

Immunolocalization of Androgen Receptor in Canine Prostatic Hyperplasia

— Effect of Antiandrogen —

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The effect of a synthetic steroidal anti-androgen, chlormadinone acetate (CMA), on spontaneous benign prostatic hyperplasia (BPH) in the dog was investigated. Old male beagle dogs (5 to 8 years old) were divided into the following experimental groups: group 1 consisting of BPH controls, and group 2 which received CMA of 0.3mg/kg/day orally for 6 months. In group 1, glandular hyperplasia of the prostate was clearly observed. The glandular epithelial cells showed uniformly intense nuclear immunostaining for androgen receptor (AR). In contrast, CMA produced marked atrophy of the glandular epithelium. The interacinar fibromuscular stroma was prominent. Furthermore, nuclear immunostaining for AR in both epithelial and stroma cells was remarkably decreased. 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.

Key Words: Chlormadinone acetate (CMA), Benign prostatic hyperplasia (BPH), Glandular hyperplasia of prostate, Androgen receptor (AR), Fibro-muscular stroma

INTRODUCTION

Benign prostatic hyperplasia (BPH) is a prevalent major disease in both the aging dog and man [1, 3, 12]. Therefore, the dog is the best available model for studying BPH since the disease occurs spontaneously or experimentally on treatment with steroid hormones [1, 3, 12]. Furthermore, it is known that canine BPH can be completely cured by either anti-androgen therapy or castration.

Several anti-androgens such as chlormadinone acetate (CMA) or cyproterone acetate (CPA) have been used in the medical management of human BPH or prostatic carcinoma [1, 6]. The atrophic effects of CMA and CPA on the prostate have been reported by several authors. However, studies on the effect of CMA on androgen receptor (AR) levels in canine prostatic tissues are rare.

In the present study, we attempted to observe immunohistochemical localization of AR in order to clarify the atrophic effect of CMA administration on canine BPH.

MATERIALS AND METHODS

Animals

Eight male beagle dogs were used. They received dry dog food (CD-1, CLEA Japan, Inc.) and water *ad libitum*. They were 5-8 years old and considered to have BPH on the basis of biopsy.

Experiments

Four old animals in group 1 were used as untreated controls. Four old animals in group 2 were administered 0.3mg/kg/day of CMA orally as 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

The prostates were 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 for androgen receptor (AR)

The prostates were frozen in dry-ice-cooled ethanol. Frozen sections ($6\ \mu\text{m}$ in thickness) were prepared in a cryostat and mounted on glass slides (APS-coated glass slide, Matsunami Co.). The sections were fixed for 10 min at 4°C in Zamboni's fixative [13]. After washing in 0.01M 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 0.01M PBS, the sections were incubated overnight at 4°C with NH27, a rabbit polyclonal anti-androgen receptor antibody (1:1000). After washing in 0.01M PBS, the sections were covered with biotin-conjugated goat anti-rabbit IgG for 1hr, washed in 0.01M PBS and then treated with streptavidin-biotin-peroxidase complex (Histofine, SAB-PO (R) Kit, Nichirei, Tokyo) for 1hr. After the incubation was completed, the sections were treated for 5 to 10 min at room temperature with Graham-Karnovsky's reaction medium [4], which contained 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, pH4.2.

RESULTS

(1) Prostatic weight

The BPH controls in group 1 showed a significant mean increase in prostatic weight ($18.9 \pm 2.1\text{g}$). However, administration of CMA to group 2 resulted in marked reduction of prostatic weight ($8.3 \pm 1.5\text{g}$) in comparison with group 1.

(2) Light microscopic findings

Group 1 (BPH control) : Representative light microscopic features are shown in Fig.

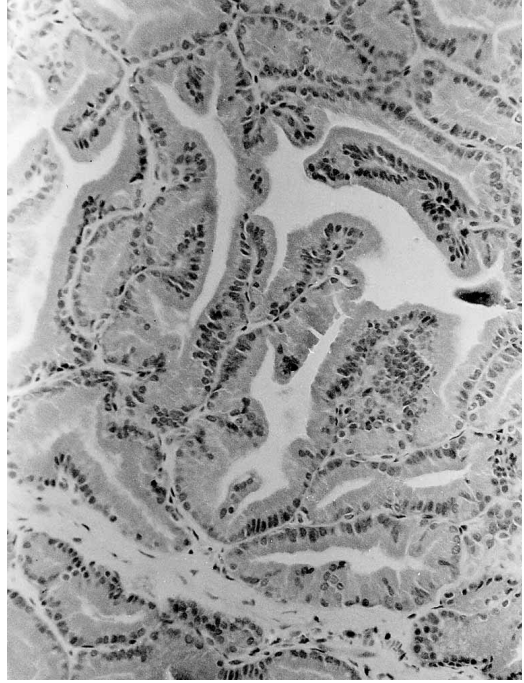


Fig. 1 Prostate of dog with spontaneous BPH. Glandular hyperplasia is dominant. HE, $\times 150$.



