THE EFFECT OF POLYENE MACROLIDES ON THE PROSTATE GLAND AND CANINE PROSTATIC HYPERPLASIA

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Candicidin,[1-2] one of the polyene macrolide antifungal antibiotics, has been known for 15 years as a potent and effective therapeutic agent active against a wide variety of yeast and fungi, including numerous animal pathogens. Produced by a strain of *Streptomyces griseus* isolated in 1948, the first *in vivo* evaluation of candicidin[3] was made in 1953. As is characteristic of all polyene macrolide antifungal antibiotics, candicidin is far less toxic by oral administration than by parenteral routes. Generally, very little, if any, absorption from the gastrointestinal tract is believed to occur. As a result, most clinical applications of these antibiotics are largely limited to topical use including the vaginal area. The sole oral application of these antibiotics to date has been the treatment of yeast and fungal infections of the intestinal tract.

In a series of studies designed to determine the extent and nature of the oral toxicity of candicidin in dogs and other laboratory animals, a rather interesting observation was made. With daily oral administration of candicidin at doses of 20 mg/kg of body weight and higher for periods of 30 days and more, examination of different body organs at autopsy indicated that one principal reaction was an apparent reduction in the size of the prostate gland. This preliminary observation of the effect of orally administered candicidin on the prostate gland volume stimulated further investigations to determine the possible effects of candicidin and related polyene macrolides on canine prostatic glandular hyperplasia. The results of these studies are reported here.

In cases of old dogs with benign prostatic hypertrophy, confirmed by pretreatment needle-punch biopsy at laparotomy followed by histological examination, oral treatment with a variety of tetraene, pentaene, and heptaene macrolides produced marked reductions in the texture and volume of the glands. The heptaene macrolides, candicidin and amphotericin B, in particular, produced posttreatment needle-punch biopsies exhibiting marked histological changes involving diminutions of gland diameters and epithelial cell heights associated with reduced congestion, granularity, and papillations. It is suggested that the polyene macrolide antifungal antibiotics as a class may be active for the treatment of prostatic hypertrophy by oral route.

Materials and Methods.—Mongrel dogs, destined for the pound, preselected on the basis of age (approximately 7–15 years) and of the possibility of the presence of prostatic glandular hyperplasia as initially determined by rectal palpation, were obtained for these studies. The dogs were acclimatized to kennel conditions for 7 days. Final selection for experiment was made after surgical examination by laparotomy to establish the hypertrophic condition of the prostate glands. Anesthesia was induced by myothesia (sodium secobarbital and mephenesin, 5:3). The prostate glands were exposed by a midline abdominal incision from the pubis to the umbilicus. The peritoneum was entered,

and the prostate gland was exposed by dissection. Upon exposure, the glands were measured with a vernier caliper in three dimensions (lateral, cranial-caudal, and dorsoventral). Multiplication of the three dimensions enabled an approximation of the gland volume (cm^3). The glands were also palpated to determine their consistency, and a needle-punch biopsy of the left hemisphere was taken. The tissue was fixed in formalin for histological processing with hemotoxylin and eosin stains for microscopic examination. During the histological evaluation, the prostate was cross-sectioned. Epithelial cell heights as well as the condition of the epithelia and stroma were noted. In a few cases (dogs P-12 and P-13 treated with candicidin), no punch biopsies were taken at the time of the laparotomy, to determine any possible traumatic effect on the gland due to the biopsy procedure. For these dogs, only measurements and observations of the gland were made. After a period of 4–7 days of recovery from surgery, the animals were given the drugs. Before and after treatment, blood samples were collected for differential and total cell counts as well as hemoglobin, and for hematocrit determinations. Urine specimens were analyzed for glucose, pH, specific gravity and the presence of blood, sedimented cells, crystals, and casts. Blood and urine specimens were also taken for routine drug assay. Body weights were determined during the course of the experiment.

The polyene macrolides employed in these studies included candicidin (S. B. Penick, lot 183-NCF-1, 25%), amphotericin B (E. R. Squibb, lot 38675-001, 90.4%), nystatin (E. R. Squibb, lot 46982-013, 4230 units/mg), filipin (Upjohn, lot U5956, 96%), and fungimycin (Institute of Microbiology, Rutgers, The State University, lot E-1-1, 60%). For comparative purposes, the microbiological potencies of all compounds used in these studies were determined against *Saccharomyces cerevisiae* ATCC 9763 by a tube dilution procedure.[4] The polyene macrolides were formulated with lactose as a diluent and filled in hard gelatin capsules (size 0) to capacity.

