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Immunohistochemical Studies in Canine Prostatic Hyperplasia
— Effect of Antiandrogen —

Masanori MURAKOSHI, Rie IKEDA, Masashi TAGAWA and Takaharu NAKAYAMA

Safety Research Department, Teikoku Hormone Mfg. Co., Ltd., Kawasaki

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To investigated spontaneous benign prostatic hyperplasia (BPH) in dog the effect of a synthetic steroidal antiandrogen, chlormadinone acetate (CMA) was studied. Old male beagle dogs (5-8 years old) were divided into following experimental groups: group 1 consisted of BPH controls; group 2 received CMA 0.3mg/kg/day p.o., for 6 months. In group 1 animals, glandular hyperplasia of the prostate was clearly detected. The glandular epithelial cells showed uniformly intense immunostaining for nuclear androgen receptors (AR). AR was also localized in the nuclei of the fibro-muscular cells. Immunoreactivity of 5α-reductase type I was positive in most glandular epithelial cells. The staining was positive in the cytoplasm but not in the nuclei. No fibro-muscular cells were stained. In contrast, CMA produced marked atrophy of the glandular epithelium. The interacinar fibro-muscular stroma was prominent. Furthermore, immunostaining of nuclear AR of both epithelial and stroma cells was remarkably decreased. The intensity of staining for 5α-reductase type I in most glandular epithelial cells also decreased. Interestingly, some basal cells exhibited positive staining for 5α-reductase type I. These results indicate that the uptake of testosterone and/or its androgenic effect on the prostate may be suppressed by CMA. We further speculate that the basal cells produce sufficient dihydrotestosterone to maintain themselves even in the presence of low testosterone levels.

Keywords : Chlormadinone acetate (CMA), Benign prostatic hyperplasia (BPH), Beagle dog, Glandular hyperplasia of prostate, Androgen receptor (AR), 5α-reductase type I

INTRODUCTION

Among laboratory animals, the dog is the only species that develops spontaneously benign prostatic hyperplasia (BPH) with a high frequency [2, 26]. Although some differences exist between human and dog 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 [4, 5, 16, 17, 21, 25].

Several antiandrogens such as chlormadinone acetate (CMA) or cyproterone acetate (CPA) have been used in the medical management of human BPH or prostatic carcinoma [5, 9, 16, 17]. 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) and 5α-reductase type I in canine prostatic tissues are rare. In the present study, we attempted to observe immunohistochemical localization of AR and 5α-reductase type I 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 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 administrated orally 0.3mg/kg/day of CMA as a crystalline pow-

Masanori MURAKOSHI, Safety Research Department, Teikoku Hormone Mfg. Co., Ltd., 1604 Shimosakunobe, Takatsu-ku, Kawasaki 213-0033 Japan

der 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

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 (APS-coated glass slide, Matsunami Co.). The sections were fixed for 10 min at 4℃ in Zamboni's fixative [27]. 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 inac-

