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# Effect of Antiandrogen, Chlormadinone Acetate (CMA), in Canine Spontaneous Benign Prostatic Hyperplasia (BPH)

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**The effect of 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 three experimental groups. Group 1 consisted of untreated controls. Groups 2 and 3 received CMA 0.03, and 0.1 mg/kg/day, p.o., respectively, for 6 months. In group 1, glandular hyperplasia of the prostate was clearly detected. In groups 2 and 3, CMA produced marked atrophy of the glandular epithelium. In addition, a histopathological study showed that CMA medication for 6 months exerted no effect on the testes and adrenals or on immunoreactive LH- and ACTH- cells of the anterior pituitary glands. Therefore, it is suggested that CMA (0.03 and 0.1 mg/kg) causes regression of spontaneous canine BPH without any histopathological effects on the testes, adrenals or anterior pituitary LH- and ACTH-cells.**

**Key words : Chlormadinone acetate (CMA), Benign prostatic hyperplasia (BPH), Beagle dog, Prostate, Testis, Adrenal gland**

## INTRODUCTION

Among laboratory animals, the dog is the only species that spontaneously develops benign prostatic hyperplasia (BPH) with a high frequency [3, 15]. Although some differences exist between human and canine BPH, the dog is considered to be a good animal model of BPH to test efficacy of drugs that cause shrinkage of the hyperplastic gland [5, 6, 13, 14].

Several antiandrogens such as chlormadinone acetate (CMA) or cyproterone acetate (CPA) have been used in the medical management of human BPH or prostatic carcinoma [6, 9, 10]. The atrophic effects of CMA and CPA on the prostate have been reported by several authors.

On the other hand, it is well known that steroidal antiandrogens such as CPA inhibit gonadotropin (LH) secretion and testosterone biosynthesis, when given alone [12], unlike non-steroidal antiandrogens, which produce increases in LH and testosterone. Furthermore, the atrophic effects of CPA and CMA on the adrenal glands have been reported by several authors [2, 7].

The purpose of the present study was to further examine the effect of CMA on spontaneous canine BPH. In addition, the effects of CMA on testicular and adrenal morphology and anterior pituitary LH- and ACTH-cells were also investigated.

## MATERIALS AND METHODS

### Animals

Twelve male beagle dogs were purchased from Hazelton Research product, Inc. (Denver, PA). The animals were housed individually in stainless steel cages in a semibarrier system maintained at a room temperature of 22 ± 3℃, 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 considered to have a BPH on the basis of biopsy.

### Experiments

Four animals served as BPH untreated controls (group 1). Group 2 and 3 were administered orally 0.03 (group 2, n = 4),

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and 0.1 (group 3, n = 4) mg/kg/day of CMA as a crystalline powder in gelatin capsule for 6 months. All animals were sacrificed by exsanguination under pentobarbital anesthesia at the end of the experimental period.

**Histopathological examination**

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

**Immunohistochemical staining of anterior pituitary LH- and ACTH-cells**

Rabbit antisera against bovin LH (UCB-Bioproducta, Belgium) and porcine ACTH (Advance, Tokyo) were used. The specificity of these antisera for staining LH cells and ACTH cells have been evaluated previously [4]. The antisera at 1:1000 dilution were incubated with the sections at room temperature for 30 min. Then, the sections were incubated with horseradish peroxidase (Sigma Chemical Co., St. Louis, MO) -labeled anti-rabbit IgG (supplied by Prof. K. Watanabe, Department of Pathology, Tokai University, School of Medicine, Isehara) for 30 min at room temperature. After the incubation was completed, the sections were treated for 5 to 10 min at room temperature with Graham-Karnovsky's reaction medium [8], consisting 20mg of 3,3'-diaminobenzidine (DAB, Wako Pure Chemical Industries, Osaka) and 0.005% hydrogen peroxide in 0.05M Tris-HCl buffer, pH 7.6. The sections were finally counterstained for nuclei with 1% methyl green dissolved in veronal acetate buffer, pH 4.2.

The number of cells per visual field of light microscope was counted at × 400. Ten fields were examined and averaged for each group.

## RESULTS

**1. Organ weight**

The BPH controls in group 1 showed a significant mean increase in prostatic weight (Table 1). On the other hand, administration of CMA (groups 2 and 3) resulted in marked reduction of prostatic weight in comparison with group 1 (Table 1). Pituitary weight showed any significant differences among the groups (Table 1).

**2. Light microscopic findings**

a. Prostates

In group 1, glandular epithelial cells were markedly hypertrophic and showed an increased number of papillary extending into acini. Thus, histological features of glandular hypertrophy and/or hyperplasia were evident in this group (Table 3). The amount of interacinar stroma was variable but not extensive. In CMA-treated animals (groups 2 and 3), the glandular epithelial cells were markedly atrophic and the acini had become completely atrophic. Thus, histo-

**Table 1** Effect of Chlormadinone Acetate (CMA) on Prostatic Weight and Pituitary Weight

| Group | n | Dose (mg/kg) | Prostatic weight (g) | | Pituitary weight (mg) | |
|---|---|---|---|---|---|---|
| | | | Absolute | Relative | Absolute | Relative |
| 1 | 4 | 0 | 28.86 ± 2.15 | 1.82 ± 0.32 | 76.72 ± 4.02 | 6.52 ± 1.52 |
| 2 | 4 | 0.03 | 18.21 ± 3.05[a] | 1.02 ± 0.28 | 77.00 ± 5.18 | 6.72 ± 1.82 |
| 3 | 4 | 0.1 | 9.92 ± 1.05[a] | 0.66 ± 0.15[a] | 75.50 ± 7.65 | 7.05 ± 0.12 |

Values are means ± S.D. [a] $P<0.05$, significant difference from BPH control (Group 1: Student's t test).

