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RESEARCH ARTICLE

Effects of Qingshen granules on Janus Kinase/ signal transducer and activator of transcription signaling pathway in rats with unilateral ureteral obstruction

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Abstract

OBJECTIVE: To investigate the effects of Qingshen granules (QSG) on janus kinase/ signal transducer and activator of transcription (JAK/STAT) signaling pathway in a rat model of unilateral ureteral obstruction (UUO).

METHODS: Sixty male Sprague-Dawley rats were randomly divided into six groups, with 10 animals in each group: the untreated sham-operated normal control group; the untreated UUO model control group, the high dose QSG-treated (16 $g \cdot kg^{-1} \cdot d^{-1}$) UUO group; the medium dose QSG-treated (8 $g \cdot kg^{-1} \cdot d^{-1}$) UUO group; the low dose QSG-treated (4 $g \cdot kg^{-1} \cdot d^{-1}$) UUO group; and the valsartan-treated group (20 $mg \cdot kg^{-1} \cdot d^{-1}$). The two untreated control groups received physiological saline (1 mL/100 g per day). All the rats were sacrificed after a 4-week course of treatment. Serum creatinine and leptin; protein expressions of leptin receptor (OB-R), p-JAK2, p-STAT3, nuclear factors-κBp6 (NF-kBp65), and monocytechemotatic protein-1 (MCP-1); mRNA of JAK2, STAT3, calcium-dependent adhesion (E-cadherin), alpha-smooth muscle actin (α-SMA) in the kidney tissues; and the expressions of type Ⅳ collagen (Col-Ⅳ) and fibronectin (FN) and the pathomorphology in kidney tissues were treated.

RESULTS: Compared with the normal group, the BUN, Scr, and serum leptin levels and the expressions of MCP-1, p-JAK2, p-STAT3, NF-kBp65 and OB-R in renal tissues, and the mRNA expressions of leptin, JAK2 protein, STAT3 protein, α-SMA protein in model group were significantly higher ($P < 0.01$) in the UUO model group. These parameters were significantly reduced in all the QSG-treated groups and the valsartan-treated group than the UUO model group ($P < 0.05$ or $P < 0.01$), with the lowest levels found in the medium dose QSG-treated group ($P < 0.05$). However, the expression levels of E-cadherin, FN, and Col-Ⅳ in the renal tissues were contrary to the expressions described above. Se-

vere pathological injury was evident in the renal tissues of UUO model rats, which was alleviated in the QSG-treated and valsartan-treated groups, with the least damage found in the medium dose QSG-treated group.

CONCLUSION: Our data suggest that the leptin-mediated JAK/STAT signaling pathway is involved in the process of renal interstitial fibrosis in UUO rats. QSG inhibited the activity of the signaling pathway, reduced the activity of NF-kB and inflammatory effect, and the transdifferentiation in the renal tubular epithelial cells. Treatment with QSG may delay the renal fibrosis and protect the renal function from damage following UUO in rats.

Keywords: Renal fibrosis; Janus kinases; STAT transcription factors; Signal transduction; Qingshen granules

INTRODUCTION

Renal fibrosis is the major pathological basis of chronic renal failure (CRF), and also a clinical feature of the development and progression of chronic kidney disease (CKD), which is closely related to the degree of renal inflammation and renal function damage.[1] Renal fibrosis is mainly manifested by glomerular sclerosis, tubular atrophy and disappearance, and excessive accumulation of extracellular matrix, which is caused by continuous synthesis and degradation reduction. In our previous study, we found that the levels of serum leptin and various inflammatory factors were significantly increased in CRF patients, suggesting that leptin and inflammatory factors may be closely related to the process of renal fibrosis.[2] JAK/STAT pathway is a major signal transduction pathway mediated by leptin.[3] JAK/STAT signaling is activated in the rat model of unilateral ureteral obstruction (UUO), which accelerates the progression of renal fibrosis.[4] Furthermore, the nuclear transcription factor kappa B (NF-kB) signaling pathway, which is the downstream regulated by the JAK/STAT pathway, is the main signal transduction of the inflammatory reaction directly involved in the pathological process of renal fibrosis.[5] Therefore, it is reasonable to infer that leptin can activate JAK/STAT signaling pathway, leading to the activation of NF-kB signaling pathway which induces the production of inflammatory factors, and ultimately resulting in renal tubular epithelial-to-mesenchymal transition and renal interstitial fibrosis. Inhibition of leptin-mediated JAK/STAT activity is therefore a potential strategy to treat renal fibrosis. Previous research has confirmed that Qingshen granules (QSG), which has the effects of clearing heat and dampness and promoting blood circulation, can alleviate the inflammatory response in the kidney by reducing the levels of serum leptin, anti-renal fibrosis, and improving the kidney function. However, the mechanisms for the signal transduction pathway of leptin and the regulation of the inflammatory response in renal fibrosis are not yet clear. In this study, a rat model of UUO was established to explore the role of leptin-mediated JAK/STAT signaling pathway in NF-kB pathway activation and inflammatory effects, and the role in renal tubule transdifferentiation and renal interstitial fibrosis. Additionally, we discuss the mechanism of QSG in regulating the leptin signaling pathway.

MATERIALS AND METHODS

Experimental animals

Sixty male Sprague-Dawley rats [(200 ± 20) g] were purchased from Beijing Weitong Lihua Experimental Animal Technology Limited Company. All the animals were housed in Xin'an Medical Laboratory of Anhui University of Traditional Chinese Medicine with specific pathogen-free conditions, and had free access to water and standard rat feed.

Drugs and reagents

QSG, 10 g/bag, was provided by the First Affiliated Hospital of Anhui Medical Center (Hefei, China, batch No. 20141023). Valsartan capsules, 80 mg/capsule, were obtained from Beijing Novartis pharmaceutical factory (Beijing, China, batch No. H20040217). Serum creatinine (batch No. 20151102, Nanjing Institute of biological engineering, Nanjing, China), Leptin (batch No. 20121215A, Shanghai Biotechnology Co., Ltd., Shanghai, China), P-JAK2 (batch No. CA36131, Bioworld, Nanjing, China), p-STAT3 (batch No. 561201, Bioworld, Nanjing, China), MCP-1 (batch No. 131120w, Bioworld, Nanjing, China), NF-kBp65 (batch No. GR194772-1, Abcam, Cambridge, England), FN (batch No. AE011910, Bioss, Beijing, China), and Col-Ⅳ (batch No. AD091929, Bioss, Beijing, China) were used in this study.

Instruments

Semi-automatic biochemical analyzer (AT-648) was purchased from Shanghai Bomaijie Biotechnology Co., Ltd., (Shanghai, China). The electrophoresis apparatus (EPS-301) was from Amersham (Piscataway, NJ, USA). Gel image analyzer was from Bio-Rad Corporation (Gel Doc XR, Hercules, CA, USA). PCR instrument was provided by Biometra Company Biometra GmbH (T-Gradient, Goettingen, Germany). Pathological section machine (LEICA-213) and paraffin-embedding station (LEICA-115) were from Leica Inc. (Frankfurt, Germany).