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Inhibition effects of chlorogenic acid on benign prostatic hyperplasia in mice

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ABSTRACT

This study aimed to evaluate the inhibitory effects and explore mechanisms of chlorogenic acid against testosterone-induced benign prostatic hyperplasia (BPH) in mice. Benign prostatic hyperplasia model was induced in experimental groups by daily subcutaneous injections of testosterone propionate (7.5 mg/kg/d) consecutively for 14 d. A total of 60 mice were randomly divided into six groups: (Group 1) normal control group, (Group 2) benign prostatic hyperplasia model control group, (Group 3) benign prostatic hyperplasia mice treated with finasteride at a dose of 1 mg/kg, (Group 4) benign prostatic hyperplasia mice treated with chlorogenic acid at dose levels of 0.8 mg/kg (low dose group), (Group 5) benign prostatic hyperplasia mice treated with chlorogenic acid at dose levels of 1.6 mg/kg (medium dose group) and (Group 6) benign prostatic hyperplasia mice treated with chlorogenic acid at dose levels of 3.2 mg/kg (high dose group). Animals were sacrificed on the scheduled termination, pick out the eyeball to get blood, then prostates were weighed and prostatic index were determined. Then the serum acid phosphatase (ACP), prostatic acid phosphatase (PACP) and typeII5-alpha-reductase (SRD5A2) levels were measured and observed morphological changes of the prostate. Comparing with benign prostatic hyperplasia model group, the high and medium dose of chlorogenic acid could significantly reduce prostate index and levels of acid phosphatase, prostatic acid phosphatase and typeII5-alpha-reductase ($P < 0.05$ or $P < 0.01$). These findings were supported by histopathological observations of prostate tissues. Histopathological examination also indicated that chlorogenic acid treatment at the high and medium doses inhibited testosterone-induced prostatic hyperplasia. The results indicated that chlorogenic acid exhibited restraining effect on benign prostatic hyperplasia model animals, and its mechanism might be related to inhibit typeII5-alpha reductase activity.

1. Introduction

With a rapidly ageing population, benign prostate hyperplasia (BPH) has gradually become one of the most familiar and harmful disease in elderly men (Kassabian, 2003). Benign prostatic hyperplasia affects nearly half of all men aged 50 years and older, and more than 90% of those older than 80 years, as a chronic disease which is characterized with histological the stromal and epithelial cells in the transition zone of the prostate proliferate in clinically diagnosed, thereby causing obstruction in urinary output (Jemal and Murray, 2004). The main clinical symptoms of the benign prostatic hyperplasia can be divided into two categories of lower urinary tract symptoms and complications caused by urinary tract obstruction. Lower urinary tract symptoms usually include urine frequency, urgency and inability to empty the bladder, etc. The complications included acute urinary retention, obstructive uropathy and urinary tract infection (Barry, 2001). The clinical features lead to an array of symptoms that compromise health-related quality of life.

Many studies showed that the pathogenesis of benign prostatic hyperplasia should be associated with hormones, but the definite etiology and pathogenesis of benign prostatic hyperplasia are remains unclear (Ahmad et al., 2012). When the male animal is castrated,

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growth slows down and the prostate will quickly shrink. In the circumstances, administration of testosterone can restore the erectile response of the penis in castrated animals (Ito and Horton, 1971). Testosterone isn't the only reason for BPH, but it has been identified as playing an integral part in the disease process. Serum testosterone can catalyzes one step in the conversion of testosterone to dihydrotestosterone in the presence of 5α-reduction and increase the concentration dihydrotestosterone (Generali and Cada, 2013), and also the excessive dihydrotestosterone production will result in the benign prostatic hyperplasia development and exacerbation (Cantlay and Ni Raghallaigh, 2015). Treatment of benign prostatic hyperplasia is mainly focused on the field of the medication and surgical treatment. The efficacy of surgery is more reliable, but it can lead to many complications and sequela. Pharmacological treatment in clinic can be classified into alpha-adrenergic blocker (doxazosin, alfuzocin, tamsulosin and terazosin, et.) and 5-alpha-reductase inhibitors at present. Side effects of alpha-blockers include orthostatic hypotension, headaches, nasal congestion, fatigue, dizziness and ejaculatory dysfunction (Romer et al., 2010). Finasteride and dutasyeride has been proved effective drugs as 5α-reductase inhibitors, which can lead to a marked decreases testosterone and dihydrotestosterone levels in serum and prostate, inhibits prostate enlargement and ultimately relieves the lower urinary tract symptoms caused by benign prostatic hyperplasia (Tacklind et al., 2010), but current evidence also indicated finasteride and dutasteride are unsafe for example erectile dysfunction, ejaculatory dysfunction, decreased libido, dizziness and upper respiratory tract infection (Souverein et al., 2001). It is necessary to find alternative materials for treating BPH that has a better therapeutic effect with fewer side effects.

