Ursolic acid reduces prostate size and dihydrotestosterone level in a rat model of benign prostatic hyperplasia

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ABSTRACT

Benign prostatic hyperplasia (BPH) is characterized by hyperplasia of prostatic stromal and epithelial cells, which can lead to lower urinary tract symptoms. The prevalence of BPH increases in an age-dependent manner. We investigated the protective effect of ursolic acid in BPH development using a testosterone-induced BPH rat model. BPH was induced in experimental groups by daily subcutaneous injections of testosterone propionate (TP), for a period of four weeks. Ursolic acid was administrated daily by oral gavage at a dose level of 5 mg/kg during the four weeks of TP injections. Animals were sacrificed on the scheduled termination, before prostate were weighed and subjected to histopathological examination. TP and dihydrotestosterone (DHT) levels in the serum and prostate were also measured. BPH-induced animals displayed an increase in prostate weight with increased testosterone and DHT levels in both the serum and prostate. However, ursolic acid treatment resulted in significant reductions in prostate weight and testosterone and DHT levels in both the serum and prostate, compared with BPH-induced animals. Histopathological examination also showed that ursolic acid treatment suppressed TP-induced prostatic hyperplasia. These findings indicate that ursolic acid may effectively inhibit the development of BPH and it may be a useful agent in BPH treatment.

Keywords: Benign prostatic hyperplasia, Ursolic acid, Testosterone, Dihydrotestosterone

1. Introduction

Benign prostatic hyperplasia (BPH) is one of the commonest diseases affecting aging men. BPH is characterized by hyperplasia of the prostate stromal and epithelial cells, which results in increased prostate size (Porpiglia et al., 2006; Pais, 2010). BPH can be associated with lower urinary tract symptoms such as urinary frequency, urgency, urgency incontinence, and nocturia, and leads to an increased risk of obstruction of the urethra, urinary retention, and urinary infection (Miller and Tarter, 2009).

The mechanism underlying the pathogenesis of BPH is not completely elucidated. Most researchers consider that androgens have a permissive role in the development and growth of the prostate gland. Dihydrotestosterone (DHT) is a metabolite of testosterone, and a critical mediator of prostate growth, which is synthesized in the prostate from circulating testosterone by 5α-reductase (Andriole et al., 2004). DHT production increases with aging and induces enhanced prostate growth and hyperplasia (Carson and Rittmaster, 2003). The importance of DHT is supported by clinical studies in men with BPH who were provided with 5α-reductase inhibitors and therapy. In many cases, the 5α-reductase inhibitor significantly reduced prostate size and the DHT level in the prostate (Kumar and Wahane, 2008). The 5α-reductase inhibitors, finasteride and dutasteride, are used commercially for the treatment of BPH (Miller and Tarter, 2007; Kumar and Wahane, 2008). Conventional drugs, such as finasteride and dutasteride, have proved to be effective treatments for BPH, but their use is restricted because of associated side effects, such as erectile dysfunction, loss of libido, dizziness, and upper respiratory tract infection (Bullock and Andriole, 2006; Paba et al., 2011).

Ursolic acid is a pentacyclic triterpenoid compound found in a large range of vegetarian foods, medicinal herbs, and other plants. Recent studies show that ursolic acid suppresses cell proliferation via the induction of apoptosis in various prostate cells, including PC-3 cells, DU145 cells, and primary human prostate cancer cells (Kwon et al., 2010; Zhang et al., 2010; Kondo et al., 2011). Given these effects, we consider that ursolic acid could be an effective drug for the treatment of BPH. However, no previous study has tested the efficacy of ursolic acid using a testosterone-induced BPH rat model. Therefore, we measured the prostate weights and examined histopathological changes in prostate tissue after administering ursolic acid by oral gavage, using a testosterone-induced BPH rat model. We also evaluated the levels of testosterone and DHT in serum and prostate tissue.
2. Materials and methods

2.1. Animals

Male 12-week-old Wistar rats weighing 250–350 g (Central Lab. Animal, Inc., Seoul, Korea) were housed in a room maintained at 18–23 °C with a relative humidity of 40–60%, and an alternating 12 h light:dark cycle. Rats were provided with a standard laboratory diet and water ad libitum. All experimental procedures were carried out in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals, while animal handling followed the dictates of the National Animal Welfare Law of Korea.

