ATROPHIC EFFECTS OF ANTIANDROGEN, CHLORMADINONE ACETATE (CMA) ON DOG PROSTATE WITH SPONTANEOUS BENIGN PROSTATIC HYPERPLASIA

Masanori MURAKOSHI, Masashi TAGAWA and Rie IKEDA

Safety Research Department, Teikoku Hormone Mfg. Co., Ltd.,
1609 Shimosakunobe, Takatsu-ku, Kawasaki-city, Kanagawa 213-0033, Japan

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ABSTRACT — The atrophic 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. The glandular epithelial cells showed uniformly intense nuclear staining for androgen receptor (AR). AR was also localized in the nuclei of the fibro-muscular stromal cells. Immunoreactivity of 5 alpha-reductase type I was positive in most glandular epithelial cells. No fibro-muscular stromal cells were stained. Immunolocalization of 5 alpha-reductase type II was clearly detected in the interacinar fibro-muscular stromal cells, but not in the glandular epithelial cells. In groups 2 to 4, CMA produced marked atrophy of the glandular epithelium. The interacinar fibro-muscular stroma was prominent. The nuclear staining for AR in both epithelial and stromal cells was remarkably decreased. Furthermore, the immunoreaction for 5 alpha-reductase type I in most glandular epithelial cells was negative or very weak. The immunoreaction of 5 alpha-reductase type II in the interacinar fibro-muscular stromal cells was negative or very weak. These results indicate that the uptake of testosterone and/or its androgenic effect on the prostate may be suppressed by CMA. The decreased AR-immunostaining may be explained by the decrease in the number of AR and/or antibody binding sites for AR. Therefore, the atrophy after treatment with CMA may be due to shrinkage of both glandular and stromal compartments in the prostate tissue.

KEY WORDS: Benign prostatic hyperplasia (BPH), Beagle dog, Androgen receptor (AR), Chlormadinone acetate (CMA), 5 alpha-reductase type I, 5 alpha-reductase type II

INTRODUCTION

Among laboratory animals, the dog is the only species that spontaneously develops benign prostatic hyperplasia (BPH) with a high frequency (Walsh and Willson, 1976; DeKlerk et al., 1979). 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 (Geller et al., 1975; Okada et al., 1988; Tunn et al., 1988; Forti et al., 1989).

Several antiandrogens such as chlormadinone acetate (CMA) or cyproterone acetate (CPA) have been used in the medical management of human BPH or prostatic carcinoma (Geller et al., 1975; Ito et al., 1977; Huang et al., 1985). The atrophic effects of CMA and CPA on the prostate have been reported by several authors (Murakoshi et al., 1990, 1992, 1999; Harada et al., 1994). However, a report concerning detailed immunohistochemical and histopathological evaluation of canine BPH treated with CMA has not appeared.

In the present study, we attempted to observed immunohistochemical localization of androgen receptor (AR) as well as steroid 5 alpha-reductase type I and type II in order to clarify the atrophic effect of CMA administration on canine BPH.

Correspondence: Masanori MURAKOSHI

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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 considered to have a BPH on the basis of biopsy (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 weights of prostates were recorded (absolute weight). Weights relative to body weight (relative weight) were calculated.

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

Immunohistochemical staining
1. Androgen receptor (AR)
The prostates were frozen in dry-ice-cooled ethanol. Frozen sections (6 μm in thickness) were prepared in a cryostat and mounted on glass slides. The sections were fixed for 10 min at 4°C in Zamboni’s fixative (Zamboni and McMartin, 1967). After washing in 0.01 M phosphate-buffered saline (PBS) containing 20% sucrose, the sections were soaked in absolute methanol containing 0.3% hydrogen peroxide for 30 min at room temperature to inactivate endogenous peroxidase. After washing in 0.01 M PBS, the sections were incubated overnight at 4°C with NH 27, a rabbit polyclonal antiandrogen receptor antibody (1:1000). After washing in 0.01 M PBS, the sections were covered with biotin-conjugated goat anti-rabbit IgG for 1 hr, washed in 0.01 M PBS and then treated with streptavidin-biotin-peroxidase complex (Histofine, SAB-PO (R) Kit, Nichirei, Tokyo) for 1 hr. After the incubation was completed, the sections were treated for 5 to 10 min at room temperature with Graham-Karnovsky’s reaction medium (Graham and Karnovsky, 1966), which contained 20 mg of 3,3’-diaminobenzidine (DAB, Wako Pure Chemical Industries, Osaka) and 0.05% hydrogen peroxide in 0.05 M 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.

2. 5 alpha-reductase type I and type II staining
Formalin fixed and paraffin sections were used. Rabbit antisera against rat 5 alpha-reductase type I and human 5 alpha-reductase type II were used. The specificity of these antisera for staining 5 alpha-reductase type I and type II have been evaluated previously (Miyamoto et al., 1996a,b). The antisera at 1:1000 dilution were incubated with the sections at room temperature for 30 min. Then, the sections were covered with biotin-conjugated goat anti-rabbit IgG for 1 hr, washed in 0.01 M PBS and then treated with streptavidin-biotin-peroxidase complex (Histofine, SAB-PO (R) Kit, Nichirei, Tokyo) for 1 hr. After the incubation was completed, the immunoperoxidase staining was performed as described above.

