Effects of Testosterone, and Testosterone Plus Estrogen, in the Castrated Rat Ventral Prostate
—Histopathological and Immunocytochemical Studies—

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The effects of testosterone (T) and 17β-estradiol (E2) on the prostate of castrated rats were investigated by histopathological and immunocytochemical procedures. A significant increase in prostatic weight occurred after 6 weeks treatment with T alone and in combination with E2. The greatest increase in prostatic weight occurred after the administration of T plus E2. Histopathologically, glandular hyperplasia of the prostate was noted, and the number of bromodeoxyuridine (BrdU)-positive cells showed a significant increase over that induced by testosterone alone.

(Key Words: Ventral prostate, Testosterone, 17β-estradiol, Bromodeoxyuridine (BrdU), Glandular hyperplasia)

INTRODUCTION

Androgens control the development and function of the prostate, but other steroid hormones, such as estrogen, are also supposed to have an important role in prostate physiology (3, 9, 11).

In a previous report (10), it was shown that glandular hyperplasia of the prostate developed in castrated dogs after combined treatment with 5α-androstane-3α, 17β-diol and 17β-estradiol. It is generally accepted that estrogens play a critical role in the etiology of canine and human benign prostatic hyperplasia (2, 5, 16, 18).

The purpose of the present study was to examine the effects of testosterone, and testosterone plus 17β-estradiol, on the castrated rat prostate. In addition, an analytical examination for cell proliferation activity, using 5-bromo-2-deoxyuridine (BrdU)-positive cell ratios, was also performed.

MATERIALS AND METHODS

Animals and tissue preparation

Male Wistar rats weighing 200 to 250 g were sorted into 4 experimental groups. Group 1 consisted of untreated controls; group 2: rats were sacrificed 2 days after castration; group 3: rats were inoculated subcutaneously (SC) with 1 mg/head of testosterone (Sigma Chemical Co., St. Louis, MO) daily for 6 weeks, beginning 2 days after castration; group 4: rats were inoculated SC with 1 mg/head of testosterone plus 0.01 mg/head of 17β-estradiol (Teikoku Hormone, Mfg. Co. Ltd., Kawasaki) daily for 6 weeks, beginning 2 days after castration. One hour prior to sacrifice, rats were given an intraperitoneal injection of 5-bromo-2-deoxyuridine (BrdU, Sigma Chemical Co., St. Louis, MO), at a dose of 10 mg/kg body weight. Each rat was killed by decapitation and the ventral prostate was removed immediately. The prostates were fixed in 0.1 M phosphate-buffered 10% formalin for 4h at room temperature, then dehydrated with a graded series of ethanol and embedded in paraffin. Sections (4 μm) were cut, deparaffinized with xylene, and rinsed thoroughly with ethanol. The sections were then placed in absolute methanol containing 0.3% H2O2 for 30 min at room temperature in inactivate endogenous peroxi
dase activity.

**Immunocytochemical staining of BrdU**

Sections were washed twice in 0.01 M phosphate-buffered saline (PBS), pH 7.4, 5 min per wash. Thereafter, sections were immersed in 4N HCl in 0.01 M PBS at 37°C for 20 min to denature the DNA. To neutralize the HCl, the sections were rinsed in 0.1 M borate buffer, pH 7.6. After washing in 0.01 M PBS, the sections were blocked with 5% normal goat serum for 30 min, then incubated for 2h with 1:50 mouse monoclonal anti-BrdU antibody (Becton Dickinson Immunocytometry Systems, San Jose, CA). After washing in 0.01 M PBS, the sections were covered with biotin-conjugated goat anti-mouse IgG for 1h, washed and then incubated with streptavidin-biotin complex (Histofine SAB-PO(M) Kit, Nichirei, Tokyo) for 1h. After washing in 0.01 M PBS, the sections were incubated in Graham-Karnovsky's reaction medium (6) which contained 3,3'-diaminobenzidine (DAB, Wako Pure Chemical Co., Osaka) and 0.005% hydrogen peroxide as the substrate, for 5 to 10 min. The sections were then counterstained with hematoxylin. As a negative control, normal nonimmune mouse serum was used.

In addition, the BrdU-positive cell ratio (%) was calculated as the positive cell number in one acinus / total cell number in the acinus × 100.

**RESULTS**

1. **Prostatic weights**

The prostatic weights are listed in Table 1. Those of the intact and castrated groups are comparable, whereas statistically significant increases were noted in the treated groups. In particular, the rats of group 4 exhibited the greatest increase in prostatic weight.

2. **Histopathological and immunocytochemical findings**

a) **Intact controls**

The glandular epithelial cells appeared as single-layered, cylindrical cells (Fig. 1A). The cytoplasm of the glandular epithelial cells showed a pronounced eosinophilic staining, and the nuclei were located mainly in the basal portion of the cells. The acini of the ventral prostate were relatively large with shallow papillary projections present in the acinar lumen. A few BrdU-positive nuclei were observed in the glandular epithelium (Table 2, Fig. 2A).

b) **Castrated rats**

Two days after castration, the cells of the glandular epithelial lining were lower in height than those of the intact controls (Fig. 1B). In addition, BrdU-positive nuclei in the glandular epithelial cells were rarely seen.

c) **Castration + testosterone**

The glandular epithelial cells were hypertrophic and showed an increased number of papillary projections extending into the acini (Fig. 1C). BrdU-positive nuclei were clearly detected. There was a distinct increase in the number of BrdU-positive nuclei compared with the intact controls (Fig. 2B, Table 2).

d) **Castration + testosterone + 17β-estradiol**

The histological features of glandular hyperplasia were evident in this group (Fig. 1D). In addition, the number of BrdU-positive nuclei was greatly increased compared to the other experimental groups (Fig. 2C).

