α-spinasterol from *Melandrium firmum* attenuates benign prostatic hyperplasia in a rat model

MEE-YOUNG LEE¹, IN-SIK SHIN¹, HWANGBO KYOUNG², CHANG-SEOB SEO¹, JONG-KEUN SON² and HYEUN-KYOO SHIN¹

¹Basic Herbal Medicine Research Group, Korea Institute of Oriental Medicine, Yuseung-gu, Daejeon 305-811; ²College of Pharmacy, Yeungnam University, Gyeongsan, Gyeongsangbuk-do 712-749, Republic of Korea

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Abstract. Spinasterol, a biologically active compound, exhibits a number of pharmacological activities, including antitumor, antiulcerogenic and anticarcinogenic activity, and originates from the aerial parts of *Aster scaber* Thunb (Asteraceae). The present study investigated whether α-spinasterol isolated from *Melandrium firmum* Rohrbach could prevent benign prostatic hyperplasia (BPH) induced by testosterone propionate (TP) in rats. Male Wistar rats were randomly divided into four groups of eight rats following castration. A negative control group received subcutaneous injections of corn oil. Treatments were administered orally 1 h prior to TP injection. All the rats were sacrificed at the scheduled termination time and their prostates were removed, cleaned and weighed. The prostate size ratio (prostate weight/rat body weight) was then calculated. Additional histopathological examinations were conducted, and the levels of TP and dihydrotestosterone (DHT) in the serum and prostate were measured. TP significantly increased the prostate size ratio (P<0.01), and DHT and testosterone levels in the serum and prostate. The TP-induced increase was significantly inhibited in α-spinasterol-treated rats when compared with the negative controls (P<0.05). In addition, histopathological examination demonstrated that α-spinasterol treatment suppressed TP-induced prostatic hyperplasia. It is concluded that α-spinasterol can prevent TP-induced prostatic hyperplasia and may be beneficial in the management of BPH.

Introduction

Benign prostatic hyperplasia (BPH) is extremely common and it is clinically characterized by prostate enlargement and lower urinary tract symptoms. Despite the enormous burden of BPH on the public health, the pathogenesis of BPH is only partially understood (1,2). It is well known that enlargement of the prostate occurs in the presence of androgens (3) and that anabolic steroids increase prostatic volume and reduce urine flow, leading to increased urinary frequency (4). The prostate is an androgen-dependent organ where testosterone and its precursors of extratesticular origin are bioactivated to the more potent dihydrotestosterone (DHT) (5). The prostate gland is generally considered a significant site of DHT formation (6). The systemic effect of endocrine activity in the prostate gland is principally DHT formation and its release to the circulation. The production of DHT increases with age, which induces the enhancement of prostate growth and hyperplasia (7). The significance of DHT is supported by clinical studies where an inhibitor of 5α-reductase was administered to males with BPH. In a number of cases, therapy with a 5α-reductase inhibitor significantly reduced the DHT level of the prostate and prostate size (8). Finasteride is widely used in the treatment of androgen-dependent diseases, specifically male pattern baldness, BPH and prostate cancer (9). Finasteride is a competitive and specific inhibitor of 5α-reductase, and has a role in inhibiting the conversion of testosterone to DHT in androgen-sensitive tissues, including the prostate and hair follicles, and therefore suppressing the serum and intraprostatic DHT concentrations (10). Conventionally, drugs, including finasteride and dutasteride, have been shown to be an effective treatment for BPH, but their use is restricted due to associated side effects, including erectile dysfunction, loss of libido, dizziness and upper respiratory tract infection (11,12).

Spinasterol is a biologically active compound isolated from the aerial parts of *Aster scaber* Thunb (Asteraceae), which exhibits various pharmacological activities, including antitumor, antiulcerogenic and anticarcinogenic activities (13-15). A study has demonstrated that spinasterol also has anti-inflammatory effects (16). However, there is no previous study of the possible efficacy of using α-spinasterol to treat testosterone-induced BPH model in rats. In the present study, a testosterone-induced rat model of BPH was used (17,18) and the...
therapeutic effects of α-spinasterol were evaluated, including its inhibition of the production of androgenic hormones, such as testosterone and DHT.

Materials and methods

Plant materials containing α-spinasterol. Whole plants of Melandrium firmum Rohrbach were purchased in March 2005 from a folk medicine market, ‘Yak-yong-si’, in Daegu and the material was confirmed taxonomically by Professor Ki-Hwan Bae, of Chungnam National University (Daejeon, Republic of Korea). A voucher specimen (YNS-99-01) was preserved at the College of Pharmacy, Yeungnam University (Gyeongsan, North Gyeongsang).

