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Chlormadinone Acetate (CMA) Induces Apoptosis on Canine Spontaneous Benign Prostatic Hyperplasia (BPH)

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The effect of a synthetic steroidal antiandrogen, chlormadinone acetate (CMA), on spontaneous benign prostatic hyperplasia (BPH) in dogs was investigated. Male beagle dogs (5–8 years old) were divided into four experimental groups. Group 1 consisted of untreated controls. Groups 2 to 4 received CMA 0.03, 0.1, and 0.3 mg/kg/day, p.o., respectively, for 6 months. In group 1, glandular hyperplasia of the prostate was clearly detected. In groups 2 to 4, CMA produced marked atrophy of the glandular epithelium. The interacinar fibro-muscular stroma was prominent. To evaluate the frequency of apoptosis, we counted the positive cells stained by the nick end labeling method. In group 1, the apoptotic index was 0.76±0.03%. In groups 2 to 4, apoptotic indices were 15.41±1.26%, 2.63±0.98% and 1.45±0.85%, respectively. Apoptotic cell death was mainly observed in the glandular epithelial cells. Based on our data, regression of BPH after treatment with CMA may be apoptotic cell death.

Key words: Chlormadinone acetate (CMA), Benign prostatic hyperplasia (BPH), Canine, Apoptosis, Atrophy

I. Introduction

Biochemical and morphologic studies have demonstrated that the involution of normal prostate after castration is not the result of necrotic cell death, but is an active process brought about by the initiation of a series of specific biochemical steps that lead to the programmed cell death (apoptosis) of androgen-dependent glandular epithelial cells within the prostate [5, 13, 25]. Associated with this programmed cell death is the enhanced expression of a series of genes within the prostate [2, 16, 17, 26].

Like other systems in which programmed cell death occurs [3, 29, 31, 33], androgen ablation induced prostatic cell death initially involved fragmentation of genomic DNA. This DNA fragmentation occurs *via* activation of endonucleases within the prostatic glandular cell nucleus which degradates the genomic DNA into nucleosomal oligomers (i.e. multiples of a 180-base pair subunit) lacking internucleosomal breaks in the DNA [13, 15].

DNA fragmentation is sequentially followed by irreversible morphological changes termed apoptosis [14, 32] which characteristically involve chromatic condensation, nuclear disintegration, cell surface blebbing, and eventually cellular fragmentation, into a cluster of membrane-bound apoptotic bodies within the prostate [5, 13, 27].

Both humans and dogs spontaneously develop benign prostatic hyperplasia (BPH) with age [1, 10, 18, 30, 34]. Canine BPH is believed by many investigators to be an appropriate model for the study of human BPH, although there are important differences between the conditions in the two species [1, 10, 18, 30, 34]. Thus, the human disease is often a multinodular process thought to arise from a priurethal stromal nodule, which is then secondarily invaded by glandular elements [8]. In contrast, canine BPH is a diffuse epithelial or glandular process with less stromal involvement [4]. Although some differences exist between human and canine BPH, the dog is considered to be a good animal model of BPH to test the efficacy of drugs that cause shrinkage of the hyperplastic gland [23, 24, 28].

Chlormadinone acetate (CMA) is a steroidal antiandrogen that is widely used in the medical management of human BPH or prostatic carcinoma [8, 11]. The atrophic effect of CMA on the human prostatic hyperplasia has been reported by several authors [8, 11]. The antiandrogenic mechanisms of CMA has been evaluated biochemically and immunohistochemically, such as androgen receptor content, and steroid 5α-reductase type II activity in the prostate [11, 19–22]. However, a report concerning apoptotic cell death

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and histopathological evaluation of canine BPH treated with CMA has not appeared.

In the present study, in order to confirm the relationship between BPH regression and atrophic effect of CMA, we made histopathological and immunohistochemical analysis of the prostates of spontaneous BPH dogs administered CMA for 6 months.

II. Materials and Methods

Animals

Seventeen male beagle dogs were purchased from Hazelton Research Product, Inc. (Denver, PA). The animals were housed individually in stainless steel cages in a semi-barrier system maintained at a room temperature of 22±3°C, and relative humidity of 60±20%, with 12 hr of light (7:00–19:00). The animals were given 300 g of a standard diet (CD-1, CLEA Japan, Inc.) daily and tap water *ad libitum*. They were 5–8 years old, and BPH was confirmed by digital rectal examination, transrectal ultrasonography, and transrectal prostatic biopsy. The prostates of all the animals showed glandular hyperplasia and hypertrophy by light microscope (histological criteria of DeKlerk *et al.* [4]). Furthermore, measured prostatic weights were 13 g and more (Group 1: 14.8–24.3 g, Group 2: 15.2–33.8 g, Group 3: 13.0–14.8 g, Group 4: 13.5–35.3 g, data not shown).

