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Potential ameliorative effects of epigallocatechin-3-gallate against testosterone-induced benign prostatic hyperplasia and fibrosis in rats

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ABSTRACT

Green tea is among the most popular beverages in the world and is an important source of phytoestrogens. Epigallocatechin-3-gallate (EGCG) is the major polyphenol in green tea. The aim of this study was to investigate the anti-benign prostatic hyperplasia (BPH) activity and underling mechanisms of EGCG in testosterone-induced BPH rats and in BPH-1 cells. Prostatic levels of oxidative stress and inflammation makers, as well as angiogenesis related growth factors were measured. Additionally, the prostatic levels of sex hormonal mediators (androgen receptor (AR), estrogen receptor (ER)-α and ER-β), hypoxia-inducible factor (HIF)-1α, transforming growth factor-β1 (TGF-β1), type I TGF-β receptor (TGF-βRI), Smad3, phosphorylation-Smad3 (p-Smad3), epithelial–mesenchymal transition (EMT) markers (E-cadherin, collagen-I, fibronectin and α-SMA) and microRNA (miR)-133a/b were analyzed by immunohistochemistry assay, western blot and/or quantitative RT-PCR. It was observed that EGCG attenuated the prostatic oxidative stress and inflammatory microenvironment, ameliorated prostatic hyperplasia and collagen deposition, reduced the levels of angiogenesis related growth factors, inhibited the over-expression of AR, ER-α, HIF-1α, TGF-β1, TGF-βRI and p-Smad3, enhanced the expression of ER-β, increased the levels of miR-133a/b, as well as relieved prostatic EMT in rats. Both HIF-1α inhibitor and EGCG decreased the expression of HIF-1α and TGF-β1, as well as attenuated EMT in BPH-1 cells. It indicated that EGCG could attenuate testosterone-induced BPH and fibrosis.

1. Introduction

Benign prostatic hyperplasia (BPH) is a nonmalignant and progressive growth phenomenon of prostate gland. It is characterized by disordered proliferation of the prostatic glandular epithelial cells, stromal cells and smooth muscle cells [1,2]. BPH is one of the most common urological diseases in elderly men. Its incidence increases with age. Report indicates that about 50% of the 50 years old men suffer from BPH, and its morbidity rises to 90% in people over 80 years of age [3]. Furthermore, among the causes for lower urinary tract symptoms (LUTS) in men, BPH is believed to be the main one [4]. Owing to the increased smooth muscle resistance and bladder outlet obstruction, BPH enhances the risk of urine storage, urinary incontinence, slow urination and postmicturition symptoms [3]. Although BPH-LUTS are not life-threatening diseases in the short term, they bring extreme embarrassment and annoyance to the daily life of patients, and cause enormous economic burden [5].

Unfortunately, the exact pathological mechanisms of BPH still not be clarified. The general therapeutic approaches mainly include medical therapies and surgical interventions. Not only that the current clinical medicine 5-α-reductase inhibitors and α-adrenergic blocking agents are not completely effective, but also that they lead to several side effects including vascular deficits and sexual dysfunction [4,6]. On the one hand, α-adrenergic blockers cannot reduce prostate volume. On the other hand, about 25–30% patients have no improvement in LUTS, and 5–7% patients even become worse after using the 5-α-reductase inhibitors [7]. Meanwhile, phytotherapies for BPH have emerged as the focal point worldwide due to these side effects of the above traditional treatment [1]. Phytoestrogens are dietary compounds and are structurally similar to estrogen. They have the functions of estrogen-like and/or anti-androgen. Therefore, they may be used for the prevention and treatment of prostatic deficits [8]. For example, lignans and silymarin are reported to can ameliorate BPH through exerting phytoestrogenic activity and modulating prostate hormone metabolism related enzymes

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[9,10].

Green tea is one of the most popular beverages in the world and is an important dietary source of phytoestrogens [11]. Green tea is believed to have beneficial activities of antioxidant, anti-inflammatory, anti-angiogenic, anti-proliferative and so on [12]. Epigallocatechin-3-gallate (EGCG) is the major polyphenol in green tea [13]. It has been demonstrated that EGCG can suppress proliferation and promote apoptosis in human benign prostate hyperplasia cell line (BPH-1) cells [12]. Our previously study found that EGCG at the doses of 100 and 50 mg/kg/d attenuated prostatic epithelial cells expansion, inhibited the enhancement in the levels of prostatic acid phosphatase and prostate relative weight in BPH rats [14]. However, the exact pharmacological mechanisms of EGCG in anti-BPH still remain unclear. The aim of this study was to investigate the anti-BPH ability and underling mechanisms of EGCG in testosterone-induced BPH rats and in BPH-1 cells.

