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Resveratrol improved detrusor fibrosis induced by mast cells during progression of chronic prostatitis in rats

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ABSTRACT

To investigate the detrusor fibrosis and urinary dysfunction in chronic prostatitis (CP), and to investigate whether resveratrol can improve the urinary dysfunction and the underlying molecular mechanism. After rat model of CP is established by subcutaneously injecting DPT vaccine, rats are treated with resveratrol. Experiments of bladder pressure and volume test in rats are used to investigate the effect of resveratrol on urinary dysfunction in CP rats. To assess tissue fibrosis, picrosirius red staining is performed. H & E staining is performed to identify the histopathological changes. Western blot and immunohistochemical staining are used to examine the expression of c-kit, SCF , tryptase, TGF-β, Wnt and α-SMA. The results of bladder pressure and volume test show that the maximum capacity of the bladder, residual urine volume and maximum voiding are increased significantly in CP rats. CP rats show significantly increased collagen deposition in the detrusor. H & E staining show that detrusor muscle arranged in disorder with fracture from CP rats. The results of western blot and immunohistochemical staining demonstrate that the activity of c-kit/SCF and TGF-β/Wnt/β-catenin pathway, expression levels of tryptase and α-SMA in bladder detrusor of CP group are significantly increased compared with the control group. However, resveratrol treatment significantly improved these factors. mast cell activation induced by the increased expression of c-kit/SCF in CP rats, may promote detrusor fibrosis which have a close relationship with urinary dysfunction. Resveratrol can improve the dysfunction by downregulating the mast cell activation and the activity of TGF-β/Wnt/β-catenin pathway.

1. Introduction

Chronic prostatitis (CP) is a common disease of urology, voiding dysfunction is the main clinical manifestations (Pradeep et al., 2015), and also an important factor that affects the quality of life of CP patients. It has been reported that the presence of urinary tract obstruction was revealed in patients with CP by urodynamic examination. As reported, this urinary tract obstruction mainly includes unstable bladder, sphincter spasm (Murnaghan and Millard, 1984; Funahashi et al., 2012). As reported (Schwartz et al., 2016; Liao et al., 1999), one possible mechanism of CP inducing bladder symptoms is afferent cross-sensitization between the prostate and bladder. However, the underlying pathophysiology mechanisms was not fully elucidated. We therefore examined changes in the bladders in prostatitis rats.

Tissue fibrosis is epidemiologically associated with the subsequent development of tissue injury caused by ageing (Karsdal et al., 2016), infection (Fabre et al., 2011), tumor (Kristina et al., 2011) and other secondary disease processes in multiple organ systems, leading to dysfunction (Sangaralingham et al., 2016; Liu et al., 2016; Chen et al., 2016). Fibrosis process is mechanistically characterized by collagen deposition, extracellular matrix remodeling, and increased tissue stiffness, so that the highly collagenized tissue impairs the organ function by reducing tissue elasticity and compliance. However, the role of the detrusor fibrosis in the development of CP is poorly understood.

Mast cells (MC) have been recognized as important effector cells in tissue fibrosis (Alves et al., 2016; Cruse and Bradding, 2016; Nakayama et al., 2016; Randi, 2013). MC tryptase is associated with fibrosis in various diseases (Jesús et al., 2015; Kondo et al., 2001). However, little is known about their involvement in CP. The pleiotropic cytokine, TGF-β, has diverse physiological roles in vivo including initiation and control of fibrosis (Zerr et al., 2016). The profibrotic effects of MC are intricately linked with TGF-β (Shaun et al., 2012). Experimental studies have largely been confirmatory, linking MC numbers and staining intensity with increased TGF-β production and interstitial fibrosis.

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Moreover, the Wnt/β-catenin signaling pathway is essential for the fibrosis induced by TGF-β (Alfiya et al., 2011).

MC migration to the inflammation site and the following activation is the prerequisite for their function. In this regard, c-Kit/SCF is possibly involved. c-Kit receptor, also known as stem cell factor receptor(SCF) (Lennartsson and Ronnstrand, 2012), belongs to family tyrosine kinase receptor type III (Abu-Duhier et al., 2003). The c-Kit/SCF pathway plays an irreplaceable role in maintaining development, normal morphology and function of MC. It is reported that the expression level of c-Kit is increased in prostate cancer (Di Lorenzo et al., 2004; Jian-Hua et al., 2015), in order that, large number of MC infiltrate and promote the process of epithelial-to-mesenchymal transition (Ester et al., 2012; Huang et al., 2008; Knab et al., 2014), which is important in progressive fibrosis (Anusha et al., 2016). Therefore, we evaluate the expression level of c-Kit protein and the correlation with the MC activation in 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 a phytoalexin (Sharma et al., 2006), and then the reliable and extensive anti-inflammatory and anti-fibrotic effects of resveratrol arouse the attention of researchers (Csiszar, 2011). And it may be efficacious for the treatment of LUTD (Mehrnaz et al., 2016).

