The Effect of a New Anti-androgen on Canine Prostatic Acid Phosphatase (37177)

J. R. Brooks, R. D. Busch, B. Lopez-Ramos, W. R. Holt, S. L. Steelman, and D. J. Patanelli

Merck Institute for Therapeutic Research, Rahway, New Jersey 07065

Acid phosphatase content of the prostatic secretion has proven to be a particularly useful criterion of prostatic function in both man (1) and the dog (2). The prostatic acid phosphatase level appears to closely parallel that of circulating androgens (3). Increases or decreases in the enzyme with drug therapy would thus be expected to reflect the androgenic or anti-androgenic potency of an administered compound.

A steroid recently developed in our laboratories, 6α,7α-difluoromethylene-4',5'-dihydro-1α,2α-methylene-(17R) -spiro- [androst-4-ene-17,2'(3'H)-furan]-3-one (Compound I), proved to possess a high degree of anti-androgenic activity in the virtual absence of other hormonal properties (4). Precedent exists for the use of anti-androgens in the treatment of benign prostatic hyperplasia (5), a common disease of older men and dogs. Therefore, it was of interest of determine the effect of Compound I on canine prostatic secretion. It was administered to dogs surgically prepared for the collection of prostatic fluid. The samples obtained were analyzed for acid phosphatase as a measure of the effectiveness of treatment. The present report will document the findings and implications of those investigations.

Methods and Materials. Four castrated, cystopreputiostomized (6) beagles (2–7 yr old) were employed in the study. Prostatic function was maintained by a daily subcutaneous injection of 5 mg testosterone given as a suspension in 0.5 ml of a 1% aqueous solution of carboxymethylcellulose (3).

The experimental design was a 4 × 4 Latin square employing treatment levels of 0, 0.5, 1.0 or 2.0 mg/kg of the anti-androgen, Compound I. Each of the four treatment periods was comprised of a 6 day (Saturday through Thursday) dosing regimen. These were followed by rest periods of 16 days during which no Compound I was administered. The drug was given once daily via gelatin capsule placed in a bolus of ground meat. The dogs accepted this very readily.

Collections of prostatic fluid were made each Tuesday and Friday morning. Flow was stimulated by a subcutaneous injection of 0.7 mg/kg of pilocarpine (7). The prostatic secretion was collected into a condom fitted loosely over the penis and held securely by cords tied over the back and between the legs. The dogs were allowed freedom of movement in their individual pens during the 30 min collection period. The volume of each sample was measured to the nearest 0.1 ml and then placed on ice if it was to be analyzed immediately, or frozen if analysis was delayed. It has been reported that prostatic acid phosphatase remains stable for several days at 5° (8).

Determination of acid phosphatase in the collected samples was made according to published procedures (9). Acid phosphatase values were expressed in terms of Babson-Read units (10).

Statistical analysis of the data for the three variables of volume, acid phosphatase units per milliliter of prostatic fluid and total acid phosphatase units per sample was facilitated by transforming the data to logarithms (log 10). The inverse transformation gave the geometric means listed in the tables. The Latin square analysis of variance was used in analyzing the values thus obtained. Differences among pretreatment and treatment group means were tested using Duncan's New Multiple Range test.

TABLE I. Geometric Means[d] of Volume, Acid Phosphatase Concentration and Total Acid Phosphatase of Prostatic Fluid from Four Dogs Orally Dosed with Compound I.

Treatment (mg/kg/day)	Tuesday collections[e]					
	Vol (ml)		Concn (Babson-Read units/ml)		Total acid phosphatase[f] (Babson-Read units in thousands)	
	Pretreatment mean[g]	Treatment mean	Pretreatment mean	Treatment mean	Pretreatment mean	Treatment mean
Control	16.86[a]	7.96[a]	1119.4[a]	1207.8[a]	18.87[a]	9.59[a]
0.5	17.78[a]	9.93[a]	1202.3[a]	891.3[ab]	21.38[a]	8.85[a]
1.0	14.00[a]	9.48[a]	1183.0[a]	677.6[bc]	16.55[a]	6.44[a]
2.0	18.62[a]	9.51[a]	1047.1[a]	582.1[c]	19.49[a]	5.55[a]

[d] Each of four dogs is represented in each mean value shown.
[e] In each vertical column, means with at least one superscript letter (*a,b,c*) in common were not statistically significantly different at $p < .05$.
[f] Total acid phosphatase = volume times acid phosphatase concentration.
[g] Pretreatment values are those from collections made on Tuesday of the week preceding the treatment period.

Results. Tuesday collections. Data obtained from Tuesday collections are summarized in Table I. There was no statistically significant effect of Compound I treatment on either the volume of prostatic fluid produced or the total amount of acid phosphatase secreted. Although not statistically significant, a dose-dependent reduction in the total amount of acid phosphatase secreted was observed.

The concentration of acid phosphatase per milliliter of prostatic fluid was significantly reduced from control at the 1.0 and 2.0 mg/kg/day treatment levels.

