Epithelial and Stromal Changes after Castration and Testosterone Treatment in Rat Prostate

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Abstract: The effects of glycosaminoglycans (GAGs) and androgen on the prostatic growth were studied using tissue from intact controls, castrated, and androgen-treated castrated rats. Biochemical analysis of glycosaminoglycans revealed increased dermatan sulfate (DS) and heparan sulfate (HS) in the ventral lobe of the prostate after castration. After testosterone propionate (TP) treatment, chondroitin 4-sulfate (CS4) and chondroitin 6-sulfate (CS6) increased. An immunohistochemical observation revealed different intensities of glycosaminoglycans such as chondroitin sulfate (CS), DS and HS after castration and TP treatment. Morphologically, after castration, indentation of the nuclear membrane was remarkable in the epithelial cells, and apoptotic cells were encountered in the lamellated epithelial cell layer combined with the irregular thickening of the basal lamina and atrophic stromal cells. After androgen administration in castrated rats, the prostatic epithelial cells increased in size and the stromal volume decreased. The effects of androgens upon prostatic growth may depend on the relationship between the epithelium and stromal components mediated by not only growth factors but also glycosaminoglycans.

Key words: prostate, castration, glycosaminoglycan, testosterone, immunohistochemistry

INTRODUCTION

The interactions between prostatic epithelial and stromal cells may be influenced by the composition of the extracellular matrix.

Terry reported that glycosaminoglycans (GAGs) are regulated by androgen and there may be lobe-specific differences in their regulation following castration and testosterone treatment. GAGs can be classified into the following groups: hyaluronic acid, chondroitin sulfate, dermatan sulfate, heparan sulfate, heparin, and keratan sulfate. GAGs may influence cell proliferation by interacting with growth factors and growth factor receptors. And also different proteoglycans can function to either inhibit or promote cell proliferation. These epithelial-mesenchymal interactions have been hypothesized to play a major role during development, differentiation, and hyperplasia of the prostate. Androgen withdrawal results in the active transformation of the glands through increased stromal biosynthetic activity and epithelial regression. After castration, GAGs content decrease in prostate and androgen replacement increase the total GAGs content. However, the relationship between the epithelial proliferation and stromal components is not so clear in the prostate.

In order to clarify these relationship, the morphological changes and the GAGs components in the ventral lobe of prostate were observed using tissues from intact controls, castrated and androgen-treated castrated rats.

MATERIALS AND METHODS

Animal preparation

Thirty-six male Wistar rats, twelve weeks of age, weighing 350-400 g, each were used. Twenty-four rats were anesthetized by diethylether and then castrated transscrotally. Twelve rats at 4 weeks and the other twelve rats at 8 weeks after treatment were sacrificed. Before being sacrificed, six rats from each group were given daily injections of 4 mg/kg of testosterone propionate with 0.2 mL of sesame oil subcutaneously for a week, and the other six rats were injected only with sesame oil (0.2 mL) for a week. The remaining twelve rats were not operated on and were sacrificed as controls. During the experiment, all animals had free access to standard food and water.

Histological observation

Ventral lobes of the prostate were resected, fixed in 10% neutral formalin and embedded in paraffin and prepared into 3 μM tissue sections. Sections from each tissue were stained with hematoxylin and eosin to assess histological changes.
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**Extraction and quantification of GAG**

The isolation and quantitation of GAGs were performed as described Hata et al.\(^6\). The lyophilized samples were dissolved with distilled water up to 400 \(\mu\)g/mL and were spotted onto the cellulose acetate membrane and were electrophoresed with 0.1 M pyridine (pH 3.0) at 20 mA for 45 min and the second dimension was run with 0.2 M barium acetate at 20 mA for 5 hours. The absorbance of the dissolved solutions was measured at 677 nm to quantify the concentration of GAGs. The standard curves for GAGs concentration were constructed using chondroitin 4-sulfate, chondroitin 6-sulfate, dermatan sulfate and heparan sulfate. These standard reagents were purchased from Seikagaku Co., Ltd. (Tokyo, Japan).

