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Atrophy and apoptosis in ventral prostate of rats induced by 5α-reductase inhibitor, epristeride

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KEY WORDS epristeride; prostatic hyperplasia; apoptosis; atrophy; acid phosphatase; *in situ* nick-end labeling

ABSTRACT

AIM: To study molecular mechanism of epristeride in the treatment of benign prostatic hyperplasia and discuss the possibility of using prostate acid phosphatase (ACP) as a marker of the atrophy of prostatic gland *in vivo*. **METHOD**: Morphological changes in cells were observed by light microscope. TdT-mediated dUTP-biotin nick end labeling (TUNEL) technique and agarose gel electrophoresis were performed to detect the nucleosomal DNA fragmentation. The activity of pACP was also assayed. **RESULTS**: Apoptosis occurred in both castration- and epristeride- treatment group. Both the degree and extent of apoptosis are much larger in the group of castration than that of epristeride-treated group. Both epristeride and castration decreased the prostate wet weight and DNA content but increased the prostate DNA concentration. Maximal or near maximal decreases were seen by d 10 in both groups. The activity of ACP was decreased by both castration and epristeride treatment. Changes in the ACP activity during treatment were coincide with other changes such as the prostate wet weight and DNA content. **CONCLUSION**: Apoptosis induced by epristeride was one of mechanisms in the treatment of benign prostatic hyperplasia and the activity of ACP could be used as a marker of prostate atrophy.

INTRODUCTION

Benign prostatic hyperplasia (BPH) is a disease of aged man and over 50 % men more than 50 years old have been found histological evidence of prostatic enlargement. The medical management of BPH may be an alternative to surgical treatment. Prostate growth required the intracellular androgen. A series of observations suggested that within the androgen-dependent prostatic glandular cells, dihydrotestosterone (DHT), not the testosterone, was the active intracellular androgen, and steroid 5α-reductase was a membrane bound enzyme which catalyzed the NADPH-dependent reduction of testosterone to DHT[1]. Both castration and 5α-reductase inhibitor could lower levels of DHT and its metabolites[2].

It was reported that antiandrogen or androgen withdrawal induced apoptosis in the prostate and prostate cultures[3]. If sufficient androgen was not chronically maintained in the prostate, it would result in the involution of prostatic gland. This rapid involution occurred because androgen ablation inhibited the proliferation of the androgen-dependent prostatic glandular cells and induced these cells to undergo both cellular atrophy and activation of a cascade of biochemical events, resetting in the energy-dependent programmed cell death (PCD)[4-5].

Currently, an uncompetitive 5α-reductase inhibitor, epristeride, was an interesting drug in the treatment of benign prostatic hyperplasia. In the present study, we tested its ability to inhibit secretion and cell proliferation as well as activate the pathway of cell death in the rat prostate and compared with that of castration. In addition, the possibility of using the activity of prostate acid phosphatase (pACP) as a marker of prostate atrophy *in vivo* was also discussed.

MATERIALS AND METHODS

Drugs and reagents Epristeride was synthesized by Prof LIAO Qing-Jiang (Department of Chemistry, Chinese Pharmaceutical University, Nanjing, China). *In situ* labeling kits (TUNEL-based assay) and pACP enzyme assay kit was purchased from Genetimes Technology

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Inc. DNA molecular markers were from Sino-American Biotechnology. RNase, ABC kit, and proteinase K (PK) were purchased from Sigma. All other reagents were of analytic grade and purchased from Shanghai № 1 Reagent Plant.

Animals treatment Male Sprague Dawley rats (Grade II, Certificate № 005, 55-d old at the start of the experiment, $n = 10$ in each group) were purchased from Shanghai Experimental Animal Center, Chinese Academy of Sciences and were kept under standard conditions.

Rats were divided into three groups: intact, castrated, and epristeride-treated group. Castration was performed via the scrotal route while under pentobarbital sodium anesthesia. Both the testes and epididymis were removed. Epristeride-treated animals were given 50 mg/kg daily by oral gavage in 0.5 % (w/v) aqueous methylcellulase. The control group received the vehicle as the same volume as that given to the treated group. The rats were killed on d 4, 7, 10, and 20 of treatment. The prostates were immediately removed, weighed, and either prepared for histological examination or frozen in −80 ℃ for determination of DNA content.

