Inhibition of regrowth of prostatic glandular cells by epristeride

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KEY WORDS epristeride; prostate; castration; immunohistochemistry

ABSTRACT

AIM: To evaluate the ability of epristeride to inhibit the prostatic glandular regrowth. METHODS: Normal rats were castrated. Testosterone was injected to induce the regrowth of glandular cells. HE staining was performed. The height of the glandular epithelium and the acinar luminal areas were determined, and dihydrotestosterone (DHT) was detected by immunohistochemistry.

RESULTS: Both the height and the acinar luminal areas were reduced by 48% and 55% in epristeride-treated group compared with control group respectively. The staining of DHT was comparatively strong in the control group. After 30-d of treatment, it turned much weaker.

CONCLUSION: The regrowth of glandular cells was inhibited by epristeride via declining of the DHT concentration in the rat prostate.

INTRODUCTION

Epristeride, a novel 5α-reductase inhibitor is currently under development for the treatment of benign prostate hyperplasia. It inhibits the transformation of DHT from testosterone in an uncompetitive manner[1]. Unlike other substrate analogs, which compete at the level of testosterone interaction with enzyme and lead to not only a decrease in DHT but also an increase in the prostate testosterone concentration[2], uncompetitive ones bind to the 5α-reductase enzyme NADP+ complex, preventing its regeneration and are not affected by the level of testosterone present. In vitro studies have demonstrated such an uncompetitive 5α-reductase inhibitor do efficiently inhibit the rat and human enzyme[1].

In the present study, we also evaluate its ability to inhibit androgen-induced regrowth of the involuted ventral prostate of previously castrated rats and monitor the DHT changes using immunohistochemistry.

MATERIALS AND METHODS

Drugs and reagents Epristeride was a gift from Prof LIAO Qing-Jiang (Department of Chemistry, Chinese Pharmaceutical University, Nanjing 210009, China). Anti-DHT and ABC kits were purchased from Doka and Sino-American Biotechnology Co. All other reagents were of analytical grade.

Animal protocol Male Sprague Dawley rats (Grade II; Certificate number 005, 55-d old at the start of the experiment) were purchased from Shanghai Experimental Animal Center, Chinese academy of Sciences and were kept under standard conditions.

The volume of an individual gastric feeding was 1.0 mL where indicated, animals were anesthetized with pentobarbital and castrated via a scrotal incision. Both the testes and epididymis were removed.

Rats were castrated and maintained for 1 week before treatment. Castrated rats received epristeride 1, 3, and 10 mg/kg via oral administration in combination with subcutaneous injection of 0.5 mg testosterone in 0.1-mL olive oil. Thirty days after treatment, ventral prostate was rapidly removed at necropsy.

Morphometric analysis Prostate was fixed in 5% buffered formalin (pH 7.4), and processed for routine paraffin embedding. Tissue sections were cut (5 μm) and stained with hematoxylin and eosin (H&E). Both the height of the glandular epithelium and the acinar luminal area were determined by a videodensitometer.

Immunohistochemistry staining for DHT Each prostate was fixed with 5% formalin, embedded in paraffin, and sectioned at 5 μm. Briefly, paraffin tissue sections were placed on glass slides. Staining was performed by avidin-biotin complex (ABC) method. The section from representative paraffin-embedded tissue samples were deparaffinized, rehydrated, and preincubat-
ed with 1 % H₂O₂ to abolish endogenous peroxidase activity. The slides were incubated with the primary rabbit anti-DHT antibody for 1 h at 37 °C with brief washes using phosphate-buffered saline between each step. The secondary antibody (biotinylated goat anti-rabbit, Sino-American Co; dilution 1:50) was applied for 5 min at room temperature. The slides were treated with 0.05 % diaminobenzidine in Tris buffer 0.05 mol/L containing 0.3 % H₂O₂. Finally, the sections were dehydrated, coverslipped, and examined using a standard light microscope and the relative content (transmittance) of DHT (%) were assayed with a spectrophotometer at a wavelength of 650 nm. The transmittance from a blank space without any tissue in the same section was assigned as 100 %. The standards for DHT quantification were strong positive, 21 % - 40 %; positive, 41 % - 60 %; weak positive, 61 % - 80 %; and negative, 81 % - 100 %.

Statistics Data were expressed as x ± s and analyzed with ANOVA followed by Dunnett's procedure. P values of less than 0.05 were considered to be significant.

