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Histopathological and Immunocytochemical Studies of Chlormadinone Acetate on the Rat Prostate

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In order to confirm the relationship between glutathione-peroxidase (GSH-PO) and testosterone action in rat ventral prostate, we have studied the immunocytochemical localization of GSH-PO in glandular epithelial cells of rat ventral prostate after chlormadinone acetate CMA) as antiandrogen. In the control rat ventral prostate, GSH-PO was predominantly observed in the glandular epithelial cells and intracellular localization of GSH-PO was exclusively observed in the cytoplasmic matrix near the rough endoplasmic reticulum and it was occasionally noted as small granular structure. In CMA-administered rats, the glandular epithelial cells of the ventral prostate were markedly atrophic. The intensity of GSH-PO staining in the glandular epithelial cells was markedly decreased. Immunoelectron microscopically, GSH-PO-positive granules were hardly seen in the atrophic glandular epithelial cells. These findings strongly suggest that loss of GSH-PO staining in the glandular epithelial cells of the rat ventral prostate treated with CMA as antiandrogen is thought to be caused by inhibition of testosterone action, and that its staining pattern is useful for the effect of antiandrogens or antiprostatic agents on prostate.

Key words : Chlormadinone acetate (CMA), Glandular epithelial cells, Glutathione-peroxidase (GSH-PO), Lipid peroxidation, Rat, Ventral prostate

INTRODUCTION

In our previous reports [5, 6], immunocytochemical localization of glutathione-peroxidase (GSH-PO), which effectively reduces the lipid peroxides, was demonstrated in the rat ventral prostate under castration and testosterone-administration. We postulated that GSH-PO in the glandular epithelial cells of the rat ventral prostate was testosterone-dependent, and that its staining pattern was useful marker for testosterone action [6].

Several antiandrogens such as cyproterone acetate or chlormadinone acetate (CMA) have been used in the medical management of human benign prostatic hyperplasia or prostatic carcinoma [2-4, 9, 11-13] The atrophic effect of CMA on the human prostatic hyperplasia has been reported by several authors [3, 4, 11, 12]. The antiandrogenic mechanisms of CMA has been evaluated biochemically and immunohistochemically, such as androgen receptor content, and steroid 5 α-reductase type II activity in the prostate [4, 7, 11, 12]. However, a report concerning lipid peroxidation and immunohistochemical evaluation of prostate treated with CMA has not appeared.

In the present study, an attempt to observe immunocytochemical localization of GSH-PO was made in order to clarify the testosterone action in prostate under CMA-administration.

MATERIALS AND METHODS

Animals

Male Crj: CD(SD) IGS rats were purchased from Charles River Japan Inc. (Atsugi, Japan) at the age of five weeks. The animals were kept in a barrier-maintained animal room, which was maintained at a temperature of 22 ± 2 ℃ with a relative humidity of 55 ± 15 %. The room was ventilated twenty-one times per hr and provided with 12 hr of

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light (from 8:00 to 20:00). The animals were housed individually in plastic cages (CLEA Japan In). Solid food (CE-2, CLEA Japan Inc.) and tap water were given *ad libitum*. The animals were kept for one-week acclimation period under laboratory conditions.

Experiments

These animals were divided into three experimental groups. Group 1 consisted of untreated controls. In groups 2, rats were administered 50 mg/kg of chlormadinone acetate (CMA) orally gavage singles shot daily for three weeks. CMA was suspended in distilled water containing 2 % Tween 80. The dosages of CMA has been previously shown to cause prostatic atrophy in the rat. Animals of the group 3 were given only vehicle solution (2 mL/kg each) consisting of distilled water and 2 % Tween 80. Each group consisted of five animals, and the rats were sacrificed by exsanguinations under pentobarbital anesthesia at the end of the experimental period and the ventral prostates were removed.

The animals were cared for according to the principles outlined in the guide for the care and use of laboratory animals prepared by the Japanese Association for Laboratory Animal Science and our institution.