The encapsulated compounds were wrapped in food and were administered orally twice daily, in the morning and in the late afternoon, for a period of 30 days. Daily doses varied approximately from 5 to 20 mg/kg of body weight, as indicated in *Results*. After 30 days of drug administration, urine and blood samples were again collected for the analyses described earlier, and the dogs were killed and autopsied. Final measurements of the prostate gland were made, and a needle-punch biopsy was taken of the right hemisphere. In addition, histological specimens of the general organs were taken at necropsy to determine by microscopic examination the extent of drug toxicity, if any. Sections of the bladder, pancreas, kidneys, adrenals, liver, spleen, testes, intestines, caecum, lung, thyroid, and heart were made.

Results.—The effects of the heptaene macrolide, candicidin, on canine prostatic hyperplasia after oral administration at 5, 10, and 20 mg/kg for 30 days are reviewed in Table 1. It is quite clear that the treatment had a profound effect on the volumes of the enlarged prostate glands examined in this study. All the old dogs receiving candicidin exhibited marked reductions in gland volume and varying alterations of gland texture. At all the dose levels in these studies, the reduction in gland volume did not appear to be closely related to the dose administered. The trauma of the punch biopsy had no effect on the reduction of the gland. On the contrary, a control animal with prostatic hyperplasia exhibited an increase in gland volume after undergoing the trauma of laparotomy and punch biopsy.

The effects of oral administration of other miscellaneous polyene macrolides on canine prostatic hyperplasia are given in Table 2. It can be readily noted that all the compounds tested produced marked reductions of the prostate gland volumes. Although the number of animals treated with the tetraene (nystatin) and the heptaene (fungimycin) were small, the results clearly indicate that these compounds exhibit some effect on the glands.

TABLE 1. *Effect of candicidin on canine prostatic glandular hyperplasia.*

Animal no.	Daily dose (mg)	Body weight (kg)	Prostate Gland Volume (cm^3) At laparatomy	After treatment	Gland volume change (%)
P-1	100	14.5	26.3	20.7	−21.3
P-6	100	24.0	63.0	56.0	−11.1
P-7	100	11.4	6.0	2.9	−51.7
P-10	100	32.4	100.0	51.2	−48.8
P-3	200	21.4	11.9	5.2	−56.3
P-4	200	25.9	16.6	8.1	−51.2
P-5	200	25.0	11.3	4.9	−56.7
P-11	200	14.4	48.0	25.5	−46.9
P-12	300	17.7	31.1	28.6	− 8.0
P-13	300	15.0	24.1	19.0	−21.1
P-15	300	13.6	60.0	21.0	−65.0
P-16	300	15.5	27.4	9.0	−67.2

Candicidin was administered to three groups of four animals each at 5, 10, and 20 mg/kg of body weight by the oral route.

Not all the posttreatment glands showed the same degree of volume reduction nor alteration of tissue consistency. The dosage period of 30 days was selected arbitrarily and may have been too short. The results obtained with one dog treated orally with candicidin at 20 mg/kg for only five days clearly revealed a significant gland volume decline (26.5%). Thus, the data show that a relatively short time of administration with a relatively large dose effected a significant change in gland volume. In the case of dog P-7 treated with candicidin, drug administration was discontinued after a second laparotomy. After 20 days without further treatment, the prostate gland exhibited an increase in size of 50 per cent toward the volume noted at the beginning of the experiment.

Histopathologic evaluation of the sections prepared from the needle-punch biopsies of the prostate glands before and after treatment was made. It was

TABLE 2. *Effect of miscellaneous polyene macrolide antifungal antibiotics on canine prostatic glandular hyperplasia.*

Polyene macrolide administered	Animal no.	Daily dose (mg)	Body weight (kg)	Prostate Gland Volume (cm^3) At laparatomy	After treatment	Gland volume change (%)
Nystatin	P-17	200	6.8	9.9	7.9	−20.2
	P-18	200	6.4	12.5	12.5	0
	P-19	400	20.9	38.4	22.1	−42.5
Amphotericin B	P-23	200	12.3	19.8	13.2	−33.3
	P-67	400	11.8	25.2	12.0	−52.4
	P-68	400	10.4	25.0	17.6	−29.6
	P-69	400	10.0	35.4	25.5	−28.0
	P-28	500	22.7	210.0	120.0	−42.9
Filipin	P-36	200	6.2	46.8	29.2	−37.6
	P-32	400	19.0	22.5	9.4	−58.2
	P-35	400	21.6	35.8	12.5	−65.1
	P-70	400	10.2	21.5	19.7	−8.4
	P-71	400	13.6	34.8	25.3	−27.3
Fungimycin	P-37	100	9.1	59.4	15.0	−74.7
	P-40	100	11.8	43.2	44.8	+3.7
	P-41	100	11.8	42.0	34.1	−18.8