**Immunohistochemical observation**

The streptavidin-biotin-peroxidase (LSAB) method was used for immunohistochemical observation. Tissue sections were deparaffinized in xylene, rehydrated in graded ethanol to a phosphate buffer saline (PBS) (pH 7.4), and immersed in a solution of 0.3% hydrogen peroxide to block endogenous peroxidase activity. The sections were then incubated with 10% normal goat serum for 10 min to block nonspecific reactive sites. After a brief washing with the buffer, the sections were incubated with the primary antibody. The primary antibodies used were rabbit anti-human Von Willebrand Factor antibody (DAKO A/S Glostrup, Denmark) at a 1:500 dilution, mouse anti-human alpha-smooth muscle actin (\(\alpha\)-SMA) antibody (DAKO A/S Glostrup, Denmark) at a 1:200 dilution, mouse monoclonal anti-chondroitin sulfate (CS), Monoclonal anti proteoglycan \(\beta\) Di-4S (Seikagaku Kogyo Co., Ltd., Japan) at a 1:100 dilution, mouse monoclonal anti-human dermatan sulfate (DS) antibody (Seikagaku Kogyo Co., Ltd., Japan) at a 1:1000 dilution, mouse monoclonal anti-heparan sulfate (HS) antibody (Seikagaku Kogyo Co., Ltd., Japan) at a 1:100 dilution and mouse monoclonal anti-human androgen receptor (AR) antibody (Via Gramsci, Italy) at a 1:200 dilution.

All primary antibodies showed a cross-reaction with rat tissues. The specificities of the antibodies were confirmed in preliminary experiments. The reaction with primary antibodies was performed overnight at 4°C, then the staining procedure was completed using a Nichirei SAB-PO kit. All procedures were carried out at room temperature except for the incubation with the first antibody. The sections were then counter-stained for nuclei with hematoxylin.

As a negative control for the immunohistochemical staining, tissue sections were treated with normal rabbit serum instead of a primary antibody.

**Ultrastructural observation**

The tissues were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer solution (pH 7.4) and postfixed in 1% osmium tetroxide in 0.1 M cacodylate buffer, then dehydrated in ethanol and embedded in epoxy 812. Ultra-thin sections were cut with a diamond knife using an Ivan Sorvall MT-5000 ultra-microtome. These sections were stained with uranyl acetate and lead citrate. Some sections were stained using periodic acid thiosemicarbazide gelatin methenamine silver stain (PATSC-GMS) and were examined with a Hitachi H-7000 electron microscope under the accelerating potential of 75 kV. The staining procedure for PATSC-GMS was as follows: ultrathin sections were placed on nickel grids and preincubated in an ammonium solution (25%) for 10 min, oxidized in 1% periodic acid for 20 min, reacted with 0.1% thiosemicarbazide (TSC) for 1 min, post-incubated in ammonium solution (25%) for 5 min, and stained with gelatin methenamine silver (GMS) solution for 50 min at 50-55°C in an oven.

**RESULTS**

Experimental animals were divided into three groups. First group (C) is non-treated control animals, second group (CA) is castrated animals, and third group (CT) is androgen-treated castrated animals.

According to the biochemical analysis, a CS4 (chondroitin 4-sulfate) spot was not shown at 4 and 8 weeks after castration, but there was a significant difference between the CT and CA groups at 8-weeks (\(p<0.01\)). Additionally, there was a significant difference at 8 weeks between the CT and C groups (\(p<0.05\)) (Fig. 1a). However the CS6 (chondroitin-6-sulfate) spot was not observed at 4 and 8 weeks after castration (Fig. 1b). DS (dermatan sulfate) was increased at 4 and 8 weeks after castration. In addition, there was a significant difference between CT and CA groups (\(p<0.05\)), C and CA groups (\(p<0.01\)) and C and CT groups at 8 weeks (\(p<0.05\)) (Fig. 1c). HS (heparan sulfate) showed an increase at 4 and 8 weeks after castration and there was a significant difference at 4 weeks between the CA and CT groups (\(p<0.05\)), at 4 weeks between the C and CA groups (\(p<0.01\)) and at 8-weeks for C and CA groups (\(p<0.05\)) (Fig. 1d).