Statistical analysis
The data were expressed as mean±S.D. Homogeneity of variance was tested by Bartlett’s method, 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 (Yoshimura, 1997) was performed between the control group and the other experimental groups.

RESULTS

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 significantly (p<0.05) reduced statistically, compared to the control values.

Light microscopic findings
In group 1, glandular epithelial cells were markedly hypertrophic and showed an increased number of papillary projections extending into the acini (Photo 1A). Thus, histological features of glandular hypertro-
The effect of CMA on canine spontaneous BPH.

Phy and/or hyperplasia were evident in this group (Table 2). The amount of interacinar stroma was variable but not extensive. The glandular epithelial cells showed uniformly intense nuclear immunostaining for AR (Photo 2A). AR was also localized in the nuclei of the fibro-muscular stromal cells. Immunoreactivity of 5 alpha-reductase type I was positive in most glandular epithelial cells (Photo 3A). The staining was positive in the cytoplasm but not in the nuclei. No fibro-muscular stromal cells were stained. Immunoreactivity of 5 alpha-reductase type II was positive in interacinar fibro-muscular stromal cells, but not in the glandular epithelial cells (Photo 4A). The reaction products were diffusely distributed in the cytoplasm but not in nuclei.

In CMA-treated animals (groups 2 to 4), the glandular epithelial cells were markedly atrophic and the acini had completely atrophic. Thus, histological features of glandular atrophy were evident in this group (Photo 1B). In contrast, the interacinar fibro-muscular stroma was prominent (Table 2). By CMA administration, intensity of the immunoreaction for AR was dose-dependently decreased in both glandular epithelial cells and fibro-muscular stromal cells (Photo 2B) (Table 2). The intensity of staining of both 5 alpha-reductase type I and type II became markedly decreased (Photos 3B and 4B) (Table 2).

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

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Dose (mg/kg)</th>
<th>Prostatic weight (g)</th>
<th>Absolute</th>
<th>Relative</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>0</td>
<td>26.58 ± 1.15</td>
<td>1.67 ± 0.45</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>0.03</td>
<td>15.56 ± 2.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.02 ± 0.36</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>0.1</td>
<td>10.01 ± 1.40&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.71 ± 0.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>0.3</td>
<td>8.90 ± 0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.60 ± 0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± S.D.

<sup>a</sup>p<0.05, significant difference from BPH control (Group 1).

### Table 2. Histopathological findings.

<table>
<thead>
<tr>
<th>Organ</th>
<th>Findings</th>
<th>CMA (mg/kg)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>0.03</td>
<td>0.1</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(n=4)</td>
<td>(n=4)</td>
<td>(n=4)</td>
<td>(n=5)</td>
</tr>
<tr>
<td>Prostate</td>
<td>Glandular hypertrophy/hyperplasia</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Glandular atrophy</td>
<td>mild</td>
<td>0</td>
<td>3</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>moderate</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>severe</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Prominence of fibro-muscular stroma</td>
<td>mild</td>
<td>0</td>
<td>3</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>moderate</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>severe</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Decreased number of AR-positive cells</td>
<td>mild</td>
<td>0</td>
<td>4</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>moderate</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>severe</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Decreased number of 5 alpha-reductase type I-positive cells</td>
<td>mild</td>
<td>0</td>
<td>4</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>moderate</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>severe</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Decreased number of 5 alpha-reductase type II-positive cells</td>
<td>mild</td>
<td>0</td>
<td>4</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>moderate</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>severe</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>
Photo 1. A: Prostate of a dog with spontaneous BPH. Glandular hyperplasia is dominant. HE × 120.
B: Prostate of a dog with spontaneous BPH after treatment with CMA 0.3 mg/kg/day. The glandular epithelium is atrophic, and interacinar stroma is prominent. HE × 240.

Photo 2. A: Prostate of a dog with spontaneous BPH. The glandular epithelial cells show uniformly intense immunostaining for nuclear AR. AR is also localized in the nuclei of the fibro-muscular stromal cells (arrows). Peroxidase-labeled antibody method, × 120.
B: Prostate of a dog with spontaneous BPH after treatment with CMA 0.3 mg/kg/day. The immunoreaction for AR is negative or very weak in both glandular epithelial cells and fibro-muscular stromal cells. Peroxidase-labeled antibody method, × 240.
Effect of CMA on canine spontaneous BPH.