quite evident that particularly the treatment with the heptaene macrolides candicidin and amphotericin B produced varying volume reductions and changes in the morphologic features of the benign prostatic hyperplasia. The degree of change could not be correlated clearly with the dose level, total dosage, or length of treatment. The histological effect of a high dose of candicidin (20 mg/kg) on the prostate gland of dog P-16, exhibiting a relatively high reduction in gland volume (67.2%), is presented in Figure 1. The pretreatment biopsy of the gland (Fig. 1*a*) shows advanced glandular hyperplasia with enlarged lobular subunits filled with densely packed, papillary infolded processes of tall columnar epithelium. The posttreatment biopsy (Fig. 1*b*) shows abolition of the papillary pattern. The glands are tubular, and the stroma is moderately dense, showing sparse lymphocytic reaction. The histological effect of amphotericin B (20 mg/kg) on the prostate gland of dog P-28, exhibiting a substantially large volume reduction (42.9%), is shown in Figure 2. The pretreatment biopsy (Fig.2*a*) exhibits a mild hyperplasia. The posttreatment section (Fig. 2*b*) shows a morphologic change comparable to that obtained with candicidin shown in Figure 1. About 75 per cent of the glands are dilated, almost cystic, lined by flat epithelium, and separated from each other by a loose edematous stroma. There is no significant inflammatory reaction.

Although there had been marked gland volume reductions, the oral administration of filipin, nystatin, and fungimycin had only slightly altered the histological morphology of the prostatic glandular hyperplasia. The three dogs treated with nystatin produced biopsies at autopsy, exhibiting the least apparent histological modification of the gland. Filipin and fungimycin produced somewhat greater morphological changes with some degree of stromal loosening and less intense papillary infolding. The columnar epithelial cells were also slightly lower. The relatively poor response associated with nystatin may be due to the short duration of treatment or inadequate dose level, or both. This may also be the basis for the relatively poorer response observed with filipin and fungimycin. The purity of the compound employed in these studies may also be a factor.

In review, the experiments presented here clearly indicate that the polyene macrolide antifungal antibiotics as a class administered to dogs with established prostatic glandular hyperplasia produce marked reductions in gland volume. The heptaenes, candicidin and amphotericin B, clearly altered the hyperplastic glands with histological changes associated with decreased columnar epithelial cell heights in addition to diminished or absent granularity. Papillations were reduced or absent, and abundant and loose edematous stroma, due to reduced parenchyma, were noted.

With the exception of candicidin, there were no external signs of drug toxicity with any of the polyene macrolide drugs administered orally at various levels for 30 days. Symptoms of diarrhea, emesis, and loss of appetite occurred in some dogs at the higher dose levels with candicidin. Although these gastrointestinal effects were observed, it was not found necessary to discontinue the treatment with candicidin, nor were these effects associated with cytotoxicity.

There was no histopathologic evidence of drug toxicity in any of the dogs studied. Sections of such parenchymatous organs as the heart, lungs, liver,

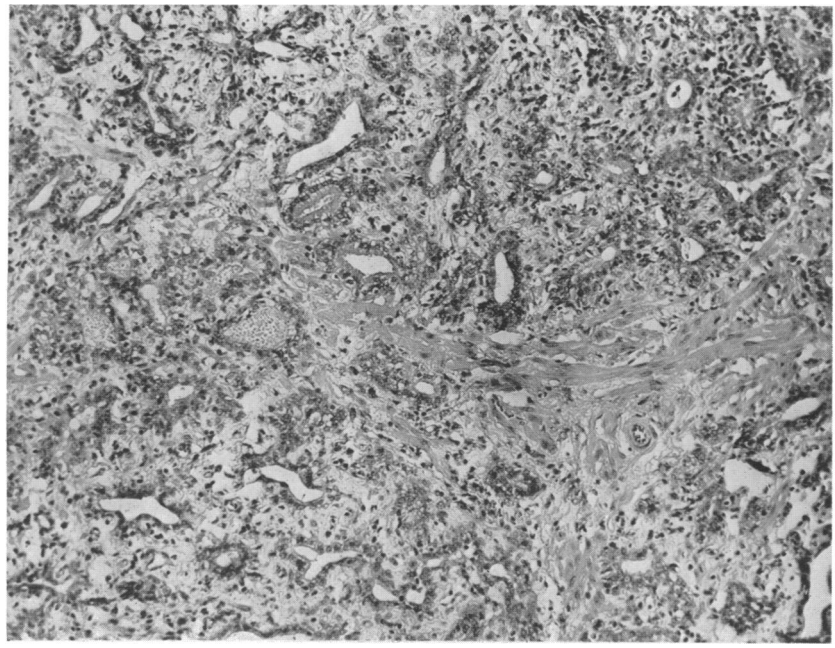

FIG. 1b.—Dog P-16. Posttreatment biopsy; candicidin, orally administered, 150 mg twice a day, 30 days, total dose 9.0 gm. Hematoxylin and eosin (×120).