2.2. Castration procedure

After one week of acclimatization, all rats were castrated while anesthetized by an intraperitoneal injection of pentobarbital three days before the beginning of the experiments to exclude the influence of intrinsic testosterone. Castration was performed by removing the testicles and epididymal fat through the scrotal sac, according to a method published previously (Coppinolle et al., 2000).

2.3. Induction of BPH and treatments

Castrated rats were randomly divided into four groups (n = 6): (A) castration group, which received PBS administered orally and corn oil injected subcutaneously; (B) BPH group, which received PBS administered orally and testosterone propionate (TP 3 mg/kg body weight) injected subcutaneously; (C) positive control group, which received finasteride (10 mg/kg body weight) administered orally and TP (3 mg/kg body weight) injected subcutaneously; (D) ursolic acid group, which received ursolic acid (5 mg/kg body weight) administered by oral gavage and TP (3 mg/kg body weight) injected subcutaneously. Ursolic acid was purchased from Across Organics (Geel, Belgium). The positive control drug, finasteride, is a 5α-reductase inhibitor used for BPH treatment. The effective dose of finasteride was based on previous reports (Huynh, 2002). All rats were treated once a day for four weeks. Body weight was measured weekly during the experiment. The application volume was calculated in advance, based on the most recent recorded body weight of individual animals.

All animals were anesthetized with pentobarbital [100 mg/kg body weight, i.p.] after the final treatment and overnight fasting. Blood samples were drawn from the caudal vena cava. Serum was separated by centrifugation and stored at −80 °C. Whole prostates were immediately removed, and weighted. Relative organ weights were calculated as the ratio of prostate weight to body weight. Sections of the ventral prostate lobe were fixed with 10% neutral buffered formalin and embedded in paraffin for histological analysis. The remainder of each prostate was stored at −70 °C and used to evaluate hormone levels.

2.4. Determination of testosterone and DHT levels in serum and prostate

Prostate tissue was homogenized (1/10 w/v) using a homogenizer in a tissue lysis/extraction reagent that contained a protease inhibitor cocktail (Sigma, MI, USA). Homogenates were centrifuged at 12,000g for 25 min at 4 °C, and the protein concentration in the supernatant fractions were determined using Bradford reagent (Bio-Rad, Hercules, CA). Testosterone and DHT levels in the serum and prostate were measured using enzyme-linked-immunosorbent assay (ELISA). DHT kits purchased from ALPCO Diagnostics (Salem, NH) and testosterone kits were from Cayman (Ann Arbor, MI). Values were expressed as per mg protein in prostate and per mL in serum.

2.5. Determination of prostate specific antigen (PSA) levels in serum

The level of PSA in the serum was quantified by ELISA (MyBiosource, Arizona, USA) according to manufacturer’s protocol. Values were expressed as per mL in serum.

2.6. Histopathological examination

To assess morphological changes in prostate, tissues were embedded in paraffin and cut into sections of 4 μm thickness and stained with H&E solution (hematoxylin, Sigma MHS-16, and eosin, Sigma HT110-1-32). Tissues were subsequently mounted and coverslipped using mounting medium (Invitrogen, Carlsbad, CA), and then examined microscopically (Nikon, Japan).

2.7. Measurement of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels in serum

ALT and AST levels were determined to assess liver function using commercial kits (BECKMAN Coulter, Inc., Fullerton, CA, USA) and an auto-analyzer (BECKMAN CX4, CA, USA).

2.8. Statistical analysis

Data were expressed as means ± standard deviation (SD). Statistical significance was determined using analysis of variance (ANOVA). Tests that showed a significant difference among groups were analyzed by a multiple comparison procedure using Dunnett’s test (1964). The level for significance was set at P < 0.05 or P < 0.01.

3. Results

3.1. Effect of ursolic acid on prostate weight

The relative prostate weight is an important indicator in BPH. TP-induced BPH group showed a significant increase in relative prostate organ weight (0.52 ± 0.02%, P < 0.01) compared with the castration group (Table 1). Finasteride-treated group showed a significant reduction in relative prostate weight (0.44 ± 0.03%, P < 0.01) compared with the TP-induced BPH group. Ursolic acid-treated group showed a significant reduction in relative prostate weight (0.45 ± 0.03%, P < 0.01, F = 2.250, degrees of freedom (DF) = 10) compared with the TP-induced BPH group.

