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Protective potential of epigallocatechin-3-gallate against benign prostatic hyperplasia in metabolic syndrome rats

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ABSTRACT

Epigallocatechin-3-gallate (EGCG) is a major catechin in green tea with functions of antioxidant, anti-proliferative, anti-inflammatory and attenuating metabolic syndrome. In this study, rat model of benign prostatic hyperplasia (BPH) accompanied with metabolic syndrome was induced by fed on high-fat diet for 12 weeks combined with testosterone injection (10 mg/kg/d) from 9th to 12th weeks. EGCG was orally given from 9th to 12th weeks. Finally, the levels of glucose, total cholesterol, triglyceride, prostate weight, insulin-like growth factors (IGFs), inflammatory cytokines, antioxidant enzymes, and prostatic expression of IGF binding protein-3 (IGFBP-3) and peroxisome proliferator activated receptors (PPARs) were evaluated. It was found that EGCG significantly decreased the levels of glucose, total cholesterol, triglyceride, IGFs, and inflammatory cytokines, normalized the activities of antioxidant enzymes, as well as increased the prostatic expression of IGFBP-3 and PPARs. These results indicated that EGCG was able to exert anti-BPH activities in metabolic syndrome rats.

1. Introduction

Benign prostatic hyperplasia (BPH) is a chronic disease in elderly men and characterized by nonmalignant enlargement of prostate gland. BPH is one of the most fourth prevalent diseases in men over 50 years age, and its incidence increases to 80% in man older than 70 ages of years (Vikram et al., 2010a; Xu et al., 2014a,b). Metabolic syndrome (MS) is another commonly encountered disease these years. MS includes obesity, dyslipidemia, hypertension and type II diabetes mellitus (Ozden et al., 2007). Recently, accumulated studies have focused on the relevance between BPH and MS. Clinical observation shows that obesity-related metabolic diseases contribute to prostatic hyperplasia. The prostate volume is larger in obese patients than in health people (Lee et al., 2006). The levels of glucose, triglyceride (TG) and prostate specific antigen in BPH patients with MS are higher than those in simple BPH patients. As a result, the patients suffered from BPH and MS are more susceptible to experience further severe hyperblastosis of prostate than the patients with BPH alone (Ozden et al., 2007; Parsons et al., 2006). MS promotes systemic inflammatory responses and stimulates oxidative stress. Chronic inflammatory state and oxidative stress have become evident as the major factors for BPH progression. Additionally, the emerged hyperinsulinemia in MS is also considered as an independent promoter for BPH. On the one hand, it can directly mediate the mitogenic effect on prostate cell *via* signal transduction mechanisms. On the other hand, hyperinsulinemia also stimulates the growth of prostate through insulin-like growth factor (IGF) axis (Jiang et al., 2011; Wang and Olumi, 2011).

Green tea (*Camellia sinensis*) is one of the most popular beverage worldwide. Due to its antioxidant properties and the capacities of modulating numerous cell signaling pathways, green tea is believed to have beneficial effects of anti-angiogenic, anti-proliferative, anti-inflammatory, improving lipid profiles, enhancing body fat loss, attenuating insulin resistance and so on (Bansal et al., 2013). The main active ingredients in green tea are catechins. Epigallocatechin-3-gallate (EGCG) combined with other catechins are responsible for the beneficial therapeutic functions of green tea (Nagle et al., 2006). And our preliminary study indicated that EGCG had potential protective effects against testosterone induced BPH in rats. EGCG at the doses of 100 and 50 mg/kg/d can inhibited testosterone induced epithelial cells expansion (Supplementary Fig. 1S in the online version at DOI: 10.1016/j.etap.2016.06.015), decreased the activity of prostatic acid phosphatase (PACP) and the levels of prostatic index, dihydrotestosterone and 5-α-reductase (Supplementary Fig. 2S in the online version at DOI: 10.1016/j.etap.2016.06.015). This study was undertaken to assess the potential protective effects of EGCG and its possible mechanisms on BPH

Abbreviations: BPH, benign prostatic hyperplasia; ELISA, enzyme linked immunosorbent assay; EGCG, epigallocatechin-3-gallate; GPx, glutathione peroxidase; HFD, high-fat diet; IGFs, insulin-like growth factors; IGFBP-3, IGF binding protein-3; IL-1β, interleukin-1β; LFD, low-fat diet; MDA, malondialdehyde; MS, metabolic syndrome; PPARs, peroxisome proliferator activated receptors; SOD, superoxide dismutase; TC, total cholesterol; TG, triglyceride; TNF-α, tumor necrosis factor-α; VEGF, vascular endothelial growth factor.

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Table 1
The composition of low-fat diet and high-fat diet.

component	low-fat diet (g/100 g)	high-fat diet (g/100 g)
casein	20	9.4
D,L-Methionine	0.3	0.141
corn starch	15	7.05
sucrose	50	33.5
cellulose powder	5	2.35
mineral mix	3.5	1.645
vitamin mix	1	0.47
choline bitartrate	0.2	0.094
corn oil	5	2.35
lard	0	20
egg yolk	0	20
cholesterol	0	2
sodium deoxycholate	0	1
kcal/100 g	386.2	–
calories from fat (%)	11.7	–
calories from carbohydrate (%)	67.3	–
calories from protein (%)	21.0	–

–: Unknown.

accompanied with MS rats induced by a combined treatment with high-fat diet (HFD) and testosterone injection.

