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Resveratrol improves prostate fibrosis during progression of urinary dysfunction in chronic prostatitis

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ABSTRACT

Aim: We investigated whether prostate fibrosis was associated with urinary dysfunction in chronic prostatitis (CP) and whether resveratrol improved urinary dysfunction and the underlying molecular mechanism.
Methods: Rat model of CP was established via subcutaneous injections of DPT vaccine and subsequently treated with resveratrol. Bladder pressure and volume tests investigated the effect of resveratrol on urinary dysfunction in CP rats. Western blotting and immunohistochemical staining examined the expression level of C-kit/SCF and TGF-β/Wnt/β-catenin.
Results: Compared to the control group, the maximum capacity of the bladder, residual urine volume and maximum voiding pressure, the activity of C-kit/SCF and TGF-β/Wnt/β-catenin pathways were increased significantly in the CP group. Resveratrol treatment significantly improved these factors.
Conclusion: CP induced significantly prostate fibrosis, which exhibits a close relationship with urinary dysfunction. Resveratrol improved fibrosis, which may be associated with the suppression of C-kit/SCF and TGF-β/Wnt/β-catenin pathway.

1. Introduction

Chronic prostatitis (CP) is a common disease in urology, and voiding dysfunction is the primary clinical manifestation (Tyagi et al., 2015) that affects the quality of life of CP patients. Urinary tract obstruction was revealed in CP patients upon urodynamic examination (Liao et al., 1999). Prostate fibrosis is a contributing factor to lower urinary tract symptom etiology (Ma et al., 2012).

Tissue fibrosis is epidemiologically associated with the subsequent development of tissue injury caused by aging (Karsdal et al., 2016), infection (Fabre et al., 2011), tumors (Trujillo et al., 2011) or other secondary disease processes in multiple organ systems, which lead to dysfunction. The process of fibrosis is mechanistically characterized by myofibroblast accumulation, collagen deposition, extracellular matrix remodeling, and increased tissue stiffness, and the highly collagenized tissue impairs organ function by reducing tissue elasticity and compliance. However, the role of fibrosis in the development of CP is poorly understood. Therefore, we investigated whether CP with urinary dysfunction was associated with collagen accumulation in the prostate.

The C-kit receptor, also known as the stem cell factor (SCF) receptor (Lennartsson and Ronnstrand, 2012), belongs to the tyrosine kinase receptor type III family (Abu-Duhier et al., 2003). The C-kit/SCF pathway have been implicated in tissue remodeling and fibrosis (Ding et al., 2013). The pleiotropic cytokine, TGF-β, plays diverse physiological roles in vivo, including the initiation and control of fibrosis (Zerr et al., 2016) .As Rojas reported (Rojas et al., 2016), there is an aberrant molecular network between the TGF-β and C-kit pathway that mediates the functional switch of TGF-β, that TGF-β signaling transcriptionally regulates expression of the C-kit receptor ligand (SCF), SCF induces TGF-β ligand expression via STAT3, thereby forming a positive feedback loop between TGF-β and SCF/C-kit signaling.Anymore, The Wnt/β-catenin signaling pathway is essential for TGF-β-induced fibrosis (Akhmetshina et al., 2011). Therefore, we also examined the potential role of the Wnt/β-catenin/TGF-β/C-kit signaling pathway in the pathogenesis of CP.

Resveratrol (Torres et al., 2010) (trans-3,4,5-trihydroxy stilbene) is widely found in many plants and foods, such as cassia, pine trees, grapes, wine, mulberry and peanuts. Resveratrol was originally used as

Abbreviations: CP, chronic prostatitis; SCF, stem cell factor; α-SMA, α-smooth muscle actin; TGF-, βtransforming growth factor-β; LUTS, lower urinary tract symptoms

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a phytoalexin (Sharma et al., 2006), and the reliable and extensive anti-inflammatory effects of resveratrol gained the attention of researchers (Csiszar, 2011). Resveratrol treatment represses and reverses prostate fibroblast to myofibroblast phenoconversion in vitro. There is no report about the effect of resveratrol on prostatitis. As reported, anti-fibrotic therapeutics may be efficacious for the treatment of lower urinary tract symptoms (LUTS) (Mehrnaz et al., 2016).

This study investigated whether prostate fibrosis was associated with urinary dysfunction in CP and whether resveratrol improved urinary dysfunction. We also evaluated the activity of the C-kit/SCF and TGF-β/Wnt/β-catenin pathways in the prostates of CP rats.

2. Materials and methods

2.1. Chemicals

Resveratrol of > 99% purity was purchased from Dalian Meilun Biotechnology Co., Ltd. (Dalian, China). DPT vaccine was obtained from the Wuhan Institute of Biological Products Co., Ltd. (Wuhan, China). All other chemicals used in this study were analytical grade and commercially available.

2.2. Animals and ethics statement

Male Sprague-Dawley (SD) rats (180 ± 20 g) were purchased from the Experimental Animal Center of Dalian Medical University (Dalian, China). Rats were allowed access to water and food ad libitum but were fasted overnight before surgery, with water available.

2.3. Ethic approval

The ethics committee of Dalian Medical University approved all animal experiments, which were performed in accordance with institutional guidelines and conducted according to the National Research Council's guidelines.