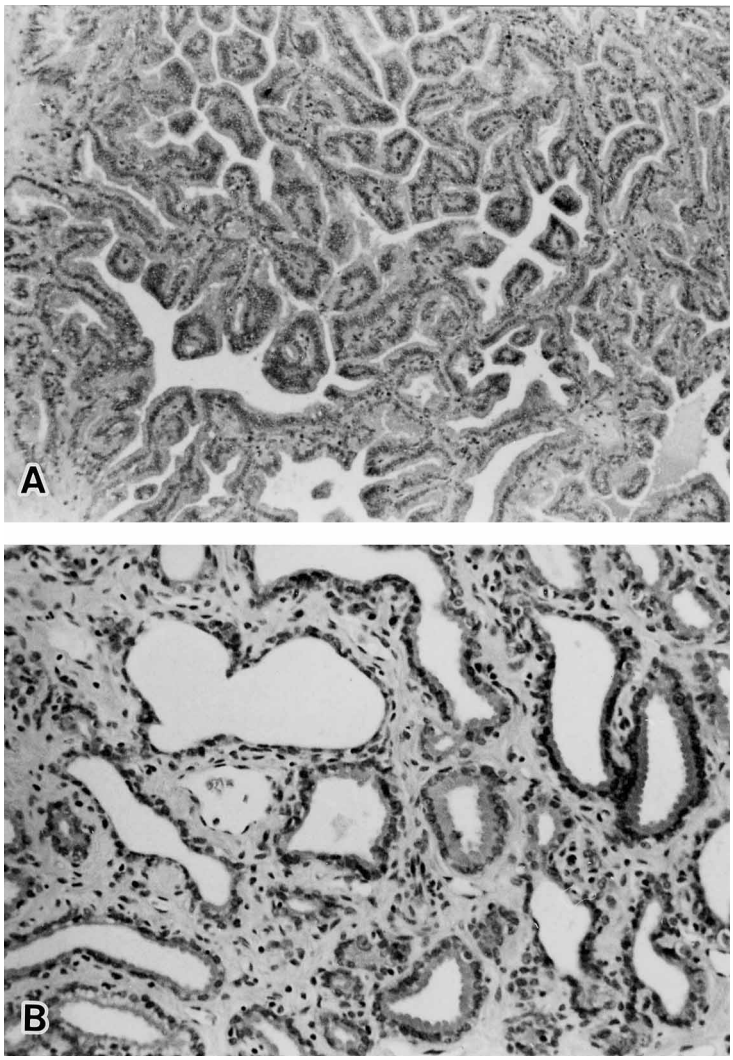

Fig. 1 **A**: Prostate of dog with spontaneous BPH. Glandular hyperplasia is dominant. HE, ×150. **B**: Prostate of dog with spontaneous BPH after treatment with CMA. The glandular epithelium is markedly atrophic, and the interacinar stroma is prominent. HE, ×150.

tivate endogenous peroxidase. After washing in 0.01M PBS, the sections were incubated overnight at 4℃ with NH 27, a rabbit polyclonal anti-androgen receptor antibody [15, 18, 19, 20]. The adjacent sections were incubated at 4℃ with rabbit polyclonal anti-5α-reductasr type I antiserum [14]. After washing in 0.0lM 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 [6], consisting of 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.

RESULTS

(1) Prostatic weight

The BPH controls in group 1 showed a significant increase in prostatic weight (18.9 ± 2.1g). However, administration of CMA to group 2 resulted in a marked reduction of prostatic weight (8.3 ± 1.5g), as compared to group 1.

(2) Light microscopical findings

Group 1 (BPH control) : Representative light microscopical features are shown in Figs. 1A, 2A and 3A. The glandular epithelial cells were markedly hypertrophic and showed an increased number of papillary projections extending into acinar lumen(Fig. 1A). 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. 2A). AR was also localized in the nuclei of the fibro-muscular cells. Immunoreactivity of 5α-reductase type I was positive in most glandular epithelial cells (Fig. 3A). The staining was positive in the cytoplasm but not in the nuclei. No fibromuscular cells were stained.

Group 2 (CMA) : The representative light microscopical features are shown in Figs. 1B, 2B and 3B. The glandular epithelial cells were markedly atrophic and the acini had become completely atrophic (Fig 1B). 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. 2B). The intensity of staining for 5α-reductase type I in most glandular epithelial cells also decreased (Fig. 3B). Interestingly, some basal cells exhibited positive staining for 5α-reductase type I (Fig. 3B). No fibro-muscular cells were stained.

DISCUSSION

The pathogenesis of BPH is not yet understood, but there are epidemiological and experimental data suggesting that androgens play an important role. In the prostate, testosterone is irreversibly converted to dihydrotestosterone (DHT) by an enzyme, 5α-reductase. DHT, the major androgen of the prostate, has a higher affinity for androgen receptors than testosterone [7], and is required for the normal development and function of the gland. DHT levels are increased in canine BPH [10], but BPH does not develop in castrated dogs.

In the present study, 5α-reductase type I was localized in the cytoplasm of most glandular epithelial cells of the hyperplastic prostate. As to the subcellular localization of 5α-reductase type I in the prostate, Miyamoto *et al.* [14] demonstrated positive staining for 5α-reductase type I on the membrane of the rough endoplasmic reticulum of glandular epithelial cells. Furthermore, the hyperplastic glandular epithelium has been characterized by well-developed rough endoplasmic reticulum and Golgi complex [16]. Therefore, it seemed likely that 5α-reductase type I exists on the membranes of rough endoplasmic reticulum. Immunostaining of nuclear AR was detected in both epithelial and stromal fibro-muscular cells. The epithelial cells of human prostate in BPH showed uniformly intense staining for nuclear AR [22]. Furthermore, intense AR staining has been observed in stromal cells of fibro-muscular hyperplasia [22].