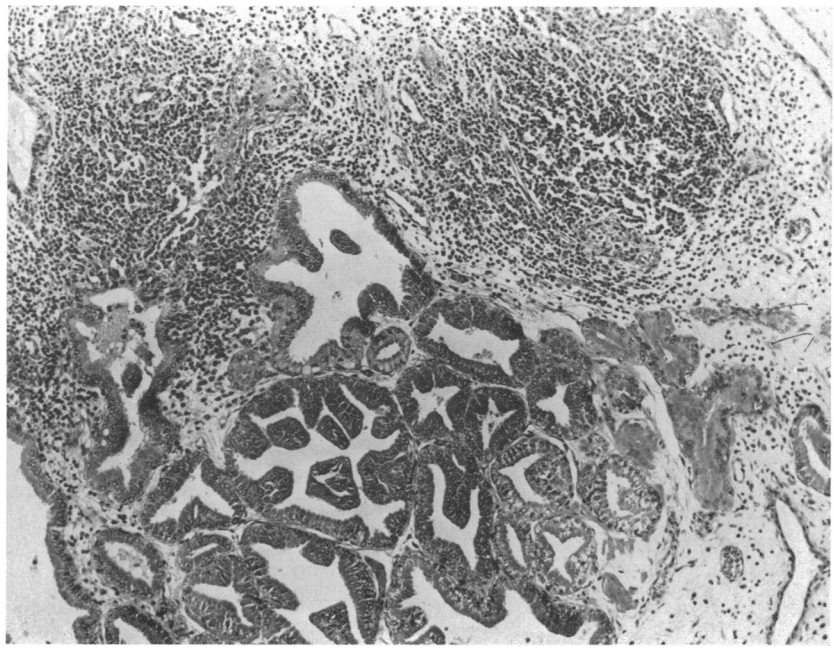

FIG. 1a.—Dog P-16. Pretreatment biopsy of the prostate gland. Hematoxylin and eosin (×120).

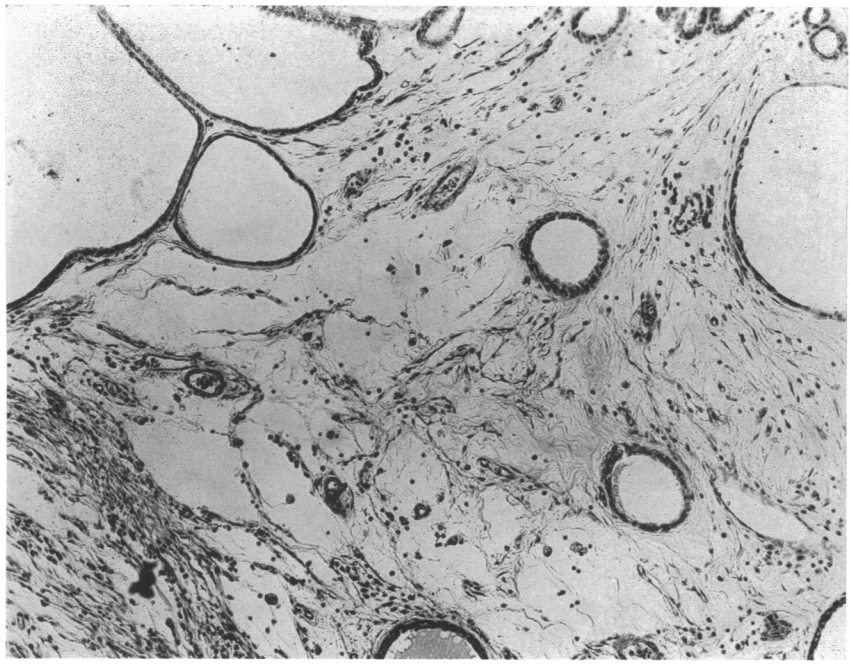

FIG. 2b.—Dog P-28. Posttreatment biopsy; amphotericin B, orally administered, 250 mg twice a day, 30 days, total dose 15.0 gm. Hematoxylin and eosin (×120).