Morphologically, in the early stage after castration, the indentation of the nuclear membrane was remarkable because epithelial cells and apoptotic cells were observed in the lamellated epithelial cell layer combined with irregular thickening of the basal lamina and atrophic stromal cells (Fig. 2a, b). After androgen was administered in the castrated rats, the prostatic epithelial cells increased in size and the stromal volume decreased. Ultrastructurally, the cytoplasm of epithelial and stromal cells contained abundant rough endoplasmic reticulum, numerous mitochondria and actin filaments (Fig. 2c).

Immunohistochemically, \(\alpha\)-smooth muscle actin was localized in smooth muscle cells surrounding acini and blood vessels (Fig. 3a). At 4 weeks after castration, the periacinar smooth muscle cells were arranged irregularly (Fig. 3b). In androgen-treated castrated animals, the layer of smooth muscle cells was thickened and irregularly arranged (Fig. 3c). Factor VIII was faintly localized in the endothelial of blood vessels (Fig. 4a). At 4 weeks after castration, the endothelial of the blood...
vessels displayed weak immunoreactivity (Fig. 4b). In androgen-treated castrated animals, Factor VIII immunoreactivity was markedly observed as the control group (Fig. 4c). According to glycosaminoglycan and androgen receptor, chondroitin sulfate (CS), dermatan sulfate (DS), heparan sulfate (HS), and androgen receptor (AR) in rat prostates showed various intensities of staining as demonstrated in Table 1. The CS was confined to the periacinar basement membranes and stroma in the prostate. It was faintly localized in the stroma and markedly localized in the periacinar basement membranes at 4 and 8 weeks in the C and CT groups. CS was not observed at 4 and 8 weeks in the CA group. CS staining intensity was observed in the basement membranes of epithelial cells of the CT group at 8 weeks after castration (Fig. 5a, b, c).

DS was faintly localized in collagenous fibers in the stroma at 4 weeks in the C and CA groups. At 4 and 8 weeks in the CT group and at 8 weeks in C and CA groups, DS markedly presented in the stroma. At 8 weeks in the CA group and at 4 weeks in the CT group, the epithelial basement membrane showed weak immunoreactivity to DS. However, at 8 weeks in the CT group, DS was markedly localized (Fig. 6a, b, c).

HS was markedly localized in the stromal and epithelial cells at 4 and 8 weeks in the C group and in epithelial cells at 4 and 8 weeks in the CT group. However, HS was localized in the stroma and epithelial
Fig. 2. Ultrastructural changes in the ventral lobe with castration and TP treatment. After castration, the lamellated epithelial cells are observed and atrophic stromal cells in edematous stroma combined with the irregular thickening of the basal lamina and blood vessels (a, b). After androgen administration, the cytoplasm of epithelial and stromal cells contain abundant rough endoplasmic reticulum, numerous mitochondria and actin filaments (c). PATSC-GMS staining. Ep: Epithelial cell, BM: Basement membrane, ST: Stromal cell, L: Vascular lumen.
cells at 4 and 8 weeks in the CA group (Fig. 7a, b, c).

Androgen receptor (AR) was faintly localized in the nuclei of epithelial cells in all three groups. No immunoreactivity was observed in the stromal cell (Fig. 8a, b, c). No immunohistochemical staining was detected when tissue sections were treated either in the absence of primary antibody or in the presence of normal mouse or rabbit serum instead of primary antibody, respectively.

