Full Length Research Paper

Qianliening capsule treats benign prostatic hyperplasia through regulating the expression of sex hormones, estrogen receptor and androgen receptor

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Accepted 12 December, 2011

The objective of the study is to evaluate the effect of Qianliening capsule (QLNC) on the expression levels of serum hormones, prostatic estrogen receptor and androgen receptor in benign prostatic hyperplasia (BPH) rats, and investigate the possible molecular mechanisms mediating its anti-BPH activity. Male Sprague-Dawley (SD) rat BPH model was generated. BPH rats were orally treated with different concentrations of QLNC. Blood and the prostatic tissues of animals were obtained. The prostatic weight (PW) and prostatic index (PI) were evaluated; the histopathological changes of prostatic tissue, the levels of serum testosterone and estradiol, the mRNA and protein expression of ER and AR in prostatic tissue were examined by microscopy with hematoxylin and eosin staining HE staining, ELISA, RT-PCR, immunohistochemistry, respectively. Compared to the model group, the PW and PI in all QLNC-treated groups have significantly lower (p<0.05) serum than T/E2 in QLNC-treated groups which was elevated significantly (p<0.05 or p< 0.01). Pathomorphism of prostatic tissue in QLNC-treated groups improved. The mRNA and protein expression of ER and AR in QLNC-treated groups decreased significantly. QLNC has significant therapeutic effect on BPH rats. Improvement of sex hormones disorder and regulation of ER and AR are one of the mechanisms by which QLNC treats BPH.

Key words: Benign prostatic hyperplasia; Qianliening capsule; sex hormones; estrogen receptor; androgen receptor.

INTRODUCTION

Benign prostatic hyperplasia (BPH) is a common disease of the urinary system among elderly men (Clement et al., 2008). Recent estimates suggest a BPH incidence of 42, 70 and 90% in males aged 51 to 60, 61 to 70, and 81 to 90 years, respectively (Nickel, 2008). More than 50% of patients with BPH experience lower urinary tract symptoms (LUTS) including urinary frequency, urgency, nocturia, and feeling of incomplete emptying of the bladder (Nickel, 2008). The pathogenesis of BPH is not fully understood. Currently, many scholars believe that the occurrence of BPH is primarily the result of the synergistic action of androgens and estrogens, as reflected by an imbalanced ratio of estrogen to androgen levels (Zhou et al., 2009). However, estrogen and androgen can only exert their biological effects after binding to the corresponding receptors. Indeed, studies have shown that the development of BPH is closely related to the levels of serum sex hormones as well as their receptors (James and Mohler, 2008; Gallardo et al., 2009).

The growth, development, hyperplasia, and malignant
transformation of the prostate gland has also been shown to be influenced by sex hormone levels (Brinkmann et al., 1992; Hull and Bostwick, 2008).

Qianliening capsule (QLNC) is a traditional Chinese medicinal formulation, which has been shown to have therapeutic effects on BPH (Zhou et al., 2010). However, the mechanism of QLNC’s anti-BPH activity remains unclear. The objective of the current study was to explore the mechanism of Qianliening capsules in the treatment of BPH, by assessing the effects of administration in a rat model of BPH.

MATERIALS AND METHODS

Experimental materials

Animals

Healthy SPF (Specific pathogen Free) grade male adult SD rats (n = 60) weighing 190 to 220 g were provided by Shanghai Slack Laboratory Animal Co., Ltd. (license number, SCXK (Shanghai) 2007-0005); batch number, 0017446). The rats were normally raised and accommodated to the laboratory environment for 5 days prior to experimentation.

Reagents and drugs

Testosterone propionate injection solution (25 mg/ml) was obtained from Shanghai GM Pharmaceutical Co., Ltd. (batch number: H31020524), while saline injection solution was obtained from Fuyao Pharmaceutical Co., Ltd. (Fuzhou, Fujian, batch number 060528A07). Finasteride was obtained from Hangzhou Merck (batch number: J20050041), while Trizol was obtained from Invitrogen; dNTP, reverse transcription (RT) Kit was obtained from Promega, and Qianliening capsules were developed by Fujian Chinese Medical University. Taq polymerase, RNase inhibitor was provided by Takara, while primers were synthesized by Shanghai Yingjun biological technology Co., Ltd. Estrogen receptor (ER) and androgen receptor (AR) primary antibody, secondary antibody, streptavidin-peroxidase (SP), 3,3′-diaminobenzidine (DAB) were obtained from Hebei Bohai Biotechnology Development Co., Ltd.