2. Materials and methods

2.1. Reagents

EGCG (purity > 98%) were purchased from Huayue Chemical Products Co., Ltd. (Henan, China). Testosterone propionate was obtained from Tianjin Kingyork Group Co, Ltd. (Tianjin, China). The commercial kits for the analysis of the antioxidant enzymes (superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT)), non-enzymatic antioxidants (reduced glutathione (GSH) and total sulfhydryl (T-SH)), as well as the malondialdehyde (MDA) were purchased from Nanjing Jiancheng Bioengineering Institute (Jiangsu, China). The ELISA kits for the analysis of inflammation makers (interleukin (IL)-1β, IL-6, tumor necrosis factor (TNF)-α and cyclooxygenase (COX)-2), as well as angiogenesis related growth factors (vascular endothelial growth factor (VEGF), basic fibroblast growth factor (βFGF) and epidermal growth factor (EGF)) were purchased from ELISA Lab, Co. Ltd. (Hubei, China). The antibodies of androgen receptor (AR), estrogen receptor (ER)-α, ER-β, NFκB-p65 (p65), phosphorylated-p65 (p-p65), transforming growth factor-β1 (TGF-β1), type I TGF-β receptor (TGF-βRI), Smad3, phosphorylation-Smad3 (p-Smad3), hypoxia-inducible factor (HIF)-1α, collagen-I (Col-I), fibronectin and α-smooth muscle actin (α-SMA) and E-cadherin were obtained from Abcam, Co. (UK). HIF-1α inhibitor YC-1 was purchased from Selleck, Co. Ltd. (USA). BPH-1 cells were purchased from BeNa Culture Collection (Beijing China). Cell culture medium was purchased from ThermoFisher Scientific, Co. Ltd. (USA).

2.2. Rat model of BPH and drug treatment

Male Wistar rats with the body weight of 180–200 g (six weeks old) were purchased from Hubei Provincial Laboratory Animal Research Center (Hubei, China) and randomly assigned into four groups (n = 6): the vehicle control group (vehicle), the BPH model group (BPH), the BPH-100 mg/kg EGCG group (100 mg/kg EGCG) and the BPH-50 mg/kg EGCG group (50 mg/kg EGCG). According to the proposed method [15], the testicles of rats from BPH and EGCG groups were removed by surgery (castration). After recovered for one week, these castrated rats were daily subcutaneous injected with 10 mg/kg testosterone (dissolved in olive oil) for 4 weeks. The animals of vehicle control were sham operated and daily injected with the same volume of olive oil. In addition, the rats from 100 or 50 mg/kg EGCG groups were daily intragastric administrated with 100 or 50 mg/kg EGCG for 4 weeks, respectively [14].

2.3. Prostate tissue samples collection

After the whole 6 weeks experimental period, the prostate tissue samples were collected, weighted and excised into three parts. One part fresh prostate tissue was used for the western blot and quantitative RT-PCR (qPCR) assay. One part tissue was fixed in 4% paraformaldehyde, embedded in paraffin and sectioned. Another part tissue was made to the 10% tissue homogenate using ice cold 0.9% sodium chloride. All the experiments were performed in accordance with the Chinese legislation and the ethical rules of NIH Guidelines for the Care and Use of Laboratory Animal.

2.4. Prostatic morphological analysis

The prostate tissue sections were stained by haematoxylin-eosin (HE) for histomorphological observation, and by masson for qualitative analysis of collagen deposition. Additionally, immunohistochemical analysis for the prostatic expression of AR, ER-α and ER-β was performed according to reported method [15]. Stained areas were visualized and photographed using Leica DMi8 microscope (×200). A 100 μm black ruler was marked in the recorded picture.

2.5. Analysis for prostatic oxidative stress, inflammation and angiogenesis

The prostatic oxidative stress was evaluated by detecting the activities of antioxidant enzymes (SOD, GPx and CAT), as well as the levels of non-enzymatic antioxidants (GSH and T-SH) and lipid peroxidation product (MDA) using commercial kits. The activity of SOD was measured by hydroxylamine method *via* detecting the absorbance at 550 nm. The final product of nitrite was purple red under the action of P-aminophenol sulfonic acid and α-naphthylamine. The activity of GPx was measured by colorimetry *via* detecting the absorbance at 412 nm. GPx catalyzed the reaction of GSH with 3-carboxy-4-nitrophenyl disulfide to produce yellow compound. The activity of CAT was measured by ammonium molybdate method *via* detecting the absorbance at 405 nm. Ammonium molybdate discontinued the reaction of CAT with H_2O_2. The remaining H_2O_2 produced a yellow complex by reacting with ammonium molybdate. The levels of GSH and T-SH were measured by spectrophotometric method *via* detecting the absorbance at 420 nm and 412 nm, respectively. The level of MDA was measured by thiobarbituric acid (TBA) method *via* detecting the absorbance at 532 nm. A pink compound formed under the reaction of MDA with TBA.

The prostatic local inflammation was estimated by measuring the levels of proinflammatory cytokines (IL-1β, IL-6 and TNF-α) and the activity of COX-2, while the prostatic angiogenesis was assessed by examining the levels of angiogenic growth factors (VEGF, βFGF and EGF) using ELISA kits. The performance was carried out in accordance with the instructions of commercial kits.

2.6. Western blot analysis

The total protein of each prostate sample was extracted after being ground in liquid nitrogen. Protein concentration was determined by BCA method. After separated by 10% SDS-polyacrylamide gel electrophoresis, the protein samples were transferred to a PVDF membrane by electrophoretic transfer and blocked for 1 h at room temperature with 5% nonfat milk in Tris-buffered saline containing 0.1% Tween 20 (TBST). The membranes were incubated with the primary antibody overnight at 4 °C, washed with TBST and incubated with horseradish peroxidase-conjugated secondary antibody in TBST with 3% nonfat milk for 30 min at room temperature. The chemiluminescence reaction was developed and the quantification of bands was determined by integrated optical density (IOD) analysis using Alpha Innotech software. The data were normalized using β-actin as an internal control.

2.7. qPCR analysis for the mRNA levels of α-SMA, fibronectin, E-cadherin and microRNA (miR)-133a/b

The total RNA of each prostate sample was extracted using Trizol reagent and quantified using gel electrophoresis. The qPCR was