DNA extraction Prostates were pulverized under liquid nitrogen and suspended in lysis buffer [Tris HCl 10 mmol/L, NaCl 10 mmol/L, edetic acid 10 mmol/L, PK 100 mg/L, 1 % SDS, pH = 8.0] and incubated at 37 ℃ till the mixture became clear. The DNA was extracted by phenol/chloroform 1∶1, precipitated overnight, and centrifuged for 30 min, 4 ℃, at 12 000 × g. The pellet was resuspended in TE buffer (Tris-HCl 0.1 moL/L, pH 8.0, edetic acid 10 mmol/L). The DNA was subsequently treated with RNAase (100 mg/L) for 1 h at 25 ℃, incubated overnight with PK (100 mg/L) at 37 ℃, and finally reextracted with phenol, phenol/chloroform, precipitated with ethanol, and resuspended in TE buffer.

Determination of DNA content The UV absorbance of extracted DNA was measured at 260 nm and 280 nm. Purity requirement was set at $OD_{260}/OD_{280} > 2$. Then, the DNA content was calculated from the OD_{260} value. Every unit of OD_{260} is equivalent to double strained DNA 50 mg/L.

Agarose gel electrophoresis DNA samples, of about 0.2 μg each, were electrophoretically separated on 1.8 % agarose gel containing ethidium bromide (0.4 mg/L). DNA was visualized by a UV (302 nm) transilluminator and the gels were photographed with a Polaroid camera.

Quantification of apoptotic cells Ventral prostates were fixed by immersing in 10 % buffered formalin, embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin (H&E).

Apoptotic cells were also identified using a modification of the TdT-mediated dUTP-biotin nick end labeling (TUNEL)[6]. The TUNEL technique involved inserting labeled nucleotides into broken ends of DNA strands. Sections 5-μm thick were deparaffinized and rehydrated. Sections were incubated with proteinase K 20 mg/L for 30 min at 37 ℃ to digest nuclear proteins, washed in distilled water four times, and immersed in 2 % hydrogen peroxidase for 5 min at 25 ℃ to inactivate endogenous peroxidase activity. The sections were rewashed as above, equilibrated in TdT buffer (Tris 30 mmol/L, pH 7.2, sodium colodylate 140 mmol/L , cobalt chloride 1 mmol/L), and covered with TdT 0.4 mg/L and biotin-dATP 12.5 μmol/L in TdT buffer for 1 h at 37 ℃ in 100 % humidity.

Assay of the pACP activity Prostates were removed and frozen sectioned at 6 μm. After that it was fixed with ice cold acetone and incubated with citrate buffer (4 mg of p-nitrophenol phosphate in 0.5 mL of H_2O, plus 0.5 mL of citrate buffer 90 mmol/L pH 4.8) at 37 ℃ for 30 min. At the end of incubation, the slides were rinsed by water and immersed in 2 % acetate acid buffer to terminate the action. Optical density at 410 nm was determined in a microspectrophotometer. After subtracting for the appropriate blank, the concentration of the reaction product, p-nitrophenol was calculated by comparing the OD_{410} of the sample to the OD_{410} of a standard curve constructed with known concentration of p-nitrophenol. The percentage of inhibition by castration and epristeride treatment was calculated as percentage of the control activity.

Statistics Results were expressed as the $\bar{x} \pm s$ and statistical significance was assessed using t-test. P values less than 0.05 were considered to be significant.

RESULTS

Prostate weight and DNA content In intact animals, the prostate wet weight gradually increased with time from (479 ± 67) g on d 4 to (539 ± 95) g on d 10 (Tab 1). Both epristeride and castration decreased prostate wet weight, but the decrease was greater in castration-treated group at all time points. Maximal or near-maximal decrease in prostate weight was seen on d 10 in both groups.

Tab 1. Epristeride 50 mg/kg changed the prostate wet weight and intraprostatic DNA content and concentration in rats. $n = 10$. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* intact group.

	Duration of experiment/d			
	4	7	10	20
Prostate weight/mg				
Intact	479 ± 67	477 ± 66	539 ± 95	539 ± 95
Epristeride	350 ± 102^b	349 ± 48^c	305 ± 48^c	290 ± 58^c
Castrate	221 ± 36^c	164 ± 25^c	65 ± 11^c	73 ± 16^c
DNA content/μg per prostate				
Intact	833 ± 41	833 ± 41	870 ± 30	870 ± 39
Epristeride	799 ± 24	783 ± 13^b	573 ± 40^c	552 ± 33^c
Castrate	388 ± 31	379 ± 33^b	172 ± 10^c	160 ± 13^c
DNA concentration/mg·g^{-1}wet tissue				
Intact	1.70 ± 0.12	1.70 ± 0.05	1.6 ± 0.3	1.6 ± 0.3
Epristeride	2.28 ± 0.21^b	2.2 ± 0.3^b	1.88 ± 0.10	1.90 ± 0.21
Castrate	1.8 ± 0.3	2.31 ± 0.12^b	2.65 ± 0.22^c	2.19 ± 0.04^b

Both castration and epristeride caused an increase in prostate DNA concentration, indicating a greater loss of cell mass than DNA content. This was consistent with cellular atrophy and occurred to a greater extent in castrated group than in epristeride-treated group. Castration diminished intraprostatic DNA content by 53 % after 4 d, and a near maximal decrease in prostate DNA content was reached by d 10 (80 % decrease). Epristeride caused little decrease in DNA content by d 4 and thereafter. The decrease was less than castration (34 % on d 10).