Experimental instruments

The following instruments were used in the current study: An E1x808 microplate reader (PET, USA), a type 9600 PCR instrument (PE), a Gel DOC type 2000 gel imaging analysis system, an APC speed refrigerated centrifuge (Eppendorf, 382E type, Japan), a DU650 type protein nucleic acid analyzer (Beckman Corporation), a true color multi-functional cell image analysis system (Image-Pro Plus, Media Cybernetics, USA), a 5417R high-speed refrigerated centrifuge (Eppendorf, Germany), an electronic balance (Switzerland METTER AE100), a drying oven (Sanyo MIR-153 type), and a low temperature refrigerator (Sanyo MDF-382E type, Japan).

Experimental methods

Preparation of rat models of BPH

Rats were anesthetized with an intraperitoneal injection of 100 mg/kg ketamine. Aseptic surgery was carried out via the scrotum, including the bilateral removal of the testes. After one week of recovery, each rat received a daily subcutaneous injection of testosterone propionate 5 mg/kg for 28 consecutive days. The body weight was measured once per week (Wu et al., 2005).

Drug administration and sample collection

After successful model construction, animals received repeated injection to prevent self-healing. Rats in the control group and the pathological model group received intragastric gavage of normal saline, meanwhile drugs were administered to the remaining groups at appropriate doses. The control group and the pathological model group received daily saline at 10 ml/kg, the Finasteride group received daily finasteride at 0.5 mg/kg, and the low-dose, mid-dose, and high-dose Qianliening capsule groups received daily Qianliening at 2.25, 4.5 and 9 g/kg, respectively. Daily intragastric gavage was used for drug administration during a continuous 28-day period.

Evaluation of drug efficacy and sample collection

During drug administration, body weight was measured once per week. After the last treatment, the rats were fasted for 24 h and then anesthetized. Blood samples were collected from the abdominal aorta; and intact prostate tissue was removed. The weight and volume of the prostate were measured and the PI was calculated as: Prostate wet weight / body weight × 100%. Prostate tissue was fixed with 10% formalin or stored in liquid nitrogen for later analyses.

Histological examination

Small pieces of the prostatic specimens were fixed with 10% buffered formalin for 24 h. Samples were then paraffin-embedded, sectioned, and stained with hematoxylin and eosin (H&E). Histopathological changes were observed under the microscope.

Detection of AR and ER mRNA expression using RT-PCR

Freshly frozen prostate tissue (80 mg) was collected and the total RNA was extracted using the Trizol kit according to the manufacturer’s instructions. A sample of 1 μg total RNA was taken and a RT reaction was carried out using the appropriate kit. The PCR reaction system consisted of 1 μl cDNA, 10 μl PCR MIX (2x), 0.4 μl each of upstream and downstream primers, and finally, DEPC water for a total system volume of 20 μl. The samples were vortexed, thoroughly mixed, and centrifuged at a high speed for 10 s. Subsequently, liquid paraffin was added and the samples were placed into the PCR thermal cycler for amplification. The amplification consisted of 35 cycles under the following conditions: 5 min of pre-denaturation at 95°C, 30 s of denaturation at 94°C, 40 s of annealing at 72°C, 30 s of extension at 72°C, and final annealing for 7 min at 72°C. The PCR products were electrophoresized in 1.5% agarose gel, and the resulting bands were scanned and analyzed using the gel image analysis system. GAPDH was selected as an internal reference gene. The primers used were: AR forward 5'-GEGGATGGTGAACAGATGC-3' and reverse 5'-GCCTTCACTGTGTAAGATC-3'; ER forward 5'-GGAGACATGATGGCTGCCA-3' and reverse 5'-CCAGCAAATGTCAAGATC-3'; GAPDH forward 5'TGCTGAATGTGTCCTGGA-3' and reverse 5'-
The grades were as follows: 0, 1 (weak positive, +), 2 (positive, ++), and 3 (strong positive, +++).

