic action by inhibiting the uptake and retention of testosterone and dihydrotestosterone by the cells and the intracellular androgen receptors of the target tissue [11, 12]. Studies were performed to determine whether flutamide exerts its antiandrogenic properties on these processes.

Peets *et al.* [13] demonstrated that flutamide exerts its antiandrogenic effects on male secondary sex structures by an inhibition of androgen uptake and/or inhibition of nuclear binding of the androgens in the target tissues. This effect is similar to that observed with CPA. Thus, when flutamide or CPA was administered p.o. at 15 mg/kg 4 days, ^{3}H-testosterone uptake and retention by prostate and prostate nuclei was inhibited when the labeled androgen was given 3 h following the last dose of drug. ^{3}H-testosterone uptake by the prostate tissue of flutamidetreated rats averaged 37% of the control level. The ^{3}H-steroid level in the purified nuclear fraction was also markedly suppressed by the drug, averaging 5·8% of con-

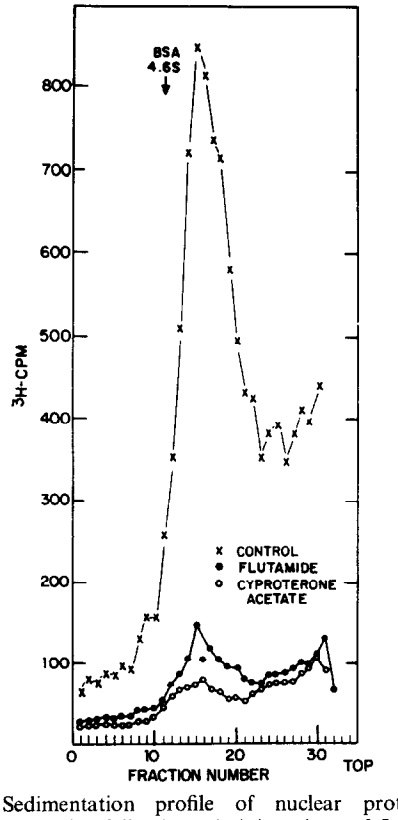

Fig. 4. Sedimentation profile of nuclear protein-^{3}H androgen complex following administrations of flutamide or CPA, and ^{3}H-testosterone to rats.
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trol, and the effect on nuclear incorporation of ^3H was greater than that on the whole tissue. CPA also reduced the uptake of ^3H-testosterone by rat ventral prostate whole tissue and nuclei. Similar to flutamide its effect on nuclear uptake of ^3H-steroid (8·5% of control) was greater than that on the whole tissue (49% of control) [13].

Sucrose density gradient centrifugation of these nuclear extracts in addition to demonstrating a reduced formation of the protein–^3H-androgen complex indicated further that flutamide, and CPA, induced a redistribution of ^3H in the nuclear extract as well (Fig. 4). While ^3H-radioactivity in the nuclear extract of the control group was localized mainly in the 3S region of the gradient, corresponding in position to that of the nuclear androgen receptor, that of flutamide, and CPA-treated groups was divided somewhat equally between the 3S region and the top of the gradient.

Similarly, a single dose of these drugs co-administered *via ip* injection with either ^3H-testosterone or ^3H-dihydrotestosterone inhibited uptake and retention of the labeled androgen by prostate whole tissue and nuclei [13]. Similar findings were reported by Liao and co-workers [14].

It is apparent from all these animal studies that flutamide possesses potent antiandrogenic activity and is devoid of other hormonal properties.

Clinical studies in prostatic hyperplasias have been initiated with flutamide. Although full scale studies have not been completed two publications have appeared showing the effect of flutamide in stage IV prostatic carcinoma.

In the first study, Stoliar and Albert[15] described their results with 18 patients with far-advanced prostatic carcinoma. These patients either had been treated with no benefit or had relapsed following successful conventional therapy. One patient was not orchiectomized nor had he received estrogen. The patients were given 750 mg of flutamide orally each day for one month in a double blind study to detect toxicity. The dose was increased to 1500 mg/day in all patients when no toxicity was observed at the lower dose. Length of treatment ranged from 3–39 weeks with an average of 20. There were 7 positive responders, 10 failures and 1 inconclusive report. Those patients who responded, did so within 1 week of instituting therapy. A positive response was defined as including 1 or more of the following criteria: (1) decrease in pain at least 3 months in duration (2) relief of urethral obstruction (3) decrease in size and induration of the primary tumor or metastases or (4) reversal of abnormal laboratory findings. One untoward effect observed in 10 patients was mild gynecomastia.

In a second report Drs. Prout and Irwin described their observations in twelve patients with stage D prostatic carcinoma who had received 250–500 mg t.i.d. orally for 1–15 months[16]. Nine of twelve responded favorably. Three of the patients were in complete remission for more than 40 weeks of treatment. Of the men who were sexually active before therapy, all remained so during therapy. Serum testosterone levels were unaffected by the drug. Mild gynecomastia developed in 6 patients but sequential determination of LH, FSH, estrone and estradiol prior to and during therapy in all patients failed to reveal any consistently altered pattern.

In conclusion, we have identified a potent antiandrogen comparable in activity to cyproterone acetate. Biological tests indicate that it is devoid of other demonstrable hormonal activity.

Initial clinical observations with flutamide in advanced cancer of the prostate are encouraging to warrant further exploration of this drug in this disease state.

Clinical studies are currently in progress determining the efficacy of flutamide in benign prostatic hyperplasia and acne.

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