H&E staining Both castration and epristeride treatment increased the number of apoptotic cells and caused epithelial cell atrophy. Four days after castration, the nuclear chromatin condensation, compactness of cytoplasmatic organelles, and the appearance of peduculated protuberances on the cell surfaces were mainly found in the epithelial cells and some of the stromal cells. Apoptotic bodies increased thereafter. Similar changes were also found in the epristeride-treated group (Fig 1).

TUNEL technique In castrated rats, TUNEL clearly revealed a distinct pattern of nuclear staining in both epithelial and stromal cells on d 4, followed by a gradual decrease in the rate of staining thereafter. With epristeride treatment, positive staining was found in both cells on d 4, and there was no marked increase thereafter (Fig 2).

DNA electrophoresis In castrated rats, DNA lysis occurred on d 4, 7, 10, 20 and DNA extract demonstrated a clear ladder with bands down to a single nucleosome's complement of DNA. While in epristeride

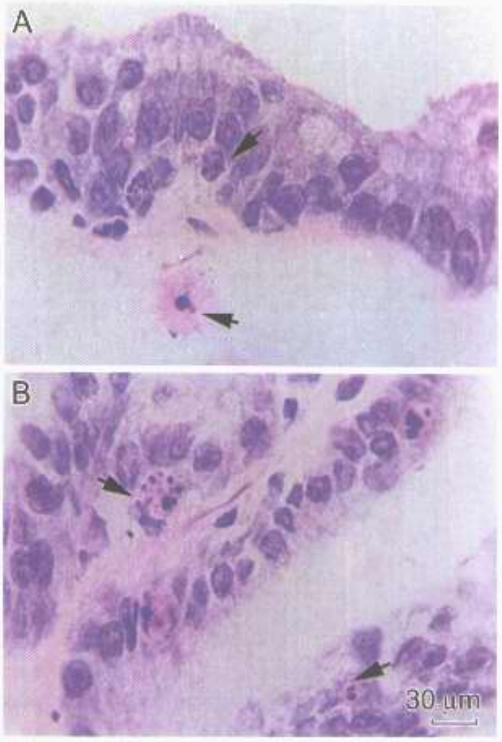

Fig 1. HE staining of prostate. Paraffine section from rat ventral prostate stained by the H&E. Apoptotic bodies are marked by arrows. (×330). (A) Prostate from rats 4 d postorchiectomy; (B) Prostate from rats treated with epristeride 50 mg/kg for 4 d.

treated group, typical ladder was found only on d 4, 7, and 10 (Fig 3).

Assay of pACP activity Seven days after castration, the activity decreased by 61 % of control. It continued to decrease thereafter. At the end of 20 d, it was only 39 % of control. Similarly, ACP activity declined in the same manner as that of castration, but in a much lesser extent in the epristeride group. The changes in the activity of ACP were coincide with those in the prostate wet weight and DNA content during treatment (Fig 4, 5, 6).

DISCUSSION

Various reports noted apoptosis occurred mainly at the glandular epithelium. We have also found positive staining in stromal cells in epristeride-treated group using TUNEL technique. This was perhaps due to the specific inhibitory activity of 5α-reductase type II [7]. This was