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**Table 1.** Influence of testosterone (T), and T plus 17β-estradiol (E₂), on the weight of the castrated rat ventral prostate.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>No. of animals</th>
<th>Prostatic weight (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Absolute (mg)</td>
</tr>
<tr>
<td>1</td>
<td>Intact</td>
<td>17</td>
<td>579 ± 114*</td>
</tr>
<tr>
<td>2</td>
<td>Castration</td>
<td>17</td>
<td>553 ± 98 **</td>
</tr>
<tr>
<td>3</td>
<td>Castration + T</td>
<td>17</td>
<td>695 ± 154 †</td>
</tr>
<tr>
<td>4</td>
<td>Castration + T + E₂</td>
<td>17</td>
<td>893 ± 180 †</td>
</tr>
</tbody>
</table>

* p< 0.05 vs intact (Dunnett's multiple comparison test).

** p< 0.01 vs intact (Dunnett's multiple comparison test).

† p< 0.01 vs castration + T (Student's t-test).
Fig. 1. Ventral prostate of control (A), castrated (B), testosterone (C)—and testosterone plus 17β-estradiol (D)—treated castrated rats. Glandular epithelial cells are atrophic (B). Following testosterone administration (C, D), glandular epithelial cells are of a highly columnar shape. In addition, glandular hyperplasia is clearly detected in D. HE stain. ×110 (A, C, D), ×220 (B).
Table 2. Influence of testosterone (T), and T plus 17β-estradiol (E2), on the bromodeoxyuridine (BrdU)-positive cell ratio in the castrated rat ventral prostate.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of Acini</th>
<th>BrdU positive cell ratio (%) mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact</td>
<td>10</td>
<td>0.54 ± 0.20</td>
</tr>
<tr>
<td>Castration + T</td>
<td>10</td>
<td>1.40 ± 0.78</td>
</tr>
<tr>
<td>Castration + T + E2</td>
<td>10</td>
<td>3.25 ± 1.00</td>
</tr>
</tbody>
</table>

*p < 0.05 vs intact (Dunnett's multiple comparison test).

**p < 0.01 vs intact (Dunnett's multiple comparison test).

†p < 0.05 vs castration + T (Student's t-test).

Fig. 2. BrdU-positive cells of control (A), testosterone (B)— and testosterone plus 17β-estradiol (C)—treated castrated rat ventral prostates. BrdU-positive cells are located mainly in the glandular epithelium. The number of positive cells are greatly increased in the testosterone plus 17β-estradiol-treated group.

Peroxidase-labeled antibody method, ×140 (A–C).

there was a significant increase in the BrdU-positive cell ratio over the group that received testosterone alone (Table 2).

DISCUSSION

The most widely used methods of measuring the labeling index (LI%, a measure of the percentage of proliferating cells in a tumor cell population) are complex and/or costly, limiting their widespread use, especially in clinical analysis. 5-bromo-2-deoxyuridine (BrdU) is a thymidine analogue that is incorporated into DNA during the replication phase (S-phase) of the cell cycle (7). In the past few years, BrdU has been used extensively to study the cell kinetics of experimental and human tumors.

In the present work, BrdU-positive cells in the rat ventral prostate were located predominantly in the glandular epithelium. In addition, it was seen that the castration-induced decrease in the number of BrdU-positive cells was reversed by testosterone. The stimulatory effects of testosterone were strongly potentiated by the addition of 17β-estradiol, resulting in the highest BrdU-positive cell ratio. Histologically, glandular hyperplasia was clearly de-
ected in the group of rats receiving the combination treatment.

The histological appearance of the prostate in the castrated animals treated with testosterone plus 17β-estradiol, resembled glandular hyperplasia (10, 17). It is known that 17β-estradiol alone would induce glandular atrophy of the prostate in normal and castrated animals (1, 2). The suppressive effects of 17β-estradiol on the male genital organs have been attributed not only to gonadotropin suppression, but also to the direct effect of 17β-estradiol on the gonads and their accessories (1, 4, 15). It is generally accepted that 17β-estradiol exerts a synergistic effect with androgen in promoting prostatic growth in the castrated dog (18), a fact of considerable importance in understanding the pathogenesis of prostatic hyperplasia.

Based on our data and the above reports, a glandular hyperplasia was thought to be the main feature of the rat prostatic hyperplasia occurring experimentally as a result of treatment with steroid hormones.

Recently, androgen and estrogen receptors have been demonstrated biochemically as well as immunocytochemically (8, 12–14). The mechanism by which 17β-estradiol enhances the androgen receptor is uncertain. In this context, immunocytochemical staining of androgen and/or estrogen receptors in the prostate would seem to be important for the clarifying this problem. Further work along these lines is now in progress in our laboratory.

REFERENCES