Detection of serum T and E2 using ELISA

Blood samples were obtained aseptically from the abdominal aorta. Serums were collected by centrifugation at 3000 × g for 20 min. The serum levels of T and E2 were measured with ELISA kits (Shanghai Xitang Biotechnology Co., Ltd), following the instructions of the manufacturer. Absorbance was read on a microplate reader and the concentrations were calculated according to the standard curve.

Detection of AR and ER protein expression using immunohistochemical methods

A 0.5 cm × 0.5 cm × 0.1 cm block of tissue was collected from the lateral lobe of the prostate gland of each rat. The tissue blocks were rinsed with phosphate buffer solution (PBS), fixed with 10% formaldehyde for 12 to 24 h, and subsequently embedded in paraffin, archived, and finally, sliced. The paraffin sections were used for ER and AR immunohistochemical staining. The primary antibodies employed were polyclonal rabbit anti-rat ER and AR. PBS were used to replace the primary antibody as a negative control.

Color was developed using 3,3'-diaminobenzidine (DAB) chromogen, as per the manufacturer’s instructions. Five different visual fields were randomly selected from each section under the microscope (×400), and the true color multi-functional cell image analysis and management system were used to obtain the surface density of positive targets and positive units. The grade of the number of positive cells multiplied by the color intensity of positive cells was used as the positive scoring criterion, with its level reflecting the amount of antigen. The grades were as follows: 0 (negative, -), 1 (weak positive, +), 2 (positive, ++), and 3 (strong positive, +++).

Statistical analysis

Statistical analysis was conducted using SPSS 12.0 software. Data were presented as mean ± standard deviation (SD). Quantitative data were analyzed using a Student’s t-test, and qualitative data were analyzed using chi-square test or the rank sum test, as appropriate. The between-group comparisons of the quantitative data used single factor analysis of variance. A P value < 0.05 indicated a statistically significant difference.

RESULTS

Effects of QLNC on the weight of prostate and the prostate index

In the model group, the wet weight of the prostate tissue and the prostate index increased significantly after model construction compared with those in the normal group (P < 0.05), and lasted for a continuous period of 28 days. This evidence indicates a successful model construction. Different doses of both Qianliening capsules and Finasteride significantly reduced the wet weight of prostate tissue and the prostate index in BPH rats compared with those in the model group (P < 0.05; Table 1). These findings suggest that Qianliening capsules have comparable efficacy to Finasteride in the treatment of BPH (Table 1).

QLNC treatment ameliorated the damage to prostate tissue

In the normal group, low columnar epithelial cells were arranged as a single layer forming secretory lumen which was filled with thin acidophilic materials (Figure 1A). In the model group, the epithelial cells proliferated obviously to develop excessive glands and cells were arranged as multiple unorganized layers (Figure 1B). In all treated groups, the cell proliferation and gland development were significantly inhibited (Figure 1C to F). In addition, QLNC treatment ameliorated the histopathological changes in a dose-dependent manner (Figure 1D to F).

Effects of QLNC on the serum testosterone (T) and estradiol (E2) levels

The serum T/E2 ratio in the model group was significantly lower than that in the control group (P < 0.05). The T/E2 ratios in the different Qianliening capsule dose groups and the Finasteride group were significantly increased (P
Figure 1. Effect of QLNC treatment on the histopathological changes in BPH rats. Small pieces of the prostatic specimens from different groups were fixed with 10% buffered formalin for 24 h. Samples were then paraffin-embedded, sectioned, and stained with hematoxylin and eosin (H&E). Histopathological changes were observed under the microscope. Images are representative photographs taken at a magnification of 100 ×. (A) control group; (B) model group; (C) Finasteride group; (D) QLNC-L group; (E) QLNC-M group; (F) QLNC-H group. QLNC-L, Qianliening capsule low dose. QLNC-M, Qianliening capsule middle dose. QLNC-H, Qianliening capsule high dose.

< 0.05). The effects of mid- and high-dose Qianliening capsules were most marked (P < 0.01; Figure 2).

**ER and AR mRNA expressions**

The ER and AR mRNA expressions in the prostate tissue of rats in the model group were significantly increased, and both Qianliening capsules and Finasteride at various doses significantly reduced these expressions (Figure 3).