3.2. Effects of ursolic acid on testosterone and DHT levels in serum

The TP-induced BPH group had significantly increased serum testosterone levels (20.81 ± 2.63 ng/mL, P < 0.01) compared with the castration group (0.14 ± 0.04 ng/mL). However, the finasteride-treated group (12.92 ± 2.24 ng/mL, P < 0.01) and the ursolic acid-treated group (13.44 ± 2.11 ng/mL, P < 0.01, F = 1.554, DF = 10) had significantly decreased serum testosterone levels compared with the TP-induced BPH group (Fig. 1A). Serum DHT levels in the TP-induced BPH group (23.95 ± 3.75 ng/mL, P < 0.01) were significantly increased compared with the castration group (0.18 ± 0.04 ng/mL). However, serum DHT levels in the finasteride-treated group (14.91 ± 3.02 ng/mL, P < 0.05) and ursolic acid-treated group (13.25 ± 2.40 ng/mL, P < 0.05, F = 2.441, DF = 12) were significantly decreased compared with the TP-induced BPH group (Fig. 1B).

3.3. Effect of ursolic acid on prostate testosterone and DHT levels in prostate

In prostate, while TP-induced BPH group increased the levels of testosterone (3.25 ± 0.23 ng/mL, P < 0.01) and DHT (301.61 ± 65.32 pg/mL, P < 0.01) more than the castration group, finasteride treated group markedly decreased testosterone (1.87 ± 0.27 ng/mL, P < 0.01) and DHT (165.39 ± 66.35 pg/mL, P < 0.05). Similar to the results of finasteride treated group, ursolic acid-treated group significantly reduced testosterone (1.97 ± 0.22 ng/mL, P < 0.05, F = 1.903, DF = 10) and DHT (135.24 ± 35.96 pg/mL, P < 0.01, F = 3.300, DF = 10) levels compared with those of the BPH group (Fig. 2A and B).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body weights (G)</th>
<th>Prostate weights</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
</tr>
<tr>
<td>Castration</td>
<td>320.6 ± 12.91</td>
<td>384.1 ± 19.43</td>
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<tr>
<td>BPH</td>
<td>316.4 ± 16.43</td>
<td>356.2 ± 13.27</td>
</tr>
<tr>
<td>Finasteride</td>
<td>322.0 ± 10.64</td>
<td>359.7 ± 21.36</td>
</tr>
<tr>
<td>Ursolic acid</td>
<td>321.3 ± 12.6</td>
<td>354.5 ± 27.14</td>
</tr>
</tbody>
</table>

Table 1

Effects of ursolic acid on body weights and prostate weights in TP treated rats.

Castration: corn oil injection (s.c) + PBS (p.o.). BPH: testosterone (s.c) + PBS (p.o.). Finasteride: finasteride (10 mg/kg, p.o.) + testosterone (s.c). Ursolic acid: ursolic acid (5 mg/kg, p.o.) + testosterone (s.c). * P < 0.01 when compared with the castration group. ** P < 0.05 when compared with the BPH group.
3.4. Effect of ursolic acid on PSA level in serum

TP-induced BPH group significantly increased the levels of PSA in serum (3.78 ± 0.36 ng/mL, \( P < 0.01 \)) compared with the castration group (2.97 ± 0.25 ng/mL) (Fig. 3). However, finasteride treated group (2.87 ± 0.27 ng/mL, \( P < 0.01 \)) decreased the level of PSA in serum more than the BPH group. Ursolic acid-treated group (3.19 ± 0.31 ng/mL, \( P < 0.05 \), \( F = 1.349 \), DF = 10) significantly reduced PSA level compared with the BPH group.

3.5. Effect of ursolic acid on prostate tissues by histopathological examination

Epithelial cell layers and stromal spaces of the prostate were larger in the TP-induced BPH group compared with the castration group (Fig. 4). Finasteride-treated group showed mild glandular hyperplasia compared with the TP-induced BPH group. Ursolic acid-treated animals also displayed a reduction of in the epithelial cell layers and stromal spaces of the prostate compared with BPH group, which similar to that observed in the finasteride-treated group.