Fig. 2 Immunolocalization of AR. The glandular epithelial cells show uniformly intense nuclear immunostaining for AR. AR is also localized in nuclei of the fibro-muscular cells (arrows). ABC methods, $\times 300$.

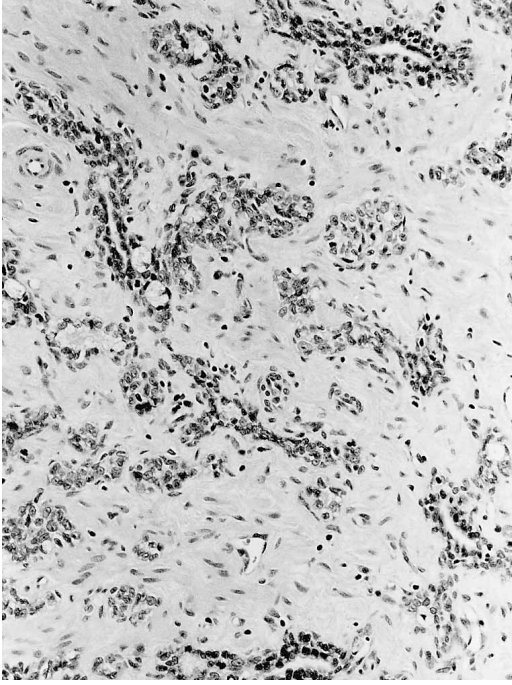


Fig. 3 Prostate of dog with spontaneous BPH after treatment with CMA. The glandular epithelium is markedly atrophic, and the interacinar stroma is prominent. HE, $\times 150$.

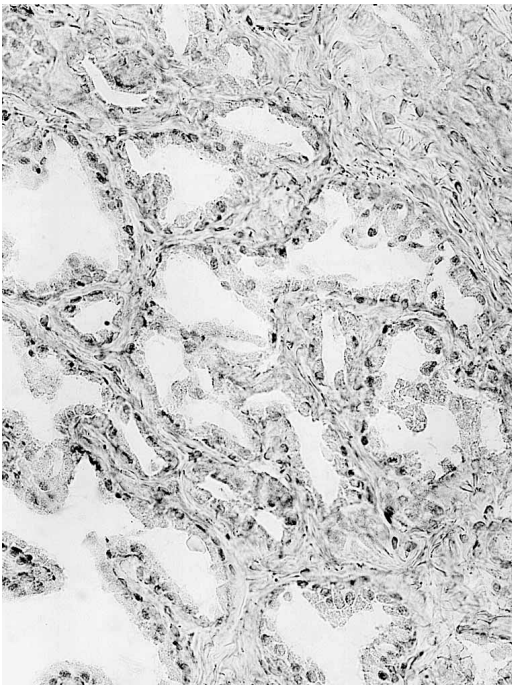


Fig. 4 Immunolocalization of AR. The immunoreaction for AR is negative or very weak in both glandular epithelial cells and fibro-muscular cells. ABC methods, $\times 300$.

1. The glandular epithelial cells were markedly hypertrophic and showed an increased number of papillary projections extending into acinar lumen. Thus, histological features of glandular hypertrophy and/or hyperplasia were evident in this group. The amount of interacinar stroma was variable but not extensive. The glandular epithelial cells showed uniformly intense nuclear immunostaining for AR (Fig. 2). AR was also localized in the nuclei of the fibro-muscular cells.

Group 2 (CMA) : The glandular epithelial cells were markedly atrophic and the acini had become completely atrophic (Fig. 3). Thus, histological features of glandular atrophy were evident in this group. In contrast, the interacinar fibro-muscular stroma was prominent. The immunoreaction for AR was negative or very weak in both glandular epithelial cells and fibro-muscular cells (Fig. 4).

DISCUSSION

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 inhibition of the binding of testosterone to cytosol 5α -dihydrotestosterone (DHT)-receptor [6]. Thus, the uptake of testosterone and/or its androgenic effect on the prostate may be suppressed by CMA.