**Table 2** Effect of Chlormadinone Acetate (CMA) on Anterior Pituitary LH-and ACTH-cells

| Group | n | Dose (mg/kg) | Anterior pituitary | |
|---|---|---|---|---|
| | | | LH cells[a] | ACTH cells[a] |
| 1 | 4 | 0 | 132.00 ± 9.95 | 89.21 ± 5.03 |
| 2 | 4 | 0.03 | 137.23 ± 9.29 | 88.82 ± 4.23 |
| 3 | 4 | 0.1 | 135.26 ± 7.71 | 90.01 ± 3.93 |

Values are means ± S.D. [a] Cell counts are expressed as the number of cells per visual field by light microscopy at × 400.

logical features of glandular atrophy were evident in this group. In contrast, the interacinar fibro-muscular stroma was prominent (Table 3).

b. Testes

No evidence of abnormal spermatogenesis was seen in the seminiferous tubules and no changes in the Leydig cell population. No evidence of Leydig cell hyperplasia or atrophy was observed in any of the experimental groups.

c. Adrenal glands

No conspicuous changes were noted in any of the experimental groups.

d. Anterior Pituitary LH- and ACTH-cells

Administration of CMA produced no significant treatment-related changes in the number of immnoreactive cells (Table 3).

## DISCUSSION

In the present study, glandular hyperplasia of the prostate was seen in spontaneous canine BPH. The histological appearance of the prostates in animals that had been treated with 5 alpha-androstane-3 alpha, 17 beta-estradiol plus 17 beta-estradiol, or castrated animals, resembled that of glandular hyperplasia [1]. Therefore, glandular-type prostatic hyperplasia was thought to be the main feature of canine BPH occurring spontaneously or experimentally as a result of treatment with steroid hormones.

Histologically, CMA produced marked atrophy of the glandular epithelium. In addition, loss of secretory and metabolic activities was evident. 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 5 alpha-dihydrotestosterone (DHT)-receptor [10]. Thus, the uptake of testosterone and/or its androgenic effect on the prostate may be suppressed by CMA. In fact, immunostaining of nuclear androgen receptor (AR) in both epithelial and stromal cells has been decreased after treatment with CMA by us [11]. Quantative analysis of the prostatic compartments after 6 months of treatment showed that all compartments were decreased when compared to control values (data not shown). The shrinkage of the prostate, therefore, results from an effect on all prostatic compartments and not only on

**Table 3** Histopathological Findings

| Organ | Findings | CMA (mg/kg) | | |
|---|---|---|---|---|
| | | 0 (n = 4) | 0.03 (n = 4) | 0.1 (n = 4) |
| Prostate | Glandular hypertrophy/hyperplasia | 4 | 0 | 0 |
| | Glandular artrophy | | | |
| | mild | 0 | 3 | 2 |
| | moderate | 0 | 1 | 1 |
| | severe | 0 | 0 | 1 |
| | Prominence of fibro-muscular stroma | | | |
| | mild | 0 | 3 | 1 |
| | moderate | 0 | 1 | 3 |
| Testis | Atrophy of seminiferous tubules | | | |
| | mild | 0 | 0 | 0 |
| | moderate | 0 | 0 | 0 |
| | severe | 0 | 0 | 0 |
| Adrenal | Atrophy of cortex | | | |
| | mild | 0 | 0 | 0 |
| | moderate | 0 | 0 | 0 |
| | severe | 0 | 0 | 0 |
| Pituitary | Decreased number of LH cells | 0 | 0 | 0 |
| | Decreased number of ACTH cells | 0 | 0 | 0 |

the epithlium. Based on our data, atrophy after treatment with CMA may be due to shrinkage of both glandular and stromal compartments in the prostate. In humans, prostatic hyperplasia is the result of an increase in both glandular and stromal compartments. Therefore, it is suggested that effects on both compartments are required to achieve the intended clinical benefits in patients treated with CMA.

It is well known that steroidal antiandrogens such as CPA inhibit gonadotropin (LH) secretion and testosterone biosynthesis [12]. The present histopathological study showed that CMA (0.03 and 0.1 mg/kg) medication for 6 months exerted to effect on the testes or anterior pituitary LH cells. Therefore, it is suggested that CMA in smaller doses (0.1 mg/kg or less) causes regression of spontaneous canine BPH without any significant histopathological changes in the testes or anterior pituitary LH cells.

It is generally accepted that CMA has some glucocorticoid-like activities in rodents, and suppression of adrenal function is evident in rodents [2, 7]. The present study showed that CMA medication for 6 months exerted no effect on the adrenal gland or anterior pituitary ACTH cells. Therefore, it is suggested that CMA causes atrophy of the prostate without any significant histopathological changes in the adrenal glands or anterior pituitary ACTH cells under the present experimental conditions.

Therefore, it is suggested that CMA (0.03 and 0.1 mg/kg) causes regression of spontaneous canine BPH without any histopathological effects on the testes, adrenals or anterior pituitary LH- and ACTH-cells.

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