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*α*-dihydrotestosterone (DHT) -receptor [12, 23, 24]. 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 stromal cells was markedly decreased after treatment with CMA. Furthermore, the intensity of staining for 5*α*-reductase type I in most glandular epithelial cells also decreased. It is a well documented fact that prostate nuclear AR contents were decreased after treatment with gonadotropin-releasing hormone (GnRH) agonist [4] as well as CPA [9], an antiandrogenic agent. Based on our data and these facts, decreased immunostaining for AR and 5*α*-reductase type I after

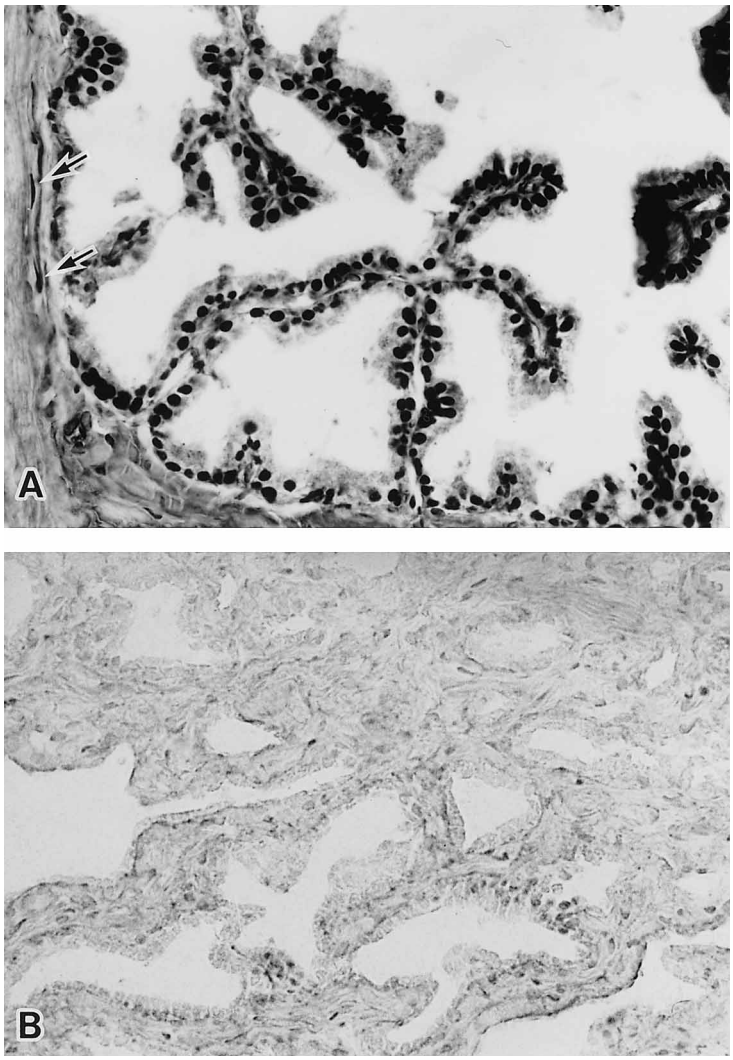

Fig. 2 **A**: Prostate of dog with spontaneous BPH. The glandular epithelial cells show uniformly intense immunostaining for nuclear AR. AR is also localized in the nuclei of the fibro-muscular cells (arrows). ABC method, ×300. **B**: Prostate of dog with spontaneous BPH after treatment with CMA. The immunoreaction for AR is negative or very weak in both glandular epithelial cells and fibro-muscular cells. ABC method, ×300.

treatment with CMA may be explained by the decrease in number of recepor and enzyme, and/or antibody binding site for AR and 5α-reductase type I.

In the present work, 5α-reductase type I was localized in some basal cells after treatment with CMA. Androgen deprivation induces atrophy or loss of the secretory epithelial cells, whereas the basal cells are maintained [3]. Isaacs *et al.* [11] reported that the basal cells persisted even in long-term castrated animals. Since both basal and secretory cells contain AR [1, 13], the difference in the persistence may dependent on the absence or presence of the enzyme which converts the steroids. The basal cells produce sufficient DHT to maintain themselves even in the presence of low testosterone level.