Instruments and reagents. Melting points were measured using Fisher-Johns melting point apparatus (Thermo Fisher Scientific Co., St. Louis, MO, USA) and they were uncorrected. Fourier transform-infrared spectroscopy (FT-IR) spectra were recorded using a JASCO FT-IR 300E spectrophotometer (Jasco, Tokyo, Japan). Nuclear magnetic resonance (NMR) spectra were recorded with a Bruker 250 MHz (DMX 250) spectrometer (Bruker, Rheinstetten, Germany) using a standard Bruker pulse program. The samples were dissolved in pyridine-d$_6$, and chemical shifts were reported in parts per million downfield of TMS. The 1H and 13C-NMR spectra were recorded with a Bruker DMX 250 MHz spectrometer (Bruker, Rheinstetten, Germany) using a standard Bruker pulse program. The samples were dissolved in pyridine-d$_6$, and chemical shifts were reported in parts per million downfield of TMS.

Extract and isolation. Whole plants of Melandrium firmum Rohrbach (10.0 kg) were extracted three times with 80% MeOH (20 liter) under reflux for 12 h, then filtered and concentrated to yield the MeOH extract (550.0 g). The MeOH extract was suspended in H$_2$O and extracted with hexane, EtOAc and then BuOH to yield the hexane-soluble fraction (75.9 g), EtOAc-soluble (47.4 g), and BuOH-soluble fractions (97.0 g), respectively. The hexane-soluble fraction (50.0 g) was subjected to column chromatography with silica gel (60x12 cm, silica gel 70-230 mesh) and eluted with hexane: EtOAc (50:1 - 1:1) and EtOAc (100:1) in a gradient manner. The resulting sections of the prostates were stored at ‑80˚C to ‑20˚C, and then stored at ‑80˚C. Whole prostates were immediately removed, and embedded in paraffin for histological analysis. The ventral prostates lobe were fixed with 10% buffered formalin, and embedded in paraffin. Histological sections of ventral prostate were stained with hematoxylin and eosin, and embedded in paraffin for histological analysis. The remaining sections of the prostates were stored at ‑80˚C to evaluate hormone levels.

Induction of BPH and treatments. The rats were randomly divided into five groups (n=8) following castration and a week of acclimatization. Experimental groups 2-5 had BPH induced via daily subcutaneous injections for four weeks with testosterone propionate (TP; 3 mg/kg) dissolved in corn oil. The animals in the experimental groups received phosphate-buffered saline (PBS), finasteride (positive control, 10 mg/kg), and α-spinasterol (5 mg/kg) by gavage during the four weeks of TP injection. The negative control groups were orally administered PBS and received daily subcutaneous injections of TP. Finasteride was used for the treatment of BPH, since it is a well-known 5α-reductase inhibitor. The effective dose of finasteride was based on previous reports. The body weight was measured weekly during the experiment. The application volume was calculated in advance, based on the most recently recorded body weight of individual animals.

Following the final treatment and overnight fasting, all animals were anesthetized with pentobarbital. The blood samples were drawn from the caudal vena cava. The serum was separated by centrifugation for 10 min at 200 x g and stored at -80˚C. Whole prostates were immediately removed, weighed and their relative organ weight was calculated as the ratio of prostate to body weight. The percentage of inhibition of prostate to relative organ weight by α-spinasterol was calculated as previously described (20). Sections of the ventral prostate lobe were fixed with 10% buffered formalin and embedded in paraffin for histological analysis.
Homogenate preparation. Prostate tissue was homogenized (1/10 w/v) in a tissue lysis/extraction reagent containing protease inhibitors (Sigma, St. Louis, MO, USA) using a homogenizer (IKA, Staufen, Germany). Homogenates were centrifuged at 12,000 x g for 25 min at 4 °C, and protein concentrations in the supernatant fractions were determined using a Bradford reagent (Bio-Rad, Hercules, CA, USA).

Measurements of testosterone and DHT in serum and prostate. The levels of testosterone and DHT in the serum and prostate were measured by an enzyme-linked immunosorbent assay. DHT kits were purchased from ALPCO Diagnostics (Salem, NH, USA) and testosterone kits were obtained from Cayman (Ann Harbor, MI, USA). The values are expressed per mg protein in the prostate and per ml in the serum.

Histopathological examination. To assess morphological changes in the prostate, the tissue was embedded in paraffin, cut into 4-µm thick sections and stained with hematoxylin and eosin solution (hematoxylin, Sigma MHS-16, and eosin, Sigma HT110-1-32). The tissues were mounted, placed under a coverslip using Dako mounting medium (Dakocytomation, Denmark, CA, USA) and examined microscopically (Olympus BX51; Olympus, Tokyo, Japan).