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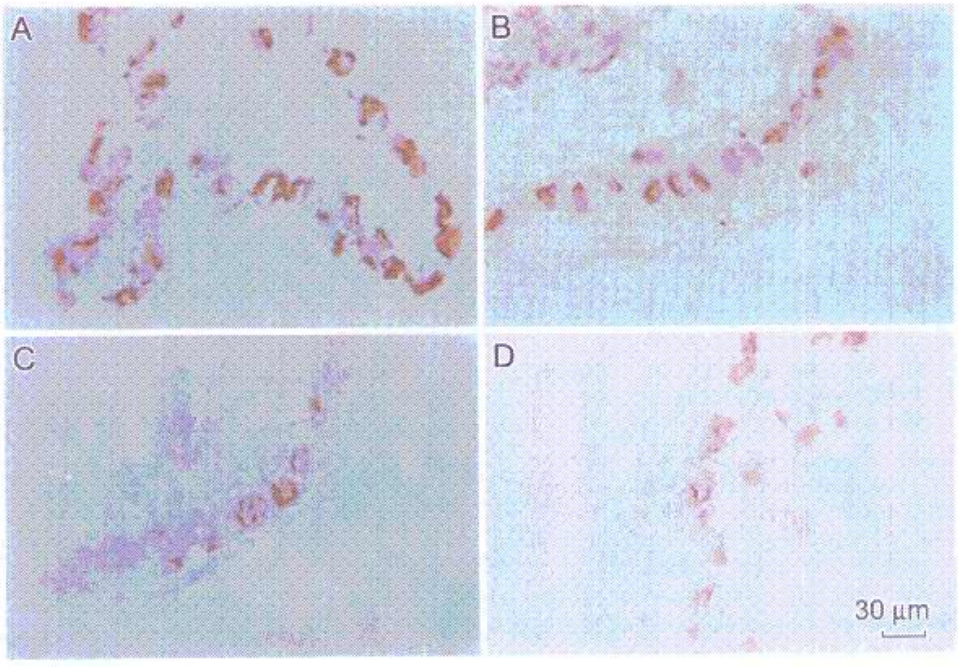

Fig 2. TUNEL of prostate. Paraffine section from rat ventral prostate stained by the TUNEL method for PCD. (×330). (A) Prostate from rats 4 d postorchiectomy; (B) Prostate from rats 10 d postorchiectomy; (C) Prostate from rats treated with epristeride 50 mg/kg for 4 d. (D) Prostate from rats treated with epristeride 50 mg/kg for 10 d.

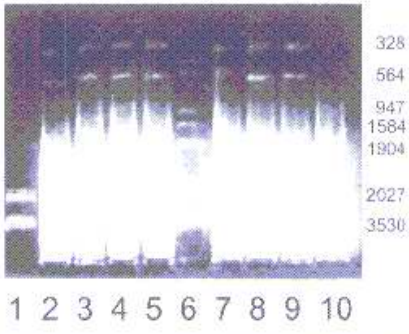

Fig 3. DNA analysis by 1 % agarose gel electrophoresis of the genomic DNA extracted from rat ventral prostate. Lane 1, 6: DNA marker; 2, 3, 4, 5: 20 d, 10 d, 7 d, and 4 d postochiectomy, respectively; 7, 8, 9, 10: 20-d, 10-d, 7-d, and 4-d treatment with epristeride 50 mg/kg, respectively.

also consistent with other reports, which demonstrated that detectable levels of 5α-reductase type Ⅱ mRNA were found in both stromal and epithelium[8].

ACP was a glycoprotein, synthesized by a series of glycosylations of the basic polypeptide chain during packaging in the golgi apparatus. There were androgenic controls at both the level of enzyme and the level of controlling the state of glycosylation[9]. Its activity may reflect the secretory ability of prostatic glandular cells. It has been reported elsewhere that acid phosphatase could be a useful marker of androgen action in prostate explant culture[10]. Our study also showed that the decrease of ACP activity was related to chemical event in PCD of prostatic gland and suggested the possibility of using it as a marker of atrophy of prostatic gland *in vivo*. The changes in the ACP activity during castration or epristeride treatment were consistent with other traditional markers of androgen action such as prostate wet weight and DNA content.

If cell death was the only mechanism causing the reduction in wet weight in the prostate of epristeride-treated animals, the prostate weight should decrease in proportion to the decrease of DNA content. But this was not the case. Prostate weight decreased more rapidly than DNA content in rats given 5α-reductase inhibitor. In the 4-d experiment, the wet weight of prostate decreased by 27 %, and DNA concentration increased by 31 %, resulting in no marked change of total prostate DNA content. However, epristeride caused a marked decrease in

