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Original article

Qi-Zhu-Xie-Zhuo-Fang reduces serum uric acid levels and ameliorates renal fibrosis in hyperuricemic nephropathy rats

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ABSTRACT

Hyperuricemia is associated with the development of chronic kidney disease. Epithelial-to-mesenchymal transition (EMT) induced by hyperuricemia is blamed for initiation of renal fibrosis, which is one of the main characters of hyperuricemic nephropathy. Qi-Zhu-Xie-Zhuo-Fang (QZXZF) has been employed clinically for many years to treat patients with hyperuricemic nephropathy, but the mechanism underlying the therapeutic potential remains unclear. In the present study, QZXZF was applied to rats treated with adenine (100 mg/kg) and potassium oxonate (300 mg/kg) and biochemical estimations, morphology and immunohistochemistry were performed to investigate whether QZXZF can improve hyperuricemia induced renal fibrosis and to explore the possible mechanisms. We found QZXZF significantly reduced serum uric acid, cystatinC and hepatic xanthine oxidase (XO) activities, meanwhile improved renal histopathologic changes of hyperuricemic nephropathy rats. Furthermore, QZXZF not only substantially decreased the protein levels of fibronectin and Collagen I but also downregulated E-cadherin and upregulated α-SMA in the kidneys of hyperuricemic nephropathy rats. In conclusion, QZXZF reduced serum uric acid levels and protected kidney against fibrosis in potassium oxonate and adenine induced hyperuricemic nephropathy rats. The mechanism might be associated with the inhibition of hepatic XO activity and the renal epithelial-to-mesenchymal transition.

1. Introduction

Numerous studies suggest that hyperuricemia (increased serum uric acid level) is associated with the development of chronic kidney disease (CKD) [1]. A study reported the prevalence of CKD patients with gout was nearly 40% [2]. Lowering serum uric acid shows potential therapeutic effect on preventing the progression of kidney disease and xanthine oxidase (XO) inhibitors were recommended for gout patients with CKD [3,4]. However the evidence for no matter allopurinol nor febuxostat, another xanthine-oxidase inhibitor, of improving kidney disease progression in gout patients with CKD was limited [5,6].

Hyperuricemic nephropathy is characterized by uric acid kidney stones, chronic interstitial nephritis and fibrosis, which can eventually lead to chronic renal failure. Of all these characters, renal fibrosis plays an important role in the injury of hyperuricemic nephropathy, with deposition of extracellular matrix (ECM) in renal interstitium [7,8]. Epithelial-to-mesenchymal transition (EMT), which means renal tubular cells lose their epithelial phenotypes and get new characteristic features of mesenchymal phenotypes, plays an important role in renal fibrosis [9]. EMT can be initiated by many factors, including transforming growth factor β1 (TGF-β1), uric acid and so on [10,11].

In the traditional Chinese medicine (TCM) theory, deficiencies in spleen and kidney are the main pathogenesis of the hyperuricemic nephropathy, leading to the dysfunction of transportation and transformation of grain and water as well as the obstruction of dampness and phlegm in meridian. Qi-Zhu-Xie-Zhuo-Fang (QZXZF, modified from Jianpi Huashi Jiedu decoction) is an empirical formula which was developed to treat hyperuricemic nephropathy by MAO Jian-Chun (Chief physician of Longhua hospital) based on the principles of TCM. QZXZF consists of eight kinds of traditional Chinese medicine, which are *Astragalus mongholicus* Bunge, (huang qi, Astragalus), *Atractylodes macrocephala* Koidz., (bai zhu, rhizome atractylodis macrocephalae), *Coix lacryma-jobi* L., (mi ren, Jobstears Seed), *Pyrrosia lingua* (Thunb.) Farw., (shi wei, Pyrrosia Leaf), *Smilax*

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glabra Roxb., (tu fu ling, Smilacis Glabrae Rhizoma), *Salvia miltiorrhiza* Bge., (dan shen, Dan-shen Root), *Cuscuta chinensis* Lam., (tu si zi, Chinese dodder Seed) and *Isaria cicadae* Miq., (jin chan hua, Fungus Sclerotia on Cicada). As principal drugs, Astragalus and rhizome atractylodis macrocephalae boost Qi and invigorate spleen to promote transportation and transformation of grain and water. As subordinate drugs, Jobstears Seed, Pyrrosia Leaf and Smilacis Glabrae Rhizoma work together to remove dampness and phlegm. As adjuvant drugs, Chinese dodder Seed and Fungus Sclerotia on Cicada invigorate kidney to help transportation and transformation of grain and water. Last but not least, as guide drugs, Dan-shen Root can activate blood and move Qi to help remove phlegm turbidity [12].

According to our previous clinical study, the overall efficacy rate of Jianpi Huashi Jiedu decoction on chronic gout patients was comparative to allopurinol and it proved better clinic response than allopurinol did [13]. In addition, Jianpi Huashi Jiedu decoction reduced the secretion of fibronectin of mesangial cells under the stimulation of uric acid, indicating its renal protection potential in hyperuricemic nephropathy [14]. After modified from Jianpi Huashi Jiedu decoction guided by TCM theory, QZXZF has been used clinically to treat chronic gout with renal dysfunction or hyperuricemic nephropathy by Dr. Mao in Longhua hospital. However, the effects of QZXZF on renal injury and potential mechanism in hyperuricemic rats have not been investigated yet. Thus we investigated the effects of QZXZF on hyperuricemia induced renal fibrosis and phenotypic transition in a rat model of hyperuricemic nephropathy in this study. We also examined the effects of QZXZF on serum uric acid, Cystatin C (CysC) and hepatic XO activity of hyperuricemic nephropathy rats.