A rabbit polyclonal antibody referred to as NH27 was raised against human AR [7]. The specificity of the antibody in immunohistochemical reactions has been described elsewhere [7-10]. In the present study, AR was also detected in the nuclei of the stromal fibro-muscular cells. Human prostate epithelial cells in BPH showed uniformly intense nuclear staining for AR [11]. Furthermore, intense AR staining has been observed in stromal cells of fibro-muscular hyperplasia [11]. The intense staining of AR in the epithelium is also in contrast with the abundance of 5α -reductase in the stroma, which converts testosterone into dihydrotestosterone in the prostate. It is, therefore, possible that the epithelium utilizes DHT supplied by the stroma.

Nuclear immunostaining of AR in both epithelial and stromal cells was remarkably decreased after treatment with CMA. It is well documented that prostatic nuclear AR contents after treatment with gonadotropin-releasing hormone (GnRH) agonist [2] as well as CPA [5], an anti-androgen agent, are decreased. Based on our present data and these facts, decreased immunostaining for AR after treatment with CMA may be explained by a decrease in the number of AR and/or antibody binding sites for AR. We further speculate that CMA binds to the prostatic AR and that oral administration causes regression of the hyperplastic prostatic weight.

REFERENCES

- 1) DeKlerk D.P., Coffey D.S., Ewing L.L., Mcdermott I.R., Reiner W.G., Robinson C.H., Talalay J.D., Walsh P.C.: Comparison of spontaneous and experimentally induced canine prostatic hyperplasia. *J Clin Invest* 64: 842-849, 1979.
- 2) Forti G., Salerno R., Moneti G., Zoppi S., Fiorelli G., Martini T., Natali A., Castantini A., Serio M., Martini L., Motta M.: Three-month treatment with a long-acting gonadotropin-releasing hormone agonist of patients with benign prostate hyperplasia. *J Clin Endocrinol Metab* 68: 461-468, 1989.
- 3) Geller J., Fishman J., Cantor T.L.: Effect of cyproterone acetate on clinical endocrine and pathological features of benign prostatic hypertrophy. *J Steroid Biochem* 6: 837-843, 1975.
- 4) Graham R.C. Karnovsky M.J.: The early stages of absorption of injected horseradish peroxidase in the proximal tubules of mouse kidney. *J Histochem Cytochem* 14: 291-302, 1966.
- 5) Huang J.K., Bartsch W., Voit K.D.: Interaction of an antiandrogen (Cyproterone acetate) with the androgen receptor system and its biological action in the rat ventral prostate. *Acta Endocrinol* 109: 569-576, 1985.
- 6) Ito Y., Kurosawa I., Yamanaka H., Koya A., Imai K., Kasaku N., Shida K.: The mechanism of antiandrogenic action of chlormadinone acetate. *Hinyokika Kyo* 68: 537-552, 1977.
- 7) Mizokami A., Masai M., Shimazaki J., Sugita A.: Production of polyclonal antibody against human androgen receptor and immunohistochemical study of human androgen receptor in prostatic tissues. *Jpn J Urol* 83: 1801-1802, 1992.
- 8) Murakoshi M., Inada R., Tagawa M., Suzuki M., Mizokami A., Watanabe K.: Histopathological and immunohistochemical studies in the rat ventral prostate. *J Toxicol Pathol* 6: 55-64, 1993.
- 9) Murakoshi M., Inada R., Tagawa M., Suzuki M., Mizokami A., Watanabe K.: Histopathological and immunohistochemical studies in the rat dorsolateral prostate. *J Toxicol Pathol* 7: 387-395, 1994.
- 10) Murakoshi M., Inada R., Tagawa M., Mizokami A., Suzuki M.: Immunohistochemical study of androgen receptor in rat prostatic hyperplasia. *Acta Histochem Cytochem* 27: 75-80, 1994.
- 11) Sar M., Lubahn D.B., French F.S., Wilson E.M.: Immunohistochemical localization of the androgen receptor in rat and human tissues. *Endocrinology* 127: 3180-3186, 1990.
- 12) Walsh P.C., Wilson J.D.: The induction of prostatic hypertrophy in the dog with androstanediol. *J Clin Invest* 57: 1093-1097, 1976.
- 13) Zamboni L., DeMartino C: Buffered picric acid formaldehyde. A new rapid fixation for electron microscopy. *J Cell Biol* 35: 148A, 1967.