Yishen Jiangzhuo Granules (益肾降浊冲剂) Affect Tubulointerstitial Fibrosis via A Mitochondrion-Mediated Apoptotic Pathway

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ABSTRACT  Objective: To investigate the effect of Yishen Jiangzhuo Granules (益肾降浊冲剂, YSJZG) on mitochondrial injury and regeneration and renal tubular epithelial cell apoptosis in chronic renal failure (CRF) rats and explore its mechanism from molecular pathology, gene, protein levels, and relative pathway. Methods: The CRF rat model was established using 5/6 nephrectomy. Sixty rats were randomly divided into six groups: sham-operation group, model (CRF) group, Niaoduqing Granules (尿毒清颗粒)-treated group [5 g/(kg.day)], low-, moderate-, and high-dose [L-YSJZG, M-YSJZG, H-YSJZG at 3, 6, and 9 g/(kg.day)] YSJZG-treated group (n=10 each). The levels of serum creatinine (Scr), blood urea nitrogen (BUN), and 24-h urine protein were assessed after 10 weeks of treatment. The tubulointerstitial injury and collagen deposition were evaluated using periodic acid-schiff stain and Masson staining. Renal tubular epithelial cell apoptosis was assessed using the terminal deoxynucleotidyl transferase dUTP nick end labeling assay, mitochondrial injury was observed using an electron microscope, and superoxide dismutase (SOD), glutathione (GSH) and malondialdehyde (MDA) levels were assessed using chromotomy. Transforming growth factor-β 1 (TGF-β 1) expression was assessed using immunohistochemistry. The expressions of Bax, Bcl-2, peroxisome proliferator-activated receptor γ co-activator-1 α (PGC-1 α), mitochondrial transcription factor A (Tfam), mitogen-activated protein kinases (MAPK) phosphorylation were evaluated by Western blot. Results: YSJZG decreased the 24-h urine protein, BUN, Scr, remnant kidney weight-to-body weight ratio, renal tubular injury, deposition of collagen, and the apoptosis of renal tubular epithelial cells in a dose-dependent manner. YSJZG dose-dependently restored the number and structure of mitochondria and the expression of Tfam and PGC-1 α, up-regulated the expression of Bcl-2, and inhibited the expression of Bax. YSJZG also dose-dependently inhibited TGF-β 1 expression, increased SOD and GSH activity, decreased the MDA level, and inhibited p38MAPK and pERK1/2 phosphorylation (all P<0.01). Conclusion: YSJZG improved the renal function in rats with CRF and inhibited the progression of tubulointerstitial fibrosis by dose-dependently alleviating mitochondrial injury, restoring the expression of Tfam and PGC-1 α, and inhibiting renal tubular epithelial cell apoptosis through inhibiting activation of reactive oxygen species-MAPK signaling.

KEYWORDS Yishen Jiangzhuo Granules, tubulointerstitial fibrosis, apoptosis, mitochondria, oxidative stress, mitogen-activated protein kinase signaling, Chinese medicine

Renal tubular atrophy and interstitial fibrosis are important pathogenic pathways that lead to end-stage renal failure in patients with chronic kidney disease (CKD). Transforming growth factor-β 1 (TGF-β 1) is one of the most important cytokines that contribute to renal interstitial fibrosis (RIF).¹) Mitochondria are involved in regulating cellular apoptosis, and mitochondrial injury is closely associated with the fate of the cells.²) Renal tubular epithelial cells with many mitochondria produce a large amount of energy to maintain physiological activity, which is easily affected by oxidative stress. The mitochondrion possesses its own genes and the ability to self-replicate. The double-stranded circular mitochondrial DNA (mtDNA) is found in vertebrate animals, which maintains the number of mitochondria in a state of equilibrium. The

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regeneration of mitochondria affects its structure and function and also plays a role in the regulation of mitochondria-mediated apoptosis.\(^{(3)}\) TGF-\(\beta\)1 promotes renal tubular atrophy and development of RIF,\(^{(4,6)}\) and this underlying mechanism has been largely explored. TGF-\(\beta\)1 induces the generation of reactive oxygen species (ROS) by activating nicotinamide adenine dinucleotide phosphate (NADPH) oxidase that activates p38 mitogen-activated protein kinases (MAPK), Jun N-terminal kinase (JNK), extracellular-signal-regulated kinase (ERK) signaling. Accumulation of ROS causes a decrease in, or a mutation of, mtDNA, a decrease in the expression of proliferator-activated receptor \(\gamma\) co-activator-1\(\alpha\) (PGC-1\(\alpha\)) and mitochondrial transcription factor A (Tfam), inhibition of generation of electron transfer chain ATP, a reduction in the mitochondrial membrane potential, and opening of mitochondrial permeability transition pores (mtPTP), which finally promote apoptosis of renal tubular cells.\(^{(4,5)}\)