2.4. Rat model of CP

For the purification of rat prostatic protein, male SD rats (240–300 g) were sacrificed, and prostate tissue was removed under sterile conditions and washed with saline solution. Prostate tissue was placed into a physiological saline solution containing 0.5% Triton X-100 and homogenized in an ice-water bath with a glass homogenizer. The homogenized liquids were centrifuged (10,000*g*) for 10 min, and protein was diluted to 15 mg/ml with PBS buffer (0.1 mol/L and pH7.2).

Rats were subcutaneously injected with a DPT vaccine (0.5 ml/kg) and a multipoint injection of a mixture (1.0 ml) of purified rat prostatic protein and Freund's Complete Adjuvant (FCA) (1:1) at 0, 15 and 30 d (22). The rat model of CP was established after 45 d. Rats were randomly divided into three groups (8 rats in each group; n = 8):

1. Control group: Normal rats orally administered saline for 10 d;
2. CP group: CP rats orally administered saline for 10 d; and
3. Resveratrol group: CP rats orally administered resveratrol (10 mg/kg) (Liu et al., 2015; Banu Sakhila et al., 2016) for 10 d.

2.5. Bladder pressure and volume tests in rats

Rats were anesthetized using an intraperitoneal injection of pentobarbital (60 mg/kg) before surgery. Rats were fixed in the operating frame, and the upper edge of the pubic symphysis skin was incised. The bladder was exposed and placed on the incision to avoid the effect of abdominal pressure on the detrusor pressure. Two 24G tubes were inserted into the bladder and fixed, and the bladder was irrigated by saline (0.4 ml/min) via one of the 24G tubes. Another tube was connected to a MedLab biological signal acquisition system via a pressure transducer. The maximum capacity of the bladder, residual urine volume and maximum voiding pressure were measured.

2.6. Morphological changes

The rats were sacrificed at the end of the bladder pressure and volume test. The prostate was removed, fixed in 10% (v/v) neutral formalin, processed using standard histological techniques, stained with hematoxylin and eosin (H & E), and examined for morphological changes. The samples were used to determinate the protein expression of C-kit, SCF, tryptase, chymase, TGF-β, Wnt and α-smooth muscle actin (α-SMA) using western blot analysis, immunohistochemical staining and immunofluorescence labeling.

2.7. Western blot analysis

Proteins were extracted from rat prostate using a protein extraction kit (KeyGen Biotech, Nanjing, China) according to the manufacturer's instructions. Protein was measured according to the bicinchoninic acid (BCA) procedure (Solarbio, Beijing, China), with bovine serum albumin as the standard. Proteins (20 μg) were resuspended in electrophoresis sample buffer containing β-mercaptoethanol and separated using electrophoresis on a pre-cast 10% SDS-polyacrylamide gel (Bio-Rad, Hercules, CA, USA), followed by electrotransfer to a PVDF membrane (Millipore, Bedford, MA, USA). Membranes were blocked using 5% non-fat milk in Tris-buffered saline with 0.1% Tween-20 (TBST) for 2 h at 37 °C. β-Actin served as the loading control. Membranes were incubated overnight at 4 °C with a 1:1000 dilution of polyclonal antibodies for C-kit, stem cell factor (SCF), transforming growth factor (TGF)-β, Wnt and α-smooth muscle actin (α-SMA) (Cruz Biotechnology, Santa Cruz, CA, USA) and a 1:1500 dilution of a monoclonal antibody for β-actin (Beyotime, China). Membranes were subsequently washed with TBST, and the blots were incubated with secondary antibodies. Blots were extensively washed with TBST and exposed to enhanced chemiluminescence-plus reagents (ECL) from Beyotime Institute of Biotechnology (Haimen, China) according to the manufacturer's protocol. Emitted light was documented using a BioSpectrum-410 multispectral imaging system with a Chemi HR camera 410(Bio-Rad, Hercules, CA, USA). Protein bands were visualized and photographed under transmitted ultraviolet light. The image was used for semi-quantitative measurements based on band densitometry.

2.8. Immunohistochemical staining

Histological sections of rat prostate (4 μm thick) were mounted on poly-L-lysine-coated slides. Slides were deparaffinized in xylene and rehydrated in graded alcohols. Sections were pretreated with citrate buffer (0.01 mol/L citric acid, pH 6.0) for 20 min at 95 °C and immersed in PBS containing 3% H_2O_2 for 10 min at room temperature. Sections were exposed to 10% normal goat serum in PBS for 30 min at room temperature and incubated at 4 °C overnight with rabbit polyclonal anti-C-kit antibody (1:100 dilution). Sections were rinsed with PBS, incubated with biotinylated goat anti-rabbit IgG for 20 min at room temperature and treated with 3,3-diaminobenzidine chromogen for 5 min at room temperature. Sections were counterstained with hematoxylin for 2 min.

2.9. Immunofluorescence labeling

Slides with rat prostate sections (4 μm thick) were rinsed with PBS, permeabilized with 0.1% Triton X-100 in PBS for 10 min, and blocked with 2% BSA in PBS for 30 min. The specimen slides were incubated with a primary anti-C-kit antibody at 4 °C overnight. The specimens were washed three times with PBS and incubated with Cy3–conjugated Affinipure Rabbit Anti-Goat IgG (H + L) (Santa Cruz Biotechnology) at