Experiments

Four animals served as BPH untreated controls (group 1). Groups 2 to 4 were administered orally 0.03 (group 2, n=4), 0.1 (group 3, n=4), and 0.3 (group 4, n=5) mg/kg/day of CMA as a crystalline powder in gelatin capsules for 6 months. All animals were sacrificed by exsanguination under pentobarbital anesthesia at the end of the experimental period.

Organ weight

The organ weights of the prostates were recorded (absolute weight). Weights relative to body weight (relative weight) were calculated.

Histopathological examination

Prostates were removed immediately, fixed in 0.1 M phosphate-buffered 10% formalin, and embedded in paraffin. Cut sections were mounted and stained with hematoxylin and eosin (HE).

Apoptosis staining

Apoptosis detection by labeling of 3'OH ends of DNA breaks using terminal deoxynucleotidyl transferase was done using the ApopTag detection Kit (Oncor, Gaitherburg, MD). Formalin fixed and paraffin sections were used. After deparaffinization and hydration, the sections were incubated with 20 μg/ml proteinase K (Sigma Chemical Co., St. Louis, MO) at room temperature for 15 min, and then inactivated by covering the sections with absolute methanol containing 0.3% hydrogen peroxide. The sections were washed with 0.01 M PBS, equibrated, and then incubated with terminal deoxynucleotidyl transferase in a reaction buffer containing digoxigenin dUTP at 37°C for 10 min. The reaction was stopped, and sections were washed for 30 min, after which the sections were incubated with anti-digoxigenin antibody coupled to peroxidase for 30 min at room temperature. After the incubation was completed, the sections were treated in Graham-Karnovsky's reaction medium [7], which contained 20 mg% 3,3'-diaminobenzidine (DAB, Wako Pure Chemical Industries, Osaka) and 0.005% hydrogen peroxide in 0.05 M Tris-HCL buffer, pH 7.6, for 5 to 10 min at room temperature. Then the sections were counterstained for nuclei with 1% methyl green dissolved in veronal acetate buffer, pH 4.2. Apoptotic index was calculated as the positive cell number in one acinus/total cell number in the acinus ×100.

Statistical analysis

The data were expressed as mean±SD. Homogeneity of variance was tested by Bartlett's methods, and when the assumption of homogeneity of variance was met, one-way layout analysis of variance was performed. When a significant difference was observed, Dunnett's multiple comparative test was performed between the BPH control group and the other experimental groups.

III. Results and Discussion

Organ weight

As shown in Table 1, absolute and relative mean prostatic weights of groups 3 and 4, and absolute mean prostatic weights of group 2 were statistically significantly ($p<0.05$) reduced, compared to the BPH control values.

Table 1. *Effect of chlormadinone acetate (CMA) on prostatic weight*

Group	N	Dose (mg/kg)	Initial age (years)	Body weight (kg)	Prostatic weight (g)	
					Absolute	Relative
1	4	0	5.0±1.8	14.3±1.9	26.58±1.15	1.67±0.45
2	4	0.03	6.0±0.9	15.0±0.7	15.56±2.25[a]	1.02±0.36
3	4	0.1	3.8±1.0	12.3±0.9	10.01±1.40[a]	0.71±0.21[a]
4	5	0.3	6.2±1.0	14.4±1.1	8.90±0.15[a]	0.60±0.11[a]

Values are the mean±S.D.

[a] $P<0.05$, significant difference from BPH control (Group 1).