Organ weight

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

Histopathological examination

The ventral prostates were fixed in 0.1 M phosphate-buffered 10 % formalin, and embedded in paraffin. Sections cut mounted and stained with hematoxylin and eosin (HE).

Immunohistochemical staining GSH-PO

The ventral prostates were fixed in 0.1 M phosphate-buffered 4 % paraformaldehyde solution, pH 7.4, for 4 to 6 hr at 4℃ under constant agitation. The fixed tissues were then washed in 0.1 M phosphate buffer. Subsequently, 6 μm-thick frozen sections were prepared from the washed tissues in a cryostate, and were placed on glass slides. The sections were washed in 0.01 M phosphate-buffered saline (PBS) and then were direct peroxidase-labeled antibody method using rabbit anti-rat GSH-PO IgG Fab fragment [8].

For light microscopic observations of GSH-PO, 6 μm-thick frozen sections were incubated with the antibody labeled with horseradish peroxidase (HRPO, Sigma Chemical Co., St. Louis, MO) for 1 hr. After the incubation was completed, the sections were treated in Graham-Karnovsky's reaction medium [1], which contained 20 mg% 3,3'-diaminobendizine (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.

For electron microscopic observations of GSH-PO, 6 μm-thick frozen sections were incubated with HRPO-labeled antibody for 6 hr. After the incubation was completed, the sections were incubated for 30 min in Graham-Karnovsky's reaction medium [1], from which the substrate hydrogen peroxide was omitted, and then they were incubated in the fully equipped reaction medium for 5 min. The sections were post-fixed in 2 % O_SO_4 in 0.1 M phosphate buffer, pH 7.4, for 90 min, dehydrated in graded ethanol series, and embedded in Quetol 812 by an inverted gelatin capsule method. Ultrathin sections were prepared with a LKB ultra-microtome and were observed under a JEOL 1200 EX electron microscope.

As an immunologic negative control, normal rabbit serum (NRS) IgG Fab fragment labeled with HRPO was applied on both light and electron microscopic investigations instead of anti-GSH-PO IgG Fab fragment labeled with HRPO.

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 intact control group and the other experimental groups.

RESULTS

1. Organ weight

The organ weights of the control and the animals after treatment are shown in Table 1. Statistically significant decrease was noted in the absolute ($p < 0.01$) and relative ($p < 0.01$) weight of ventral prostate treated with CMA 50mg/kg/day.

2. Histopathological and immunocytochemical studies

a. HE staining

In group 1, the acini of the ventral prostate were relatively large and the shallow papillary projections were present into the acinar lumen. The glandular epithelial cells were of a high columnar shape (Fig. 1A). In group 2, the cells of the glandular epithelial lining were low (Fig. 1B). In group 3, the feature was similar to that of group 1.

b. GSH-PO staining

In group 1, immunocytochemical localization of GSH-PO was predominantly observed in the glandular epithelial cells (Fig. 2A). No reaction products were seen in interstitial tissues. The control serum (NRS) was negative for immunohistochemical localization of GSH-PO in the ventral prostate. By immunoelectron microscopic investigations, GSH-PO was noted in cytoplasmic matrix near the rough endoplasmic reticulum (Fig. 3). Occasionally, GSH-PO was noted as small granular structure (Fig. 3). No reaction products were seen in other cell organelles including mitochondria or endoplasmic reticulum. In group 2, the height of the glandular epithelial cells was remarkably reduced and the intensity of GSH-PO staining was markedly decreased (Fig. 2B). By immunoelectron microscopic investigations, GSH-PO was weakly positive in cytoplasmic matrix (Fig. 4). Furthermore, GSH-PO-positive granules were hardly seen. In group 3, the feature was similar to that of group 1.