Yishen Jiangzhuo Granules (益肾降浊冲剂, YSJZG), produced by the People Hospital Affiliated to Fujian University of Traditional Chinese Medicine, has been used clinically for more than 20 years. YSJZG plays a role in invigorating Kidney (Shen)-yuan, strengthening the Spleen (Pi), replenishing qi, and resolving phlegm; these granules also inhibit oxidative stress, eliminate uremic toxins and inflammatory mediators, and improve glomerular filtration rate and renal function.\(^{(6)}\) Thus, whether YSJZG improved the renal function and delayed the progression of chronic renal failure (CRF) by inhibiting TGF-\(\beta\)1-ROS-MAPK signaling, restoring mitochondrial regeneration, improving mitochondrial structure and function, and further inhibiting renal tubular epithelial cell apoptosis was explored. Multiple possible targets for the treatment of CRF using YSJZG were explored, and these findings will provide a theoretical and experimental foundation for multilink and multitarget intervention for the progression of CRF.

**METHODS**

**Drugs Preparation**

YSJZG was provided by the People Hospital Affiliated to Fujian University of Traditional Chinese Medicine (No. Z06106052). YSJZG consist of Radix et Rhizoma Rhei, Poria, Radix Pseudostellariae, Radix Astragali, Rhizoma Atractylodis Macrocephalae, Fructus Mori, Serissa foetida, Crateagus pinnatifida, Angelica sinensis, etc. The herbs were boiled in water for 1.5 h, filtered, and concentrated to 1000 mL. Alcohol with a concentration >60% was added, allowed to stand for 24 h, centrifuged, and filtered. Alcohol was recycled and concentrated to a paste with a specific gravity of 1.38–1.40, and finally pelletized. Niaoduqing Granules (尿毒清顆粒, NDQG) were provided by Kangcheng Pharmaceutical Industry, China (No. Z10970122). NDQG consists of Radix et Rhizoma Rhei, Poria, Radix Astragalii, Rhizoma Atractylodis Macrocephalae, Sophora flavescens, Cortex Mori, Plantago asiatica, etc.

**Main Reagents**

Anti-TGF-\(\beta\)1 (ab8226), anti-\(\beta\)-actin, anti-Bax (ab5714), mitochondrial transcription factor A (Tfam) and anti-Bcl-2 (ab7973) were obtained from Abcam Inc. (Cambridge, MA, USA). Anti-PGC-1\(\alpha\) (sc-13067), anti-p38MAPK, and phosphorylated extracellular-signal-regulated kinase (pERK1/2) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Superoxide dismutase (SOD) and malondialdehyde (MDA) were obtained from Jiahefang Biotechnology (Nanjing, China). Glutathione (GSH) and terminal deoxynucleotidyl transferase-mediated dUTP nick translation end labeling (TUNEL) cell apoptosis detection kit were purchased from Beyotime Institute of Biotechnology (Haimen, Jiangsu, China).

**Animal Model and Treatment**

Sixty Sprague-Dawley male rats (weight: 220–250 g, specific pathogen free degree) were purchased from Shanghai SLAC Laboratory Animal Co., Ltd., China (Certificate No. SCXK-2007-0005). All the animal experiments were performed in accordance with an animal protocol approved by Laboratory Animal Center of Fujian Medical University. Animals were adaptively housed for 1 week and divided into six groups: (1) sham operation control (CTL) group (n=10); (2) model (CRF) group (n=10); (3) NDQG group (n=10); (4) low-dose YSJZG (L-YSJZG) group (n=10); (5) moderate-dose YSJZG (M-YSJZG) group (n=10); (6) high-dose YSJZG (H-YSJZG) group (n=10). The rats were anesthetized using an intraperitoneal injection of pentobarbital sodium at a dose of 40 mg/kg. Then, the rats were subjected either to 5/6 nephrectomy by performing a right nephrectomy with surgical resection of two-thirds of the left kidney or to sham operation.\(^{(7)}\) Two weeks after the CRF model was established, rats in the NDQG group were intragastrically administered