**Expressions of ER and AR proteins**

The immunohistochemical results showed that the positive expressions of ER and AR proteins in the prostate tissue of rats in the model group were significantly higher than those in the normal group (P < 0.05). The positive expressions of ER and AR proteins in all Qianliening capsule dose groups and the Finasteride group were significantly lower than those observed in the model group (P < 0.05) (Table 2 and Figures 4 and 5).

**DISCUSSION**

The prostate gland is an androgen dependent organ. In the body, T is converted into dihydrotestosterone (DHT) in the prostate stroma under the action of the 5-α reductase and exerts its effects through the specific receptor on the nuclear membrane of the prostate stromal cells. In turn, the stromal cells act on the prostate epithelial cells through paracrine signalling. Evidence suggests that DHT plays a unique role in the occurrence of BPH (Andriole et al., 2004), and that both T and DHT (Roberts et al., 2004; Cochrane et al., 2007) have complementary roles in physiological regulation of males. However, the physiological functions of both T and DHT are mediated by androgen receptors (AR), that is, androgen-dependent transcription factors that belong to the nuclear receptor super-family of steroid hormones. AR can regulate genes and cause cell proliferation and differentiation, and their biological activity is mediated by the intracellular nuclear receptors. While AR are expressed in both the glandular epithelium and the stroma, in cases of BPH, their expression in the glandular epithelium (particularly in the nucleus) is significantly higher than that in the stroma. AR plays a key role in the androgen signal transduction system, mediating the biological activity of androgens and playing an important role in the occurrence and development of BPH. Prior studies have shown that AR expressions in the glandular epithelium of elderly men increase with age. These findings suggest that the maintenance of AR in the prostate gland of elderly men ensures the androgen-dependent growth of the prostate even if the serum T levels decline (Xia et al., 2001; Collins et al., 1994). Therefore, androgens and androgen receptors play an important role in the occurrence and development of BPH.

However, the occurrence and development of BPH appears to be induced by the synergetic action of a variety of human sex hormones rather than the action of any single one. The synergetic action of estrogens and androgens in the development of BPH is realized through increasing AR levels and sensitivity in the tissue (Trachtenberg et al., 1980; Suzuki et al., 1992). With increasing age, testicular function among elderly men is decreased, as are the in vivo androgen levels. Additionally, the conversion of androgen to estrogen is
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Figure 2. Effect of QLNC on serum T/E2 levels. Means±S.D., n = 10; *P < 0.05 vs. control group, **P < 0.05 vs. model group, ***P < 0.01 vs. model group. T/E2 levels in serum were measure using ELISA. T, Testosterone; E2, estradiol.

Figure 3. Effect of QLNC on the mRNA expression of ER and AR in prostatic tissue. The ER/AR mRNA expression was determined by RT-PCR, and GAPDH was used as an internal control. AR, Androgen receptor; ER, estrogen receptor.

Table 2. Effect of QLNC treatment on the protein expression of AR and ER in prostatic tissue.

<table>
<thead>
<tr>
<th>Group</th>
<th>Number</th>
<th>Estrogen receptor (ER)</th>
<th>Androgen receptor (AR)</th>
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<tr>
<td>Control</td>
<td>10</td>
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<tr>
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<tr>
<td>Finasteride</td>
<td>10</td>
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<tr>
<td>QLNC-L</td>
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<td>QLNC-M</td>
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<tr>
<td>QLNC-H</td>
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Means±S.D. n = 10; *P < 0.05 vs. control group, **P < 0.05 vs. model group. QLNC-L, Qianliening capsule low dose; QLNC-M, Qianliening capsule middle dose; QLNC-H, Qianliening capsule high dose.

Increased. While the estrogen levels remain unchanged with increasing age, the plasma estrogen to androgen ratio increases, resulting in decreased inhibition of androgens on estrogen release. The released estrogens cause increased stimulation of the prostatic stroma, resulting in excessive proliferation of the prostate and occurrence of BPH (Krieg et al., 1995; Shibata et al., 2000). Indeed, it has been suggested that estrogen is a
Figure 4. Effect of QLNC treatment on the protein expression of AR in prostatic tissue. Prostatic tissue from different groups was fixed, paraffin-embedded and sectioned; and expression of AR was observed using immunocytochemical staining. Images are representative photographs. (A) control group; (B) model group; (C) Finasteride group; (D) QLNC-L group; (E) QLNC-M group; (F) QLNC-H group.