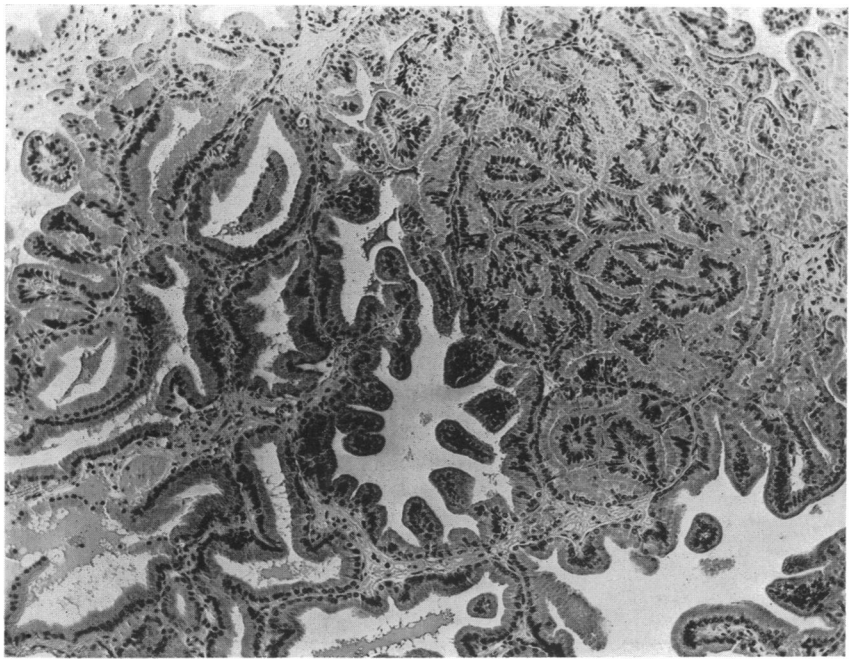

FIG. 2a.—Dog P-28. Pretreatment biopsy of the prostate gland. Hematoxylin and eosin (×120).

spleen, kidneys, testes, and adrenal glands showed no significant histopathologic changes. There were no lesions or crystalline deposits that could not readily be characterized as common in any group of aging dogs. Hence, the oral administration of all these polyene macrolide antibiotics in the doses used and for the period tested did not result in any toxic effect of a generalized or specific nature to any of the organs studied, both grossly and histologically. No abnormal changes in the blood and urine could be detected. Traces of antibiotic activity (< 2 μg/ml) could be detected in the blood and urine of those dogs treated with filipin. However, none of the other polyene drugs could be found in the blood and urine of the animals.

Discussion.—The interesting results of these studies indicating the marked effect of polyene macrolide antifungal antibiotics on naturally occurring canine prostatic glandular hyperplasia are difficult to explain at the present time. It is well known that these substances are generally very poorly absorbed, if at all, from the gastrointestinal tract. How then can these substances alter the condition of canine hyperplasia by oral route?

Possibly, the physiological effects of the polyene macrolides on the prostate gland can be related to their recently described[5] hypocholesterolemic properties by the oral route. Our experience with these compounds has suggested that some relationship may exist between their antimonilial effect and their influence on the prostate gland and hypocholesterolemic action. The results obtained here with the orally administered polyene macrolides strongly indicate a relationship to a physiochemical phenomenon rather than an antimicrobial action.

It has been postulated that prostatic glandular hyperplasia in the dog results from excessive or prolonged androgenic stimulation.[6] Recently,[7] this postulation was tested and confirmed in dogs with normal prostates receiving large doses of androgen (testosterone propionate). Whether the reduction of serum cholesterol levels can be related to altered androgen levels during oral treatment with polyene macrolides remains to be established. Recent approaches[7, 8] to the treatment of benign prostatic hyperplasia in man and dog have involved the application of progestational agents such as hydroxyprogesterone acetate and antiandrogens, SH 714 (6-chlor Δ^6-1,2α-methylen-17α-hydroxy-progesterone acetate). The side effects of these drugs, however, have deterred their widespread use.

Summary.—Oral administration of polyene macrolides at approximate dose levels of 5–20 mg/kg of body weight for 30 days produced varying reductions in the size and texture of the prostate gland, especially in cases of benign prostatic hypertrophy. The heptaene macrolides, candicidin and amphotericin B, in particular, produced marked changes in the histological appearance as well. These effects were not associated with pathological changes in the blood or urine, nor with any histopathology referable to drug toxicity.

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