2. Materials and methods

2.1. Reagents

The antibodies of peroxisome proliferator activated receptor (PPAR)-α and PPAR-γ were purchased from Santa Cruz Biotechnology, Inc. (Dallas, TX, USA). The antibody of IGF binding protein (IGFBP)-3 was purchased from ProteinTech Group, Inc. (Wuhan, Hubei, China). The commercial kits for the analysis of superoxide dismutase (SOD), glutathione peroxidase (GPx), malondialdehyde (MDA), glucose, total cholesterol (TC) and TG were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, Jiangsu, China). The commercial enzyme linked immunosorbent assay (ELISA) kits used for the determination of interleukin (IL)-1β, IL-6, tumor necrosis factor (TNF)-α, vascular endothelial growth factor (VEGF), IGF-I and IGF-II were purchased from Boster, Co. Ltd. (Wuhan, Hubei, China). Finasteride tablets were purchased from Merck Sharp & Dohme, Ltd. (Hoddesdon, Hertfordshire, UK). Testosterone propionate was purchased from Shanghai General Pharmaceutical Co Ltd. (Shanghai, China). EGCG (purity > 98%, determined by HPLC) was purchased from Huayue Reagents and Chemical Co. Ltd. (Zhengzhou, Henan, China). All other solvents and chemicals used in the study were of analytical grade and purchased from Sinopharm Chemical Reagent, Co. Ltd. (Shanghai, China).

2.2. Animals and administration

The 9 weeks old male Sprague-Dawley rats (license number: SCXKe2010-0009) were supplied by the Centers of Disease Control and Prevention of Hubei province, China. The animals were housed at a controlled room (temperature 22 ± 3 °C and humidity 50 ± 10%) and were kept on a 12 h light:12 h dark cycle. The rats were fed with standard diet and water ad libitum, and acclimated 7 days before they were used for the study. After acclimatization to the laboratory conditions, the experimental rat model of MS accompanied with BPH was induced according to the reported methods with a little change (Rahman et al., 2007; Vikram et al., 2010b). Briefly, the rats were fed on a HFD consisting of low-fat diet (LFD) 470 g/kg, sucrose 100 g/kg, egg yolk 200 g/kg, lard 200 g/kg, cholesterol 20 g/kg and sodium deoxycholate 10 g/kg for 12 weeks. The composition of HFD and LFD was showed in Table 1. At the end of the 8th week these rats were randomly divided into four groups (n = 8): the HFD-BPH model group (HFD-BPH), the positive control group (PC), the high dose EGCG treated group (H) and the low dose EGCG treated group (L). From 9th to 12th weeks, these HFD-fed rats were subcutaneous injected 10 mg/kg/d testosterone (dissolved in olive oil) for 4 weeks. Another 8 rats were fed on HFD for 12 weeks but did not give testosterone injection. They were set as the HFD control group (HFD). Other rats were fed on LFD for 12 weeks and randomly divided into two groups (n = 8): vehicle control group (VC) and the LFD-fed BPH control group (LFD-BPH). From 9th to 12th weeks, the rats from VC were daily subcutaneous injected the same volume olive oil for 4 weeks. While the rats from LFD-BPH were subcutaneous injected 10 mg/kg/d testosterone for 4 weeks.

One hour after the testosterone injection, the rats of H and L were orally treated with 100 and 50 mg/kg/d EGCG for 4 weeks, respectively. The rats of PC were orally treated with 5 mg/kg/d finasteride. The other rats were orally treated with the same volume of physiological saline.

At the end of the 12 weeks experimental period, the blood samples were prepared for the analysis of glucose, TC, TG, SOD, GPx, MDA, IL-1β, IL-6, TNF-α and VEGF. The prostatic lateral lobes samples were rapidly removed, cleaned with PBS to remove residual blood, blotted dry and weighted. Then the tissue was excised and one part was stored at -80 °C for the subsequent western blot assay for the prostatic expression of IGFBP-3, PPAR-α and PPAR-γ. Another part of each sample was fixed in 4% paraformaldehyde for 3 day. Then they were embedded in paraffin. The rest tissue was made to the tissue homogenate in 10 volumes of ice cold physiological saline and then stored at -80 °C for the subsequent assay of prostatic activities of SOD and GPx, as well as the levels of MDA, IL-1β, IL-6, TNF-α, VEGF, IGF-I and IGF-II.

All experiments were performed in compliance with the Chinese legislation on the use and care of laboratory animals and were approved by the Institutional Animal Care and Use Committee at Tongji Medical College, Huazhong University of Science and Technology.

2.3. Experimental model evaluation and EGCG pharmacodynamic assessment

The experimental rat model of BPH accompanied with MS, and the potential protective function of EGCG were evaluated by measuring the levels of glucose, TC, TG, prostate weight and body weight, as well as by performing prostatic histological analysis. The levels of glucose, TC and TG were determined using commercial available ultraviolet spectrophotometry kits. The prostate paraffin was serially sectioned and then stained for the haematoxylin-eosin and masson. Stained areas of the prostatic tissue sections were visualized using an optical microscope at × 200.

2.4. Systemic and prostatic local oxidative stress and chronic inflammatory

The systemic and prostatic local oxidative stress extent was measured by detecting the level of lipid peroxidation marker MDA while the enzymatic antioxidant status was evaluated by detecting the activities of SOD and GPx using commercial available ultraviolet spectrophotometry kits. The systemic and prostatic local chronic inflammatory responses were analysis by detecting the levels of pro-inflammatory cytokines of IL-1β, IL-6 and TNF-α as well as the key pro-angiogenic factor VEGF by ELISA kits according to the manufacturer's instructions.

2.5. Analysis for IGF axis and PPARs

The prostatic levels of IGF-I and IGF-II were measured by ELISA. The prostatic expression of IGFBP-3, PPAR-α and PPAR-γ were evaluated by western blot analysis. Tissue protein samples (50 μg) were