2. Materials and methods

2.1. Chemicals and reagents

All chemicals and reagents were purchased from Sigma–Aldrich (USA) unless otherwise specified. Hyperuricemic nephropathy rats were induced by 100 mg/kg adenine and 300 mg/kg potassium oxonate (Aladdin Industrial Corporation, China). Allopurinol was purchased from Dalian Meilun Biological Technology Co., Ltd. (China). QZXZF was developed in a granular form by SICHUAN NEO-GREEN pharmaceutical technology CO., LTD, which was commercially available in the hospital. The medicinal slices were boiled in water, separated and then concentrated into extract paste. With instant dry, physical extrusion and crush, the extract paste was finally made into granular form. Antibodies against fibronectin and collagen I were purchased from Abcam (USA). Antibodies against slug, CD68 and GAPDH were purchased from CST (USA). Antibodies against α-smooth muscle actin (α-SMA), snail, twist, E-cadherin and α-Tubulin were purchased from Proteintech (USA). Polyviny lidene fluoride (PVDF) membrane was purchased from Millipore (USA). Enhanced chemiluminescence (ECL) reagents were purchased from CST (USA). Bicinchoninic acid (BCA) protein assay kit was purchased from Shennengbocai Biotech (China). XO activity, uric acid and cysteine C detection kits were purchased from Nanjing Jiancheng Biotech (China).

2.2. Animal model and administration of chemicals

Male Wistar rats (5 weeks old) were purchased from Shanghai SLAC laboratory animal Co., Ltd. (China). The rats were housed in a temperature-controlled room under a 12-h light/dark cycle with available food and water ad libitum. The rats were allowed one week to adapt to the environment before experiments. Experimental protocols were approved by the ethics committee for experimental research, Shanghai University of TCM.

In this study, forty rats were randomly divided into four groups: Control group (normal rats), Model group (Control treated with adenine and potassium oxonate), Allopurinol group (Model treated with allopurinol), QZXZF group (Model treated with QZXZF granules). Except for Control group, rats were treated with the 2 ml suspension containing 100 mg/kg adenine and 300 mg/kg potassium oxonate once daily. After 1 h treated with adenine and potassium oxonate, rats were administered with QZXZF, allopurinol or water by gavage once daily. Rats were treated for 3 weeks. Elevated serum uric acid and CysC with the renal histopathologic changes of Model group rats proved the success of this hyperuricemic nephropathy model (Fig. 1). The ACR guidance about the daily dose of allopurinol to treat hyperuricemia was 200–300 mg and not more than 600 mg [15]. The daily dose of QZXZF used for adults was shown in Table 1.

Adult patients take QZXZF granules at a daily dose of 12 g. The dosage of allopurinol and QZXZF taken by rats was calculated based on body surface area [16]. Thus allopurinol and QZXZF granules were given to rats at 35 mg/kg per day and 1.4 g/kg per day respectively.

2.3. Blood and tissue samples collection

After treated with drugs for 3 weeks, the rats were euthanized 1 h after the last drugs administration, and the blood samples were collected from abdominal aortic. The serum samples were obtained by centrifuge at 3500 rpm for 10 min after coagulated in 4 °C overnight. The serum samples were used for the determination of uric acid and CysC level and stored at −80 °C until biochemical assays were performed. The right kidney and liver tissues were rapidly separated on ice plate, frozen in liquid nitrogen, and stored at −80 °C. The left kidney was separated and fixed in paraformaldehyde for morphological and immunohistochemistry examination.

2.4. Assessment of serum uric acid, CysC and hepatic XO activity

For hepatic XO activity assay, enzyme extraction of liver tissues was performed as protocol provided by the manufacturer. Briefly, liver tissues were sufficiently homogenized in 9 volumes of saline in the ice bath. Subsequently, the homogenate was centrifuged at 3000 rpm for 10 min at 4 °C. The supernatant was used for XO activity assay after protein concentration testing. Serum uric acid and CysC were examined according to the protocols provided by the manufacturer (Nanjing Jiancheng Biotech, China).

2.5. Renal morphology and immunohistochemistry

Paraformaldehyde-fixed kidneys were embedded in paraffin and cut into 5-μm sections. The sections were deparaffinized, rehydrated and stained with hematoxylin and eosin and Masson's trichrome. To measure the renal collagen positive area (blue), we took bright field images (original magnification, 200×) on eight random microscopic views of each Masson trichrome sections and drew a line around the positive staining area using ImageJ 1.40g software, then calculated the average positive area ratio to the whole microscopic field and graphed.

For immunohistochemistry, the sections were deparaffinized, rehydrated and treated with 10 mM citric acid buffer (pH = 6) in a pressure cooker for antigen repair. After being blocked with 3% hydrogen peroxide for 10 min at room temperature, the sections were blocked with 5% goat serum in PBS for 10 min and then incubated with anti-CD68, anti-E-cadherin and anti-α-SMA (diluted 1:200) antibody over night at 4 °C. The sections were