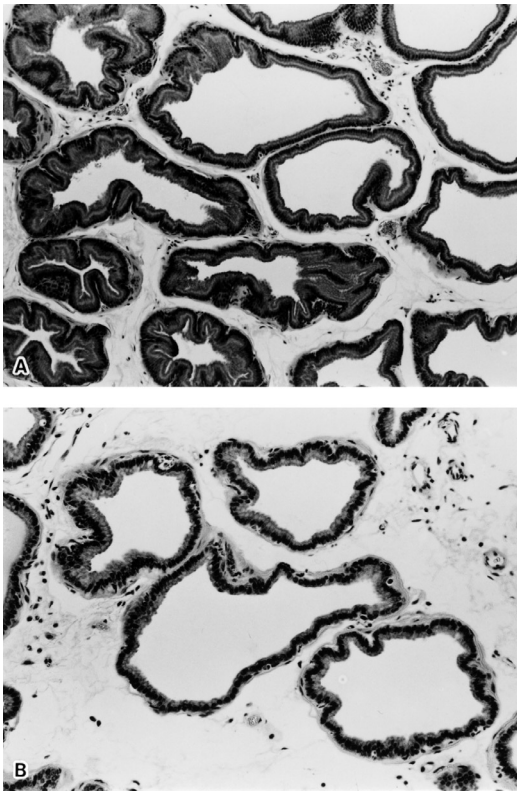

Fig. 1 Light microscopic view of intact control (A) and CMA-treated (B) rat ventral prostate. By CMA-treatment, the height of the glandular epithelial cells are markedly decreased. HE, × 150 (A, B).

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

Group		n	Prostatic weight (g)	
			Absolute	Relative
1	Intact control	5	0.51 ± 0.12	0.15 ± 0.04
2	CMA 50mg/kg	5	0.25 ± 0.08 **	0.06 ± 0.01 **
3	Tween 80	5	0.52 ± 0.11	0.16 ± 0.03

Values are mean ± S.D.
**, $p < 0.01$ *vs* intact control (Dunnett's multiple comparison test)

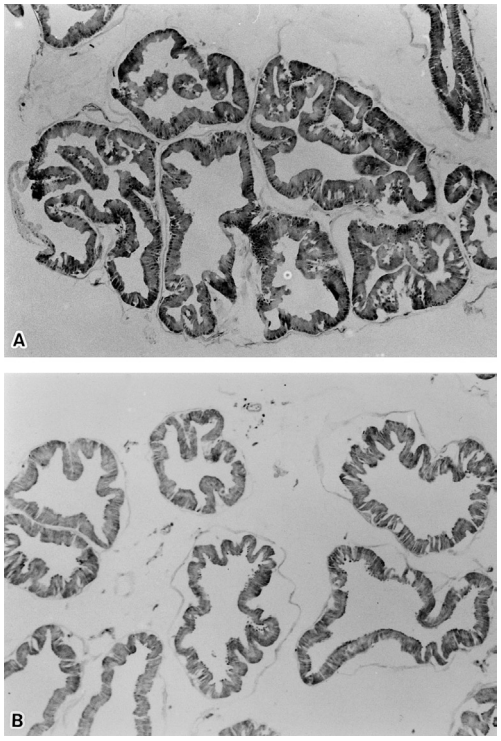

Fig. 2 Immunohistochemical localization of GSH-PO in intact control (A) and CMA-treated (B) rat ventral prostate. GSH-PO is mainly observed in cytoplasm of the glandular epithelial cells. By CMA-treatment, the intensity of the GSH-PO staining is weaker than that of the intact control. Peroxidase-labeled antibody method, × 100 (A, B).

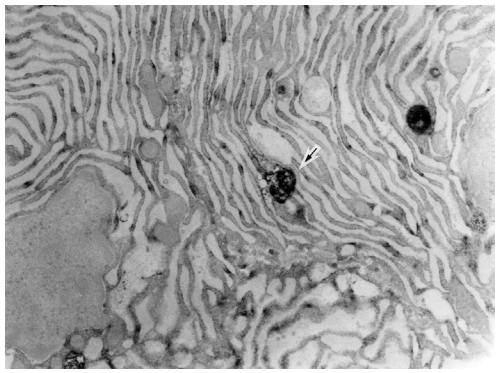

Fig. 3 Immunocytochemical localization of GSH-PO in the glandular epithelial cells of a intact control rat ventral prostate. GSH-PO is mainly observed in cytoplasmic matrix near the rough endoplasmic reticulum or mitochondria. In addition, GSH-PO-positive granules (arrow) are also noted in supranuclear region. Peroxidase-labeled antibody method, × 10,000.