**DISCUSSION**

The differentiation, growth, and function of epithelial and stromal cells in the prostate are controlled by various factors in association with the proliferation of blood vessels. Moreover, androgen and proteoglycan play an important role in the proliferation and differentiation of epithelial, stromal and vascular cells in the prostate\(^5\). Fibroblast growth factor (FGF) stimulate proliferative activities of many kinds of cells by combin-
growth factor matrix interactions can alter growth factor potency, retain either active or inactive growth factors in the vicinity of the cell, modify growth factor stability and protect growth factors from proteolytic degradation. In this study, ultrastructurally, after castration basal lamina showed extensive folding and these folding may occur under active action of remaining epithelial and stromal cells. GAG chains are responsible for the regulatory mechanisms of the movement of various types of molecules and cells. GAGs in vivo may be related to the activity of growth factor and growth plays an important role in regulating cellular processes in response to tissue injury and repair by controlling the expression of extracellular matrix components. Biochemical and immunohistochemical findings revealed decreased CS-4 and 6 in the ventral lobe after castration. The effect of castration on CS could be attributed to the presence of degradative enzymes for glycosaminoglycans, which are known to increase after castration. Androgen replacement increased the total glycosaminoglycans (GAGs) content in the three prostate lobes. The results demonstrated that the CS4 content was significantly higher for the TP-treated animals compared to the control group. Terry reported that hyaluronic acid (HA) and CS may be produced by epithelial cells, while HS and DS are produced by both epithelial and stromal cells. Extracellular matrix roles range from the sustaining of cell-cell contact, migratory and proliferative activity of cells to their biological metabolism. These findings may indicate that all GAGs are regulated by androgen and that there may be lobe-specific differences in their regulation, and also the alterations of extracellular matrix components and the morphological changes may represent early markers for prostatic bioactivities.

It is concluded that biochemical and immunohistochemical analysis may be very useful in studying the effects of androgen treatment of the prostate. These results suggest that protein synthesis is involved in the mechanism of testosterone-promoted AR regulation and that AR in the prostate may be independent of androgen. This protein synthesis-dependent mechanism may be involved in the translation of AR mRNA in the prostate. In the ventral prostate of intact rats, positive staining for androgen receptors was observed in the nuclei of glandular epithelial cells. In addition, immunodetectable AR may decrease within 2 days after castration, but return to intact levels after the administering of testosterone. The detection of androgen receptor and its transcripts in the basal cells supports the hypothesis that these cells are androgen responsive. Not only hormone, but also multiple growth factor-receptor systems participate in the androgen-dependent regrowth of castrated rat prostates. In summary, this study identified the changes in the quantity, structure and the mobilization of GAGs related with the morphological changes.

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### Table 1 Immunohistochemical localization of chondroitin sulfate, dermatan sulfate, heparan sulfate and androgen receptor in the ventral lobe of rat prostate

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E: Epithelial cell, BM: Basement membrane, St: Stromal tissue.
Fig. 5. Immunohistochemical localization of CS in the ventral lobe with castration and TP treatment.

a. CS is faintly localized in the stroma at 4 weeks in the C group, at 4 weeks in the CA group (b) and at 4 weeks for CT group (c). CS staining intensity is localized in the basement membranes of epithelial cells in the CT group. ×200 Sub method

Fig. 6. Immunohistochemical localization of DS in the ventral lobe with castration and TP treatment.

a. DS is faintly localized in the stroma at 4 weeks in the C group. b. DS is markedly localized in the atrophic glands and in the stromal cells at 8 weeks in the CA group. c. DS is mildly localized in the stromal cells at 8-weeks in the CT group. ×200 Sab method
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Fig. 7. Immunohistochemical localization of HS in the ventral lobe with castration and TP treatment.
- a. HS is faintly localized in the stroma and the epithelial cells at 4 weeks in the C group.
- b. HS is markedly localized in the epithelial and stromal cells at 8 weeks in the CA group.
- c. HS is faintly localized in some stromal and epithelial cells at 4 weeks in the CT group. ×200 Sab method

Fig. 8. Immunohistochemical localization of AR in the ventral lobe with castration and TP treatment.
- a. AR is faintly localized in the epithelial and stromal cells at 8 weeks in the C group.
- b. AR is markedly present in the epithelial cells including basal cells of atrophic glands and in the stromal cells at 4 weeks in the CA group.
- c. AR is faintly localized in the epithelial cells and in some stromal cells at 4 weeks in the CT group. ×200 Sab method
References