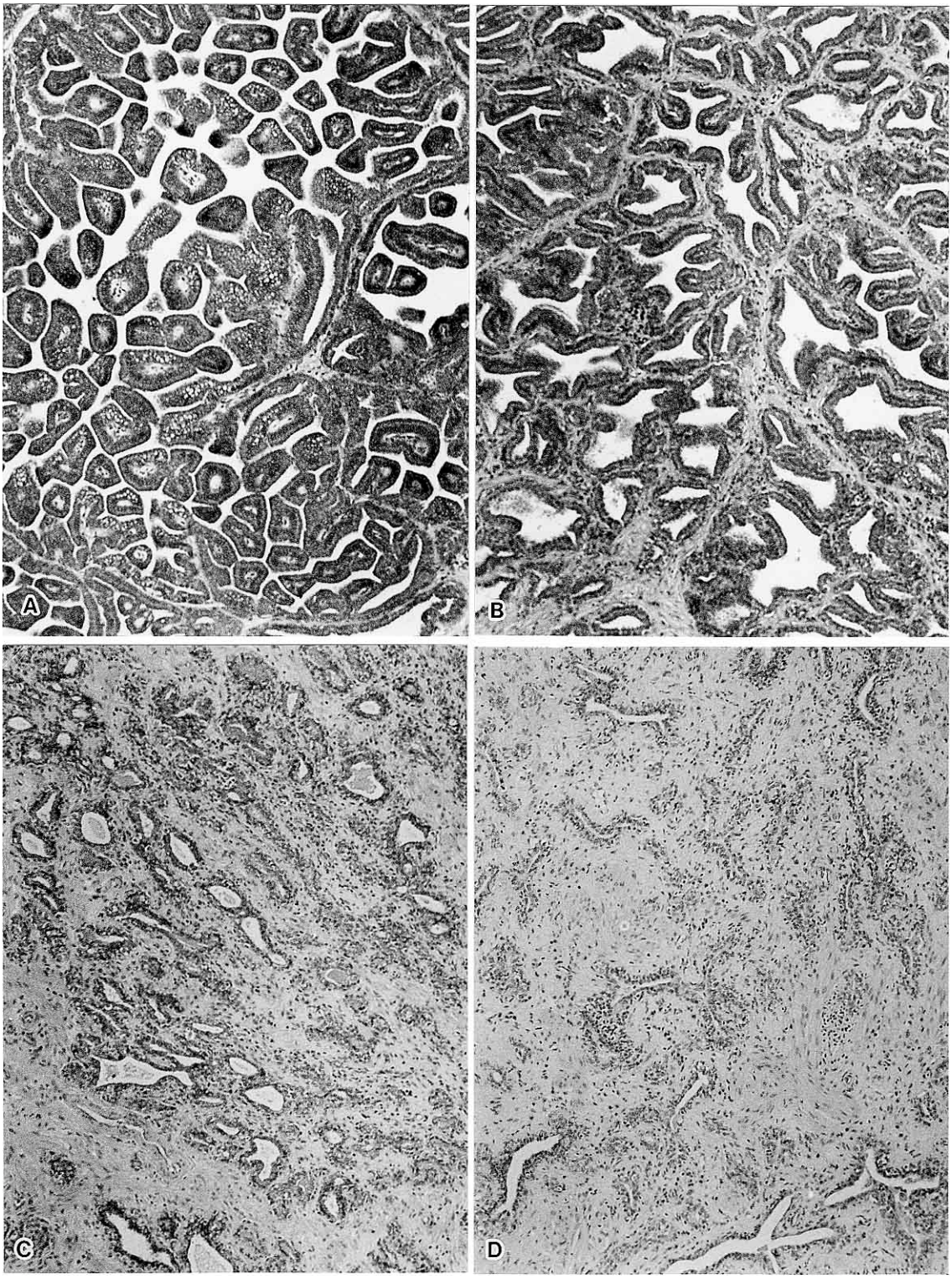

Fig. 1. **A**: Prostate of a dog with spontaneous BPH. Glandular hyperplasia is dominant. **B**–**D**: Prostate of a dog with spontaneous BPH after treatment with CMA 0.03 (**B**), 0.1 (**C**) and 0.3 (**D**) mg/kg/day. The glandular epithelium is dose-dependently atrophied. In contrast, interacinar stroma is prominent. HE ×150 (**A**–**D**).

Light microscopic findings

HE staining

In group 1, glandular epithelial cells were prominent and showed papillary projections extending into the acini (Fig. 1A). Thus, histological features of glandular hypertrophy and/or hyperplasia were evident in this group

Table 2. *Histopathological findings*

Organ	Findings	CMA (mg/kg)			
		0 (n=4)	0.03 (n=4)	0.1 (n=4)	0.3 (n=5)
Prostate					
	Glandular hypertrophy/hyperplasia	4	0	0	0
	Glandular atrophy				
	mild	0	3	2	0
	moderate	0	1	1	2
	severe	0	0	1	3
	Prominence of fibro-muscular stroma				
	mild	0	3	2	0
	moderate	0	1	1	2
	severe	0	0	1	3

Table 3. *Effect of CMA on apoptosis of BPH dog prostate*

BPH control	BPH+CMA 0.03 mg/kg	BPH+CMA 0.1 mg/kg	BPH+CMA 0.3 mg/kg
0.76±0.03%	15.41±1.26%**	2.63±0.98%*	1.45±0.85%*

Apoptotic index is calculated as the positive cell number in one acinus/total cell number in the acinus ×10. Values are the mean±S.D.
*, P<0.05; **, P<0.01, significant difference from BPH control.
CMA, Chlormadinone acetate; BPH, Benign prostatic hyperplasia.

(Table 2). The amount of interacinar stroma was variable but not extensive. In CMA-treated animals (groups 2 to 4), the glandular epithelial cells were dose-dependently atrophic and the acini became completely atrophic (Fig. 1B–D). Thus, histological features of glandular atrophy were evident in this group. In contrast, the interacinar fibro-muscular stroma was prominent (Table 2).