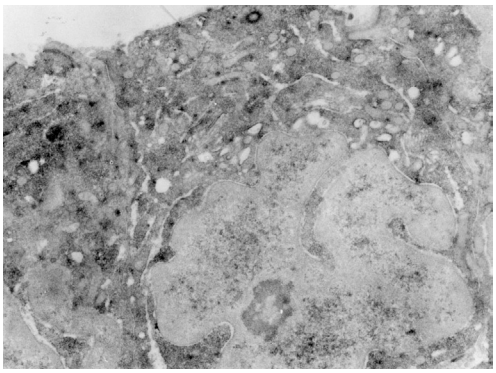

Fig. 4 Immunocytochemical localization of GSH-PO in the glandular epithelial cells of CMA-treated rat ventral prostate. The intensity of the GSH-PO staining is decreased. Furthermore, GSH-PO-positive granules are hardly seen. Peroxidase-labeled antibody method, × 15,000.

DISCUSSION

CMA as steroidal antiandrogen produced a decrease of prostatic weight. Histologically, CMA produced marked atrophy of the glandular epithelial cells. Ultrastructurally, loss of the secretory and metabolic activities was evident. CMA-induced features were considered to be resembled chemical castration. It is a well documented fact CMA inhibits the uptake of testosterone in the prostate and is selectively incorporated into prostate cells, inhibiting the binding of the cytosol 5 α-dihydrotestosterone-receptor [4, 11, 12]. Thus, the uptake of testosterone and /or its androgenic effect on the prostate may be suppressed by CMA. In addition, CMA inhibits the binding of androgen to androgen receptor (AR) competitively (data not shown). Therefore, CMA binds competitively to the AR from the prostate, and oral administration causes regression of the prostatic weight.

In our previous reports, immunocytochemical localization of GSH-PO, which effectively reduces the lipid peroxides, was demonstrated in the rat ventral prostate [5, 6]. As a results, GSH-PO was predominantly observed in the glandular epithelial cells. Furthermore, the intensity of GSH-PO staining was decreased by castration and it was clearly recovered by testosterone-administration to the castrated rats. In the present study, we found that the intensity of GSH-PO staining in the glandular epithelial cells of the rat

ventral prostate was remarkably decreased after treatment of CMA as antiandrogen. It is well recognized that the metabolic and secretory activities of the prostate are regulated by testosterone. Based on our data and these facts, GSH-PO in the rat prostate suggest a very close relationship to the status of testosterone action of the glandular epithelial cells.

Intracellular localization of GSH-PO in the glandular epithelial cells of the rats ventral prostate was mainly observed in cytoplasmic matrix near the rough endoplasmic reticulum. In this regard, lipid peroxidation may occur in the microsomes including rough endoplasmic reticulum. In fact, the microsomal membranes contain a relatively large amount of polyunsaturated fatty acid in their phospholipid, and the microsomes were very liable to lipid peroxidation and concurrent damage [10, 13]. Therefore, prostate GSH-PO might play an important role in prevention of damage to the microsome including rough endoplasmic reticulum with lipid peroxidation. In addition, we further speculated that lipid peroxidation could be continue even during the process of the degradation within the lysosome-like structure. Therefore, GSH-PO-positive granules are thought to be part of the degradation process of the peroxidized materials. Thus, it is strongly suggested that GSH-PO-positive granules in the glandular epithelial cells of the rat ventral prostate were testosterone-dependent.

In conclusion, immunocytochemical localization of GSH-PO in the glandular epithelial cells of the rat ventral prostate may well reflect the functional state of the cells, especially testosterone action. Therefore, GSH-PO staining pattern is thought to be a useful marker for the effect of antiandrogen or antiprostatic agents on the prostate.

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