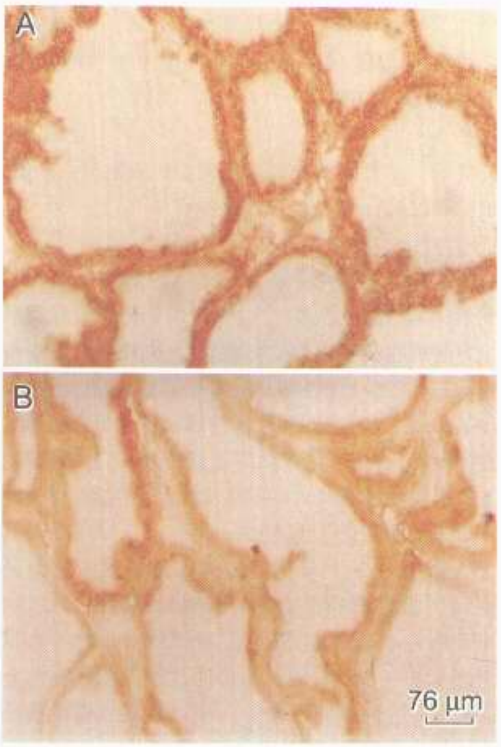

Fig 4. Prostate ACP of rats (× 132). (A) Prostate from intact rats; (B) Prostate from rats treated with epristeride for 20 d.

ACP activity. Therefore, a 4-d epristeride treatment in the rat markedly decreased epithelial cell secretory activity but caused little or no cell death. After 10 d, the continued reduction in the wet weight of the prostate appeared to be due to both cell loss and decreases of cell secretory activity. Our results provided much insight to the contribution of both the atrophy indicated by DNA content and ACP activity and cell death via apoptosis due to the epristeride-induced decrease in prostate weight.

Both the rate and the degree of inhibition of prostatic secretion and apoptotic prostatic glandular cells by epristeride treatment were smaller than those induced by surgical castration. Various reports showed that epristeride treatment induced an increase in the concentration of testosterone[11]. And an increased concentration of testosterone could compensate for the weaker interaction of testosterone with the androgen receptor[12]. This could explain why epristeride did not induce the same degree of prostatic cell death, inhibition of prostatic cell proliferation, and ACP activity as surgical castration.

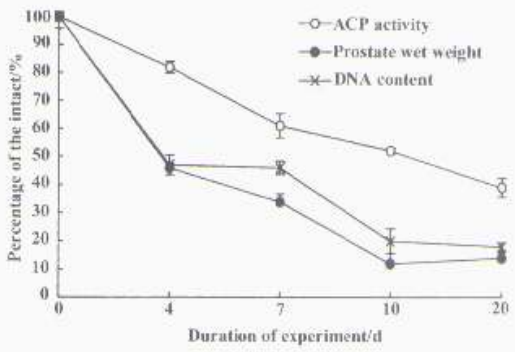

Fig 5. Changes in the prostate ACP activity of castrated rats and compared with those in the wet weight of prostate and DNA content during 20-d treatment. All data were calculated as the percentage of that of the intact. $n = 10$. $\bar{x} \pm s$.

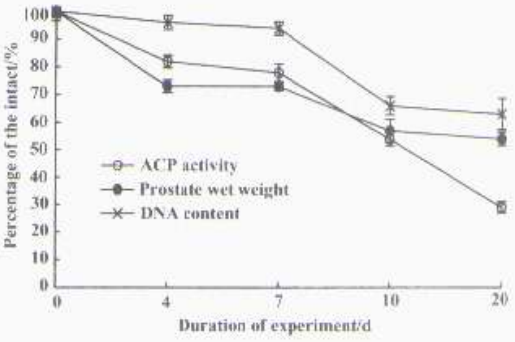

Fig 6. Changes in the prostate ACP activity of rats treated with epristeride 50 mg/kg and compared with those in the wet weight of prostate and DNA content during 20-d treatment. All data were calculated as the percentage of that of the intact. $n = 10$. $\bar{x} \pm s$.

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5α-还原酶抑制剂爱普列特诱导大鼠腹侧前列腺萎缩和细胞凋亡

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关键词 爱普列特；前列腺增生；细胞凋亡；萎缩；酸性磷酸酶；原位切口末端标记

目的：研究爱普列特是否通过诱导前列腺细胞凋亡来治疗前列腺良性增生．探讨以前列腺酸性磷酸酶作为前列腺萎缩标志的可行性．**方法**：光镜观察细胞形态变化．TUNEL 法和琼脂糖凝胶电泳检测 DNA 断裂．测定前列腺酸性磷酸酶的活性．**结果**：去势和爱普列特均诱发前列腺细胞发生细胞凋亡．去势引发的细胞凋亡的程度大于爱普列特．爱普列特和去势均降低了前列腺湿重和 DNA 含量，升高了 DNA 浓度．最大或接近最大的抑制发生在给药后 10 天．爱普列特抑制了前列腺酸性磷酸酶的活性，其变化与给药或去势后前列腺湿重和 DNA 含量的变化一致．**结论**：爱普列特通过诱发前列腺细胞凋亡来治疗前列腺良性增生．前列腺酸性磷酸酶的活性可作为前列腺萎缩的标志．

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