RESULTS

Effect of episteride on the weight of prostate After 30 d of episteride treatment, prostatic weight decreased dramatically. A minimum value of 57 % of the control was attained after 30-d treatment with episteride 10 mg/kg (Tab 1).

Morphometric analysis There was a decrease in prostatic glandular cell height by about 26 % and in acinar luminal area by about 30 % after 30 d of episteride 1 mg/kg treatment. These effects were observed to be dose-dependent (Tab 1, Fig 1, 2).

Immunohistochemistry for DHT Sections of prostate gland were immunohistochemically stained for DHT. The positive staining of the epithelial cells was observed in the control glands. Significant changes in prostate DHT were observed in the treatment group. The relative expression of intraprostate DHT was quantitatively estimated with a spectrophotometer. The staining of DHT was positive in the control group respectively. After 30 d of treatment, the transmittance of DHT decreased to weakly positive (Tab 1, Fig 3).

DISCUSSION

It is known that the prostatic tissue is androgen-dependent. After castration, the prostatic epithelium regresses. The regressive changes can be prevented by the addition of testosterone[5]. Morphometric analysis is performed to determine the glandular cell height as an index of the ability of the cells to synthesize secretory proteins and acinar luminal area as an index of the ability of the cells to secrete the synthesized products[5]. It confirmed that the decrease in the prostate weight observed after 30 d of episteride treatment was not solely due to loss in glandular cell number. Episteride treatment rapidly induced atrophy of the prostatic glandular cells, ie decreased cell height as well as a decrease in the secretory ability of the cells (reflected by a decreased luminal area).

The cellular availability of sufficient amounts of DHT is thought to be a prerequisite for the normal growth and function of the human prostate[6]. DHT, the 5α-reduced metabolite of testosterone, is the active molecule triggering androgen action. As a consequence, the

Tab 1. Effect of episteride on the wet weight, glandular cell height, acinar luminal diameter, and DHT content of prostate. n = 10. x ± s. *P > 0.05, **P < 0.05, ***P < 0.01 vs control.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose/ mg·kg⁻¹</th>
<th>DHT (%)</th>
<th>Wet weight/g</th>
<th>Acinar luminal area/ μm²</th>
<th>Glandular cell height/μm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>55 ± 3</td>
<td>0.89 ± 0.13</td>
<td>62277 ± 4309</td>
<td>20 ± 4</td>
</tr>
<tr>
<td>Episteride</td>
<td>1</td>
<td>71 ± 10⁶</td>
<td>0.77 ± 0.19⁶</td>
<td>44246 ± 10135³</td>
<td>15 ± 4³</td>
</tr>
<tr>
<td>Episteride</td>
<td>3</td>
<td>76 ± 6⁶</td>
<td>87 %²¹</td>
<td>71 %²¹</td>
<td>74 %²¹</td>
</tr>
<tr>
<td>Episteride</td>
<td>10</td>
<td>81 ± 9⁶</td>
<td>0.0 ± 0.06²¹</td>
<td>42165 ± 12231⁶</td>
<td>12 ± 3³</td>
</tr>
<tr>
<td>Episteride</td>
<td>10</td>
<td>57 %²¹</td>
<td>55 %²¹</td>
<td>34448 ± 10409³</td>
<td>9.9 ± 2.1¹</td>
</tr>
</tbody>
</table>

1) The transmittance of DHT was measured at 650 nm.
2) Percentage of that of control group.
conversion of testosterone to DHT by 5α-reductase is a key step in this mechanism, and the target tissue concentration rather than the plasma DHT level is the deciding parameter. Thus, in our study, immunohistochemistry was performed to detect the DHT level in the prostate. The DHT content of the prostate of the control rats were positive suggesting the exogenous testosterone was transformed to DHT by endogenous 5α-reductase. It markedly declined after epiristride treatment. All those changes in the morphology and weight of prostate seemed to be the direct results of this large decrease suggesting that all the effect of epiristride are due to its uncompetitive inhibitory ability on 5α-reductase.

In conclusion, our results demonstrated that epiristride treatment not only induced cell atrophy in the epithelium of normal rat, but also inhibited the regrowth of castrated rats via lowering the intraepithelial DHT concentration.

REFERENCES


Fig 3. Immunohistochemistry of DHT in prostate. (A) Testosterone 0.5 mg. (B) Testosterone 0.5 mg + eprimisteride 3 mg/kg. × 330.