3.6. Toxicity of ursolic acid in a rat model of BPH

As shown in Fig. 5, ursolic acid did not promote activities of the serum toxicity marker enzymes (ALT and AST), indicating the normal liver function.

4. Discussion

We evaluated the effects of ursolic acid on prostate size and DHT and testosterone levels in both prostate tissue and serum in
a TP-induced BPH rat model. TP-induced rats showed increases in prostate size and DHT levels in the serum and prostate compared with the castration group and prostatic hyperplasia was observed during histopathological examinations. However, ursolic acid-treated rats displayed a decrease in prostate size, DHT or testosterone levels in both the serum and prostate, and PSA level in serum compared with the TP-induced rats. Histopathological examination also showed that oral administration of ursolic acid attenuated TP-induced prostatic hyperplasia.

Prostatic enlargement is used as one of important marker of BPH (Veeresh Babu et al., 2010; Pais, 2010). The relative prostate weight used in this study was the prostate weight to body weight ratio. In previous studies, animals with BPH had a significantly increased the relative prostate weight compared with vehicle control, whereas the relative prostate weight of animals treated with finasteride or lauric acid/myristic acid was decreased when compared with BPH animals (Veeresh Babu et al., 2010). In the present study, BPH animals experienced significant increases in relative prostatic weight compared with the castration animals. In contrast, oral administration of ursolic acid resulted in a significant reduction in relative prostate weight compared with BPH animals. These results were consistent with histopathological examinations of prostate tissues. BPH animals experienced stromal proliferation and glandular hyperplasia in the prostate, whereas animals treated with ursolic acid showed mild glandular hyperplasia. These findings and the results of with the results of the hormone assay suggest that ursolic acid is an effective treatment for BPH.

Testosterone and DHT have a key role in the development of male reproductive organs and these hormones are commonly associated with BPH (Andriole et al., 2004; Miller and Tarter, 2009). DHT is synthesized primarily in the prostate, testes, and hair follicles, from circulating testosterone. DHT has three times greater affinity for androgen receptors than testosterone and it has 15–30 times greater affinity than adrenal androgen (Andriole et al., 2004). Excessive production of DHT with aging leads to the development and exacerbation of BPH (Miller and Tarter, 2009). DHT is transformed from testosterone by 5α-reductase, so many researchers have conducted the studies to reduce the DHT level via the inhibition of 5α-reductase. Finasteride is a 5α-reductase inhibitor and an elective drug used for the treatment of BPH. Finasteride reduces the testosterone and DHT level in serum and prostate, which results in a reduction in prostate size and ultimately provides relief from the lower urinary tract symptoms related to BPH (Andriole et al., 2004; Nickel et al., 2011). However, finasteride also produces serious side effects (Bullock and Andriole, 2006; Paba et al., 2011), which has led researchers to investigate alternative materials for treating BPH with fewer side effects. Previous studies report the
protective effects of many materials, such as *Serenoa repens*, lauric acid, and myristic acid, which protect from prostatic hyperplasia via their 5α-reductase inhibitory activity (Bent et al., 2006; Suzuki et al., 2009; Veeresh Babu et al., 2010). The present study found that finasteride reduced testosterone and DHT levels in the serum and prostate as described previously (Suzuki et al., 1998). Ursolic acid also decreased the levels of testosterone and DHT in serum and prostate. In addition, the level of PSA increased in BPH animals, but significantly reduced in ursolic acid-treated animals compared with the BPH animals. PSA is produced in prostate and normally present in small quantities in the serum (Lilja, 2003). However, the level of PSA is elevated under the condition of BPH and prostate cancer (Levitt and Slawin, 2007). So, the reduction of PSA level indicates usefulness of test material in the treatment of BPH (Rick et al., 2011). These findings, the relative prostate weights, and the prostate histopathological examinations, together indicate that ursolic acid inhibits the development of BPH in rats and these effects were closely associated with a reduction in of DHT level.

In conclusion, oral administration of ursolic acid in a BPH rat model significantly decreased the prostate size, prostatic hyperplasia, and DHT levels in the serum and prostate. These findings indicate that ursolic acid may effectively inhibit the development of BPH. Our results strongly suggest that ursolic acid may be a useful agent in BPH treatment.

**Conflicts of Interest**

The authors declare that there are no conflicts of interest.

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**References**