Photo 3. A: Prostate of a dog with spontaneous BPH. 5 alpha-reductase type I is localized in glandular epithelial cells. No interacinar stromal cells are stained. Peroxidase-labeled antibody method, ×240.
B: Prostate of a dog with spontaneous BPH after treatment with CMA 0.3 mg/kg/day. The immunoreaction for 5 alpha-reductase type I is negative or very weak in glandular epithelial cells. Peroxidase-labeled antibody method, ×240.

B: Prostate of a dog with spontaneous BPH after treatment with CMA 0.3 mg/kg/day. The immunoreaction for 5 alpha-reductase type II is negative or very weak in interacinar fibro-muscular cells. Peroxidase-labeled antibody method, ×240.
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-diol plus 17 beta-estradiol, or castrated animals, resembled that of glandular hyperplasia (Bartsch et al., 1987). 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.

The pathogenesis of BPH is not yet understood, but there are epidemiological and experimental data suggesting that testosterone and androgens play an important role. In the prostate, testosterone is irreversibly converted to dihydrotestosterone (DHT) by an enzyme, 5 alpha-reductase. DHT, the major androgen of the prostate, has a higher affinity for androgen receptors than testosterone (Grino et al., 1990), and is required for the normal development and function of the gland. DHT levels are increased in canine BPH (Isaacs and Coffey, 1981), but BPH does not develop in castrated dogs.

Rabbit polyclonal antibody referred to as NH 27 was raised against human AR (Mizokami et al., 1992). The specificity of the antibody in immunohistochemical reaction has been described elsewhere (Mizokami et al., 1992; Murakoshi et al., 1993, 1994). In the present study, AR was detected in the nuclei in both glandular epithelial and fibro-muscular stromal cells. Human prostate epithelial cells in BPH showed uniformly intense nuclear staining for AR (Sar et al., 1990). Furthermore, intense AR staining has been observed in stromal cells of fibro-muscular hyperplasia (Sar et al., 1990). The intense staining of AR in the epithelium is also in contrast with the abundance of 5 alpha-reductase in the stroma, which converts testosterone into dihydrotestosterone (DHT) in the prostate. It is, therefore, possible that the epithelium utilized DHT supplied by the stroma.

In the present study, 5 alpha-reductase type I was localized in the cytoplasm of most glandular epithelial cells of the hyperplastic prostate. As to the subcellular localization of 5 alpha-reductase type I in the prostate, Miyamoto et al. (1996a) demonstrated positive staining for 5 alpha-reductase type I on the membrane of the rough endoplasmic reticulum of the glandular epithelial cells. Furthermore, the hyperplastic glandular epithelium has been characterized by well-developed rough endoplasmic reticulum and Golgi complexes (Murakoshi et al., 1990). Therefore, it seemed likely that 5 alpha-reductase type I exists on the membranes of rough endoplasmic reticulum. In addition, 5 alpha-reductase type II was localized in the cytoplasm of the interacinar fibro-muscular stromal cells, but not in the glandular epithelial cells. Miyamoto et al. (1996b) demonstrated that muscle-specific actin-positive cells in the prostate also stained 5 alpha-reductase type II. Furthermore, these cells contained AR and shrink following exposure to 5 alpha-reductase inhibitor (Miyamoto et al., 1996b). These findings indicate that DHT synthesized by this enzyme in smooth muscle cells acts in an autocrine fashion to direct cell growth. Based on our data and these facts, immunoreactive cells of 5 alpha-reductase type II are considered to be interacinar smooth muscle cells.

CMA produced marked atrophy of the glandular epithelium. In addition, loss of secretory and metabolic activities was evident. These findings were good agreement with the previous report (Murakoshi et al., 1999). 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-DHT-receptor (Ito et al., 1977; Takezawa et al., 1992, 1995). Thus, the uptake of testosterone and/or its androgenic effect on the prostate may be suppressed by CMA. In fact, immunostaining of nuclear AR in both epithelial and fibro-muscular stromal cells was markedly decreased after treatment with CMA. Based on our present data and these facts, decreased immunostaining of AR after treatment with CMA may be explained by a decrease in the number of AR and/or antibody binding site for AR. We further speculated that CMA binds to the prostatic AR and that oral administration causes regression of the hyperplastic prostatic weight.

The immunoreactivity of both 5 alpha-reductase type I in the glandular epithelial cells and of 5 alpha-reductase type II in the interacinar smooth muscle cells was markedly decreased. Quantitative analysis of the prostatic compartments in the high-dose group (0.3 mg/kg) 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 epithelium. 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 (Wilson, 1980). Therefore, it is
suggested that effects on both compartments are required to achieve the intended clinical benefits in patients treated with CMA.

In conclusion, immunostainable AR and steroid 5 alpha-reductase isozyme in the canine prostate is testosterone-dependent, and its staining pattern is a useful marker for biological testosterone action in the prostatic cells.

REFERENCES


Grino, P.B., Griffin, J.E. and Wilson, J.D. (1990): Testosterone at high concentrations with the human androgen receptors acts similarly to dihydrotestosterone. Endocrinology, 126, 1165-1172.


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