Friday collections. A summary of data obtained from Friday collections is shown in Table II. A statistically significant drop from control in prostatic fluid volume occurred in the 2.0 mg/kg/day treatment group. A statistically nonsignificant decrease in volume was seen at the 0.5 and 1.0 mg/kg/day dosage levels.

For each of the Compound I treatment levels the acid phosphatase concentration was significantly reduced from control. Moreover, in addition to the fact that the greatest effect was seen with 2.0 mg/kg/day, the acid phosphatase concentration obtained at that level was significantly lower than was observed with either the 0.5 or 1.0 mg/kg/day doses.

Total acid phosphatase was significantly lowered from control by the 1.0 and 2.0 mg/kg/day treatment levels and, again, the greatest reduction was noted in the group which received the highest dosage of Compound I.

Discussion. A number of years ago Huggins and Clark (11) reported that either castration or estrogen therapy caused cessation of prostatic secretion in dogs. In the first instance androgen depletion undoubtedly occurred, and in the latter a direct antagonism to injected testosterone was noted. In 1941, Huggins and Hodges (12) observed a drop in serum acid phosphatase levels in humans suffering from disseminated prostatic carcinoma when androgen production was curtailed by castration or estrogen administration. Those reports are helpful in interpreting the results of studies such as the present one where the prostatic function of castrate dogs

is maintained by a given level of androgen. It would seem reasonable to assume that any drug-related decrease in the amount of prostatic fluid secreted or its acid phosphatase content may be attributable to an inherent anti-androgenic property of the compound administered.

Thus it is noteworthy that at each dose level employed Compound I caused a signficant reduction in acid phosphatase concentration of the prostatic secretion. This was true in all cases except for the Tuesday value with the 0.5 mg/kg/day dose level. Total content of the enzyme present in Tuesday collections, taken at the midpoint of the treatment period, was not different from control values but by the Friday collection a significant decrease had been established at both the 1.0 and 2.0 mg/kg/day dose levels. The failure to show a lowering of total acid phosphatase in the Tuesday collection is a reflection of the amount of fluid secreted. Volume of the prostatic secretions collected on Tuesday showed no decrease from control with treatment, whereas that of Friday's collection was significantly lower in the 2.0 mg/kg/day treatment group. Rosenkrantz and Mason (2) reported acid phosphatase levels declined before volume decreased. In their studies a drop in acid phosphatase was generally noted by the second day following initiation of daily estrogen administration. A decrease in volume was usually not observed until several days later. It is obvious that a very similar pattern of results was obtained in the present study.

Compound I has now been shown capable of reducing secretory capacity of the canine prostate. It will be of interest to determine whether its seeming potential for the relief of prostatic hyperplasia can be realized.

Summary. A 4 × 4 Latin square design was used in determining the effect of daily oral doses of 0, 0.5, 1.0 or 2.0 mg/kg of 6α,7α-difluoromethylene-4′,5′-dihydro-1α,2α-methylene- (17*R*) - spiro- [androst-4-ene-17,2′ (3′*H*)-furan]-3-one (Compound I), on the volume, acid phosphatase concentration and total acid phosphatase of prostatic fluid collected from four cystopreputiostomized beagles.

After the 6 day treatment period, dogs

TABLE II. Geometric Means[d] of Volume, Acid Phosphatase Concentration and Total Acid Phosphatase of Prostatic Fluid from Four Dogs Orally Dosed with Compound I.

Treatment (mg/kg/day)	Friday collections[e]					
	Vol (ml)		Concn (Babson-Read units/ml)		Total acid phosphatase[f] (Babson-Read units in thousands)	
	Pretreatment mean[g]	Treatment mean	Pretreatment mean	Treatment mean	Pretreatment mean	Treatment mean
Control	18.36[a]	21.93[a]	984.0[a]	1066.6[a]	18.07[a]	23.44[a]
0.5	19.14[a]	11.96[ab]	879.0[a]	635.3[b]	16.82[a]	7.60[ab]
1.0	20.41[a]	9.66[ab]	957.0[a]	539.5[b]	19.95[a]	5.21[bc]
2.0	20.18[a]	5.98[b]	1009.3[a]	276.7[c]	20.37[a]	1.66[c]

[d] Each of four dogs is represented in each mean value shown.
[e] In each vertical column, means with at least one superscript letter in common were not statistically different at $p < .05$.
[f] Total acid phosphatase = volume times acid phosphatase concentration.
[g] Pretreatment values are those from collections made on Friday of the week preceding the treatment period.

given 0.5 mg/kg showed a significant reduction in concentration of prostatic acid phosphatase. The 1.0 mg/kg level caused significant decreases in both concentration and total amount of acid phosphatase. In dogs which received the 2.0 mg/kg dose, all three parameters measured were significantly reduced by treatment.

The results of this experiment are believed to reflect the anti-androgenic activity of Compound I.

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Received Dec. 4, 1972. P.S.E.B.M., 1973, Vol. 142.