Statistical analysis. Data are expressed as the mean ± standard deviation. Statistical significance was determined using Student's two-tailed t-test to compare independent means with Microsoft Excel (Microsoft, London, UK). $P<0.01$ or $P<0.05$ were considered to indicate a statistically significant difference.

Results

Effect of α-spinasterol on prostate weight. The relative prostate weights of TP-induced BPH animals were significantly higher compared with the negative control group. The body weight of animals treated with α-spinasterol was not significantly different from that of BPH animals (Fig. 1A). However, a significant reduction in the relative prostate weight was identified in animals treated with α-spinasterol when compared with the TP-induced BPH group (Fig. 1B).

Effects of α-spinasterol on testosterone and DHT levels in the serum. The TP-induced BPH animals had significantly higher serum testosterone levels (20.80±2.63 ng/ml) compared with the negative controls (0.14±0.18 ng/ml). However, animals treated with α-spinasterol (10.53±2.39 ng/ml, $P<0.05$) had significantly lower serum testosterone levels compared with TP-induced BPH animals (Fig. 2A). The DHT level in animals treated with α-spinasterol (14.74±2.38 ng/ml, $P<0.01$) was also significantly lower compared with TP-induced BPH animals (23.95±3.74 ng/ml, $P<0.01$) (Fig. 2B).

Effect of α-spinasterol on testosterone and DHT levels in the prostate. The TP-induced BPH animals had higher levels of prostate testosterone (3.25±0.23 ng/ml, $P<0.01$) and DHT (426.01±144.05 pg/ml, $P<0.01$) compared with the castrated group. The group treated with finasteride had significantly lower testosterone levels (1.87±0.27 ng/ml, $P<0.01$) and DHT levels (126.22±34.66 pg/ml, $P<0.01$) compared with BPH animals. As with the finasteride-treated group, animals treated with α-spinasterol had significantly lower testosterone (1.47±0.27 ng/ml, $P<0.01$) and DHT levels (133.31±35.27 pg/ml, $P<0.01$) compared with BPH animals (Fig. 3A and 3B).
Effects of α-spinasterol on the histomorphology of prostate tissue. The epithelial cell layers of the prostate were larger in TP-induced BPH animals compared with the negative controls. Notably, this histological analysis revealed that administration of α-spinasterol marginally altered the histological architecture of rats with BPH (Fig. 4).

Discussion

In the present study, a rat model was used to examine the effect of α-spinasterol isolated from Melandrium firmum Rohrbach on the progression of induced prostatic epithelial hyperplasia. The results demonstrated that relative prostate weight, androgen (DHT and testosterone) levels in prostate and serum, and histopathological changes associated with prostatic hyperplasia were significantly reduced by administration of α-spinasterol.

The reduction of prostate weight following the administration of α-spinasterol in the present study may be due to decreased levels of DHT and testosterone. According to previous studies, testosterone and DHT exhibit key role in the development of the male reproductive organs, and these hormones are commonly associated with BPH (21,22). Aging leads to excessive production of DHT, which causes the development and exacerbation of BPH (21). The relative prostatic weight of animals with BPH is significantly increased compared with a vehicle control, whereas the relative prostatic weight is reduced in animals treated with finasteride or lauric/myristic acid (20).

The results of the present study were consistent with the histopathological examination of prostate tissue. BPH animals exhibited glandular hyperplasia in the prostate, whereas animals treated with α-spinasterol demonstrated only mild glandular hyperplasia. Therefore, these findings and the results of the hormone examination indicate that α-spinasterol is effective in the treatment of BPH.

α-spinasterol also decreased the levels of testosterone and DHT in serum and prostate gland. These findings, and the results of the relative weight and histopathological examination of prostates, indicate that α-spinasterol inhibits the development of BPH in rats, and these effects are closely associated with a reduction in DHT level. Therefore, higher serum and prostate DHT levels may be associated with a larger prostate volume and a higher prevalence of BPH.
In conclusion, oral administration of α-spinasterol in a BPH rat model significantly decreased prostate size, prostatic hyperplasia and DHT levels in the serum and prostate. This information may provide an improved understanding of the pathogenesis of age-dependent conditions, including BPH. In addition, it may aid in the identification of improved targets and the development of more specific therapeutic agents to treat prostate conditions. The results of the present study indicate that α-spinasterol may be a useful agent in BPH treatment.

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References