The precise mechanism by which CMA produces atrophy of the prostatic hyperpla-

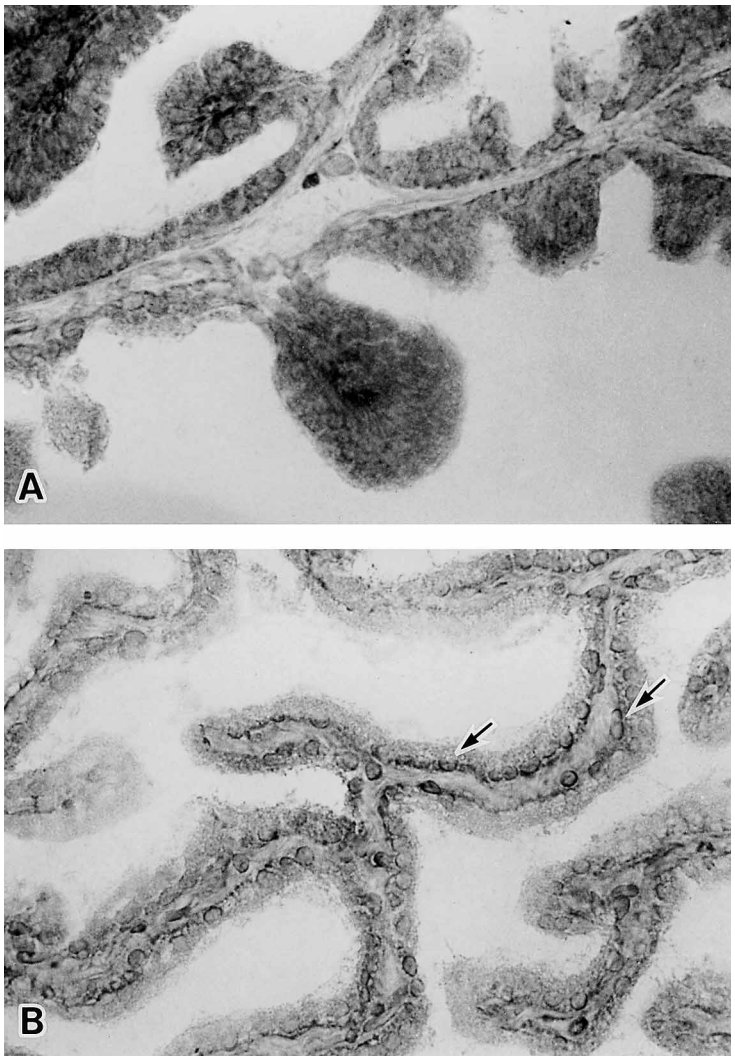

Fig. 3 **A**: Prostate of dog with spontaneous BPH. Immunoreactivity for 5α-reductase type I is positive in most glandular epithelial cells. The staining is positive in the cytoplasm but not in the nuclei. ABC method, ×300. **B**: Prostate of dog with spontaneous BPH after treatment with CMA. The intensity of staining for 5α-reductase type I is decreased in most glandular epithelial cells. Some basal cells stained positive for 5α-reductase type I (arrows). ABC method, ×300.

sia is not yet clear from the present investigation. In this context, quantitative evaluation of prostatic compartments by morphometric analysis, and immunohistochemistry of 5α-reductase type II in the prostate would seem to be important to clarify this problem. Further work along this line is now in progress in our laboratory.

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ERRATA

Per Capita Gross National Product and Summarized Odds Ratio for Epidemiologic Studies on the Relationship between Passive Smoking and Lung Cancer

Minoru SUGITA, Takashi IZUNO, Masao KANAMRI, Yumi OTAHARA* and Hitoshi KASUGA**

Department of Environmental and Occupational Health, Toho University School of Medicine,
**Department of Public Health, Hamamatsu University School of Medicine,*
***Tokai University School of Medicine*

Vol.23, No.5, p.236: Figure 1 Should have appeared as follows;

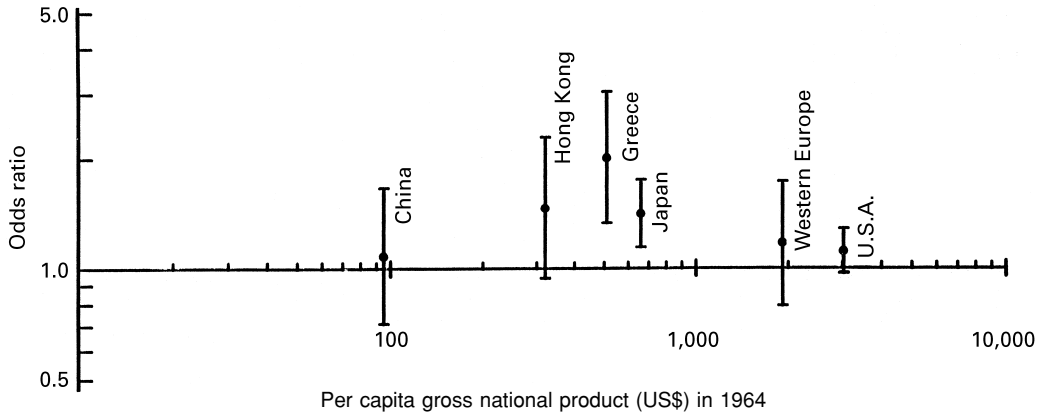

Fig. 1 Summarized odds ratios with 95% confidence intervals of epidemiologic studies on the relationship between environmental tobacco smoke and lung cancer and per capita gross national products in 1964 by country