Chlorogenic acid (5-caffeoylquinic acid, CGA), formed by esterification of caffeic and quinic acids, is one of the most abundant polyhenol compounds found in fruits, vegetables and even plants like honeysuckles (Clifford, 2000). Massive research indicated that chlorogenic acid has numerous pharmacological activities such as antioxidant, anti-inflammatory (Wang et al., 2009), antiviral (Shi et al., 2013) and may reduce the risk of some chronic diseases (Zhao et al., 2012). Some research suggests that chlorogenic acid might be a potential, natural anti-tumor activity. For instance, it has been shown that chlorogenic acid has the activity of induced human hepatoma and glioma cell apoptosis (Belkaid et al., 2006; Granado-Serrano et al., 2007). Another study indicated that chlorogenic acid can guard against gastric cancer, colon cancer and even suppress related carcinogenic factors (Matsunaga et al., 2002; Shao et al., 2015). Furthermore, Rachel M. Sortino et al. (Sortino et al., 2015) showed that chlorogenic acid nanoassemblies may have potential to be developed as drug delivery vehicles for tumor targeting. Meanwhile, our previous studies suggested chlorogenic acid might be the main active component affecting anti-benign prostatic hyperplasia bioactivity of *S. stolonifera* (Wu et al., 2015). Although the pharmacological activities of chlorogenic acid have been extensively studied, there are no reports on the efficacy of chlorogenic acid on BPH. Therefore, the effect of chlorogenic acid at different concentration levels (0.8, 1.6 and 3.2 mg/kg) on inhibition BPH were investigated, to verify on inhibiting effect and explore the mechanism of chlorogenic acid with testosterone-induced BPH model in castrated mice, so that for further experimental research and future clinical applications provide a theoretical basis and experimental evidence.

2. Materials and methods

2.1. Materials

Chlorogenic acid (purity≥98.0%) was purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Finasteride was obtained from Merck (Hangzhou, China). Testosterone propionate was manufactured by Shanghai GM Pharmaceutical Co., Ltd (Shanghai, China). In the present study, all the enzyme-linked immunosorbent assay (ELISA) kits were obtained from Shanghai MLBIO Biotechnology Co., Ltd (Shanghai, China).

2.2. Animals

Male Chinese KM mice weighing 18–22 g were purchased from Changsha Tianqin Biotechnology Co., Ltd. (Changsha, China) for this study. The animals were housed 6 mice per cage in a laminar air flow room maintained at 22 ± 2 °C with relative humidity of 55% ± 5%. An alternating 12 h light-darkness cycle, mice were provided with a standard laboratory diet and water ad libitum. Mice were cared and treated in accordance with the guidelines established by the Chinese Council on Animal Care and approved by the Guizhou Normal University Animal Care and Use Committee.

2.3. Castration procedure

After 7 day of acclimatization, all mice except for twelve in the blank group were anesthetized by inhalation of isoflurane and Castrated after intramuscular injection of penicillin (7.14×104 IU/kg body weight) 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 previously published method (Kannel et al., 2007).

2.4. Induction of BPH and treatments

The mice can be classified into two categories: unsexed mice and normal mice. The normal mice served as blank control group, which was given the normal saline (NS) administered orally and olive oil injected subcutaneously (s.c.). Unsexed mice were randomly divided into five groups with 10 mice for per group. (1) BPH model group, which received NS administered orally and testosterone propionate (TP) (7.5 mg/kg body weight, s.c.); (2) Positive control group and treated with finasteride (1 mg/kg body weight) administered orally and TP (7.5 mg/kg body weight, s.c.); (3–5) Chlorogenic acid group: there were administered with chlorogenic acid at dose levels of 0.8, 1.6 and 3.2 mg/kg (low, middle and high does group, respectively) body weight and TP (7.5 mg/kg body weight, s.c.), respectively. The positive control drug, finasteride, is a well-known 5α-reductase inhibitor used for BPH treatment. The effective dose of finasteride was based on adult dose of 12 times. All mice were treated once a day for two consecutive weeks. During the experiment, mice body weight was measured every three days to adjust the dosage. Mice were fasted for 12 h after the last administration. Blood samples were collected from the eye socket and then the mice were euthanized. The prostate gland was freed from connective tissues, excised and weighed. The prostate organs were immediately fixed with 10% neutral buffered formalin and embedded in paraffin for histological analysis.

2.5. Determination of prostate index (PI)

At the fifteenth day, each mouse was weighed, animals were killed by put to death by cervical dislocation and prostates were removed and weighed immediately. The prostatic index (PI) was calculated by following equations:

PI = prostate wet weight / mouse weight

2.6. Enzyme-linked immunosorbent assay (ELISA) analysis

Blood samples were collected from the eye socket, centrifuged at 5000*g* for 10 min to obtain serum at 4 °C. Acid phosphatase, prostatic acid phosphatase and typeII 5-alpha-reductase levels in the serum were quantified using ELISA in accordance with manufacturer's protocol.