Apoptosis

To evaluate the frequency of apoptosis, we counted the positive cells stained by the nick end labeling method (Table 3). In group 1, the apoptotic index was about 0.76±0.03%. In

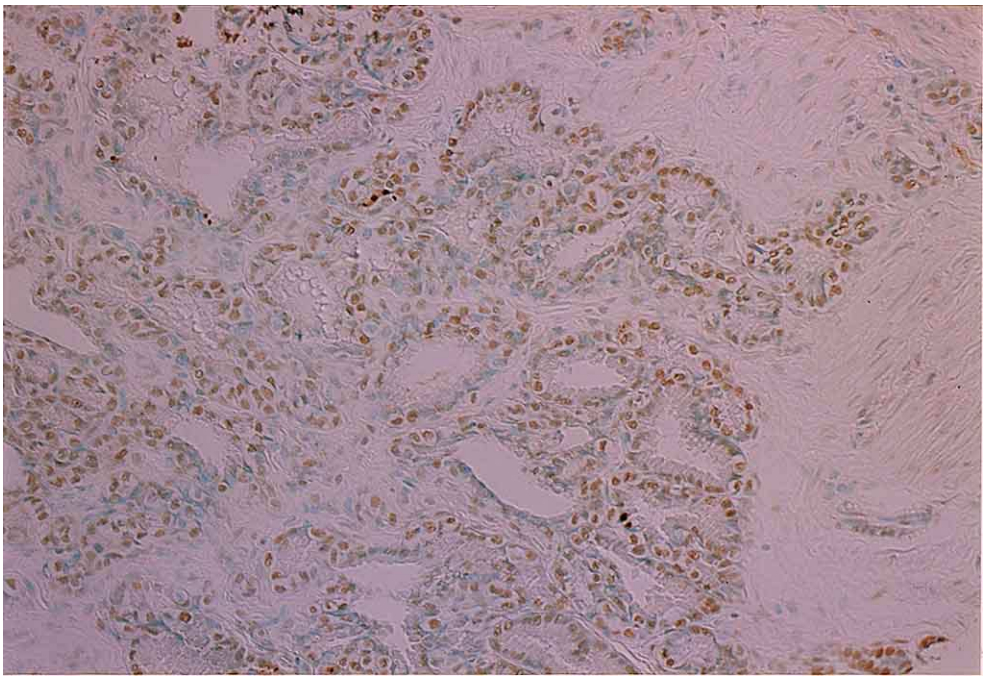

Fig. 2. Prostate of a dog with spontaneous BPH after treatment with CMA 0.03 mg/kg/day. Apoptosis measured by terminal deoxynucleotidyl transferase assay. They are labeled with the ApopTag kit. Peroxidase-labeled antibody method, ×400.

groups 2 to 4, apoptotic index were 15.41±1.26% ($p<0.01$) (Fig. 2), 2.63±0.98% ($p<0.05$) and 1.45±0.85% ($p<0.05$), respectively. In addition, positive cells were mainly observed in the glandular epithelial cells.

Histologically, CMA produced marked atrophy of the glandular epithelium. It is a well documented fact that CMA inhibits the uptake of testosterone in the prostate and is selectively incorporated into prostate cells, resulting in inhibiting testosterone binding to the cytosol 5α-dihydrotestosterone (DHT)-receptor [11, 19–22]. In our previous reports [19–22], the intensity of the immunostaining of nuclear androgen receptor in both epithelial and fibromuscular stromal cells was dose-dependently weakened by treatment with CMA. It is a well documented fact that prostatic nuclear androgen receptor contents were decreased after treatment with gonadotropin-releasing hormone (GnRH) agonist [6] as well as cyproterone acetate [9], an antiandrogenic agent. We postulated that, decreased immunostaining of androgen receptor after treatment with CMA may be explained by a decrease in the number of AR. In fact, CMA inhibits the binding to androgen and androgen receptor competitively (data not shown). Therefore, CMA binds competitively to the androgen receptor from the prostate, and dose-dependent oral administration causes regression of the hyperplastic prostatic weight.

Changes in the BPH size reflect the balance between cell proliferation and death. It has been accepted that certain antiandrogens cause the suppression of DNA synthesis by competitive inhibition against the forming complex of androgen and androge receptor, possibly resulting in BPH regression [12]. In other words, antiandrogen is considered to show static action against BPH. Thus, the pharmacological effects of antiandrogen seem to be complex, i.e. the agent causes apoptosis as an acute effect and also suppression of cell proliferation as a subacute effect. Present experiments clearly show that apoptosis of the hyperplastic prostatic cells occurs after CMA administration for 6 months. Based on our data and these facts, regression of BPH after treatment with CMA may be apoptotic cell death.

V. References

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