Figure 5. Effect of QLNC treatment on the protein expression of ER in prostatic tissue. Prostatic tissue from different groups was fixed, paraffin-embedded and sectioned; and expression of ER was observed using immunocytochemical staining. Images are representative photographs. (A) control group; (B) model group; (C) Finasteride group; (D) QLNC-L group; (E) QLNC-M group; (F) QLNC-H group.

major risk factor of BPH (Seppelt, 1978; Van Coppenolle et al., 2001). Although the mechanism by which estrogen leads to the development of BPH has not been fully understood, available studies have shown that estrogen has direct and indirect effects at the cellular level on the prostate. For example, estrogen can stimulate the pituitary to release prolactin (PRL), which acts directly on the prostate and causes proliferation, or it can induce the
production and increase of prostate androgen receptors and enhance the action of androgens (Gallardo et al., 2009). When estrogen levels are elevated, the strongly positive expressions of estrogen receptors, namely the ER-α and ER-β, increase with increasing age. It has been found that in the normal prostate (Royuela et al., 2001), ER-α is not expressed in the epithelial cells, while ER-β is not expressed in the stromal components. However, expressions of ER-α and ER-β are elevated in the epithelial components in BPH, indicating that estrogen exerts its effects mainly through the epithelial components during the pathological process of BPH.

Qianliening capsule is a traditional Chinese medicine formulation, which is composed of Radix et Rhizoma Rhei, leech, astragalus root, achyranthes, and dodder seed. Its main effects include clearing the heat and detoxification, promoting blood circulation and removing blood stasis, and toning up the kidney and nourishing vitality (replenishing qi in Chinese) (Cao and Zhao, 2009; Lin et al., 2003). Considering the roles of sex hormones and their receptors in the pathogenesis of BPH, studying the effects of Qianliening capsules on the sex hormones and their receptors in BPH is of great importance. The results of this study indicate that Qianliening capsules had marked efficacy in the treatment of BPH in rats. Compared with the model group, Qianliening capsules significantly reduced the weight of prostate in rats and decreased the prostate index. In this study, serum T levels in the Qianliening capsule treatment groups, the Finasteride group, and the control group were significantly lower than those of the model group. Serum E2 levels were not significantly changed in any of the groups. In the Qianliening capsule treatment groups, the serum T/E2 ratio increased significantly compared to the model group, displaying a dose-response relationship. These results suggest that Qianliening capsules can increase serum androgen levels in rats with BPH and regulate the T/E2 ratio, but have little effect on serum estrogen. Further studies are required to understand whether Qianliening capsules can suppress the conversion of T into DHT.

The RT-PCR results revealed that ER and AR mRNA expressions in the prostate tissue in the Qianliening capsule treatment groups were significantly decreased compared with those in the model group. The immunohistochemistry results showed that the positive expressions of AR and ER were significantly increased in the model group. Further, the positive expressions of AR and ER were significantly decreased in the Qianliening capsule treatment groups, suggesting that Qianliening capsules can significantly down-regulate the gene and protein expressions of AR and ER in BPH. Therefore, we conclude that Qianliening capsules have marked efficacy for the treatment of BPH in a rodent model. Our findings suggest that these therapeutic effects are achieved through the regulation of the serum T/E2 ratio and the inhibition of ER and AR genes and protein expressions within the prostate tissue.

ACKNOWLEDGMENT

This work was supported by the Nature Science Foundation of China (81072927), the Natural Science Foundation of Fujian Province of China (2009J01169, 2010J01199), and the Research Foundation of Education Bureau of Fujian Province of China (JA09135).

Abbreviations: BPH, Benign prostatic hyperplasia; QLNC, Qianliening capsule; PW, prostatic weight; PI, prostatic index; ER, estrogen receptor; AR, androgen receptor; T, testosterone; E2, estradiol; BW, body weight.

REFERENCES


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