In this study, we aim to investigate whether detrusor fibrosis is associated with urinary dysfunction in CP, and to investigate whether resveratrol can improve the urinary dysfunction. Furthermore, we evaluate the MC activation, the activity of c-kit/SCF and TGF-β/Wnt/β-catenin pathway in CP rats.

2. Materials and methods

2.1. Chemicals

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

2.2. Animals and ethics statement

Male Sprague-Dawley (SD) rats (180 ± 20 g) are purchased from the Experimental Animal Center of Dalian Medical University (Dalian, China). Rats are allowed access to water and food ad libitum, but fast overnight with water available before surgery. All animal experiments are approved by the ethics committee of Dalian Medical University and performed in accordance with the institutional guidelines.

2.3. Rat model of CP

Purification of rat prostatic protein: After male SD rats (240–300 g) are killed, prostate tissue is removed under sterile conditions and washed with saline solution. Then, prostate tissue is put into a physiological saline solution containing 0. 5% Triton X-100 and homogenized in an ice-water bath with a glass homogenizer. Subsequently, the homogenized liquids are centrifuged (10,000 × g) for 10 min, and protein is diluted to 15 mg/ml with PBS buffer (0. 1 mol/l and pH7. 2).

Rats are subcutaneously injected DPT vaccine (0.5 ml/kg) and intradermally multipoint injected the mixture (1.0 ml) of purification of rat prostatic protein and Freund's Complete Adjuvant (FCA) (1:1) at 0, 15 and 30 d. After rat model of CP is completed at 45 d, rats are randomly divided into three groups (8 rats in each group; n = 8):

1. Control group: Normal rats are orally administered saline for 10 d.
2. CP group: CP rats are orally administered saline for 10 d.
3. Resveratrol group: CP rats are orally administered resveratrol (10 mg/kg) for 10 d.

2.4. Bladder pressure and volume test in rats

Rats are anesthetized with an intraperitoneal injection of pentobarbital (60 mg/kg) before surgery. Then, the rats are fixed on the operating frame, and the upper edge of the pubic symphysis skin is bladed. The bladder is exposed and placed in the incision to avoid affecting the abdominal pressure on the detrusor pressure. A 24G catheter is inserted into the bladder, connected to the three-way tube and fixed. One end was connected with micro-infusion pump is irrigated by saline (0.4 ml/min). and the other end was connected to MedLab biological signal acquisition and processing system through pressure transducer. The maximum capacity of the bladder, residual urine volume and maximum voiding pressure are measured. Then rats are killed.

2.5. Morphological changes

After the end of the bladder pressure and volume test, the rats are killed. The prostate and bladder detrusor of rats are removed, and fixed in 10% (v/v) neutral formalin processed by standard histological techniques, then stained with haematoxylin and eosin (H & E), and then they are examined for morphological changes of prostate and bladder detrusor. Inflammation is quantified by assessing the density of infiltrated inflammatory cells within the stroma. Five randomized fields of were analyzed at different magnifications, and the average of the 5 fields was calculated for each animal. The samples are used to determinate expression of protein of C-kit (CD-117), SCF , tryptase, chymase, TGF-β , Wnt and α-SMA by western blot analysis and immunohistochemical staining.

2.6. Western blot analysis

According to the manufacturer's instructions, proteins were extracted from rat bladder detrusor with protein extraction kit (KeyGen Biotech, Nanjing, China). Protein was measured according to the procedure of bicinchoninic acid (BCA) (Solarbio, Beijing, China), with bovine serum albumin as the standard. Proteins (20 μg) were resuspended in electrophoresis sample buffer containing β-mercaptoethanol and separated by electrophoresis on a pre-cast 10% SDS-polyacrylamide gel (Bio-Rad, Hercules, CA), followed by electrotransfer to a PVDF membrane (Millipore, Bedford, MA). 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 loading control. Membranes were incubated overnight at 4 °C with a 1:1000 dilution of polyclonal antibody for c-Kit, SCF, tryptase, TGF-β , Wnt and α-SMA, respectively (Cruz Biotechnology, Santa Cruz, USA), and with a 1:1500 dilution of monoclonal antibody for β-actin (Beyotime, China). After subsequent washing with TBST, the blots were then incubated with secondary antibodies. After extensive washing with TBST, membranes were exposed to the enhanced chemiluminescence-plus reagents (ECL) from Beyotime Institute of Biotechnology (Haimen, China) according to the manufacturer's protocol. Emitted light was documented with 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 semiquantitative measurements based on band densitometry.

2.7. Immunohistochemical staining

Histological sections of rat detrusor (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. Then, at room temperature they were immersed in PBS containing 3% H_2O_2 for

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