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Siwu decoction attenuates oxonate-induced hyperuricemia and kidney inflammation in mice

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[ABSTRACT] The aim of the study was to investigate the effects of Siwu decoction on hyperuricemia, kidney inflammation, and dysfunction in hyperuricemic mice. Siwu decoction at 363.8, 727.5, and 1 455 mg·kg^{-1} was orally administered to potassium oxonate-induced hyperuricemic mice for 7 days. Serum urate, creatinine, and blood urea nitrogen levels and hepatic xanthine oxidase (XOD) activity were measured. The protein levels of hepatic XOD and renal urate transporter 1 (URAT1), glucose transporter 9 (GLUT9), organic anion transporters 1 (OAT1), ATP-binding cassette subfamily G member 2 (ABCG2), organic cation transporter 1 (OCT1), OCT2, organic cation/carnitine transporter 1 (OCTN1), OCNT2, Nod-like receptor family, pyrin domain containing 3 (NLRP3), apoptosis-associated speck-like protein (ASC), Caspase-1, and interleukin-1β (IL-1β) were determined by Western blotting. Renal histopathology change was obtained following hematoxylin-eosin staining. Our results indicated that Siwu decoction significantly reduced serum urate, creatinine and blood urea nitrogen levels and increased fractional excretion of uric acid in hyperuricemic mice. It effectively reduced hepatic XOD activity and protein levels in this animal model. Furthermore, Siwu decoction down-regulated URAT1 and GLUT9 protein levels, and up-regulated the protein levels of OAT1, ABCG2, OCT1, OCT2, OCTN1, and OCTN2 in the kidney of the hyperuricemic mice. Additionally, Siwu decoction remarkably reduced renal protein levels of NLRP3, ASC, Caspase-1, and IL-1β in the hyperuricemic mice. These results suggested that Siwu decoction exhibited anti-hyperuricemic and anti-inflammatory effects by inhibiting hepatic XOD activity, regulating renal organic ion transporter expression, and suppressing renal NLRP3 inflammasome activation, providing the evidence for its use in the treatment of hyperuricemia and associated kidney inflammation.

[KEY WORDS] Siwu decoction; Hyperuricemia; Renal organic ion transporter; NLRP3 inflammasome; Kidney inflammation

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Introduction

Hyperuricemia is an important risk factor for the development of gout, insulin resistance, coronary heart disease, diabetes, and metabolic syndrome [1-3]. Generally, xanthine oxidase (XOD) oxidizes xanthine to uric acid mainly in liver, through the purine metabolic pathway; its hyperactivity causes high levels of serum uric acid [4]. Increasing evidence supports the notion that kidney urate transport-related proteins mediate kidney urate excretion to maintain blood urate balance [5-9]. Among them, urate transporter 1 (URAT1) and glucose transporter 9 (GLUT9) regulate kidney uric acid reabsorption [5-7], while organic anion transporter 1 (OAT1) and native ATP-binding cassette subfamily G member 2 (ABCG2) regulate renal urate secretion [8-9]. Of note, dysregulation of these urate transporters alters urate handling in humans [10-11].

Hyperuricemia aggravates kidney dysfunction. Organic cation transporter 1 (OCT1), OCT2, organic cation and carnitine transporter 1 (OCTN1), and OCTN2 mediate the excretion of organic cations and carnitine in renal proximal tubules [7]. Down-regulation of these kidney organic ion transporters may increase the risk for kidney dysfunction in hyperuricemia [12-14]. On the other hand, the elevated serum urate levels cause kidney inflammation [15]. Nod-like receptor family, pyrin domain containing 3 (NLRP3), interacts with the bridging molecule apoptosis-associated speck-like protein (ASC) to activate caspase-1, leading to mature interleukin-1β (IL-1β) production [16-17]. Uric acid as a proinflammatory molecule activates the NLRP3 inflammasome, causing

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inflammatory response [16]. Therefore, regulation of renal organic ion transporter system and suppression of the NLRP3 inflammasome activation may reduce serum urate levels and alleviate kidney inflammation and dysfunction in hyperuricemia.

Siwu decoction, composed of Angelica sinensis radix (*Angelica sinensis* (Oliv.) Diels), Chuanxiong rhizome (*Ligusticum chuanxiong* Hort.), Paeoniae radix alba (*Paeonia lactiflora* Pall.) and Rehmanniae radix praeparata (*Rehmannia glutinosa* Libosch.), has long been used in traditional Chinese medicine. According to Xian-shou-li-shang-xu-duan-mi-fang in Tang dynasty and Tai-ping-hui-min-he-ji-ju-fang in Song dynasty, Siwu decoction is prescribed to alleviate trauma and ecchymosis, promote blood circulation, and treat gynecological and obstetrical diseases [18-19]. The combination of Siwu and Ermiao decoction is reported to markedly decrease serum uric acid levels and inhibit hepatic XOD activity in hyperuricemic rats [20]. A recent study shows that Siwu decoction inhibits triphasic skin reaction in passively sensitized mice with inflammation [21]. In the present study, we investigated the effects and possible mechanisms of Siwu decoction on hyperuricemia, renal inflammation, and dysfunction in the potassium oxonate-induced hyperuricemic mice.

Materials and Methods

Chemicals and reagents

Potassium oxonate, allopurinol, hematoxylin & eosin reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA). The ingredients of Siwu decoction, including Angelica sinensis granule (batch number 1303102, 8 g), ligusticum chuanxiong granule (batch number 1304033, 8 g), paeonia lactiflora granule (batch number 1302031, 4 g) and, rehmannia glutinosa granule (batch number 1309107, 8 g), were purchased from Jiangyin Tianjiang Pharmaceutical Co., Ltd. (Jiangyin, China).

Standards of ferulic acid, paeoniflorin, and ligustrazine hydrochloride were obtained from National Institutes for Food and Drug Control (Beijing, China). Assay kits for uric acid, creatinine, blood urea nitrogen (BUN), and XOD activity were purchased from Jiancheng Biotech (Nanjing, China). Rabbit anti-mouse XOD and Caspase-1 antibodies and mouse anti-β-actin monoclonal antibody were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). Rabbit anti-mouse URAT1, GLUT9, OCT1, and OCT2 antibodies were obtained from Cellchip Biotech (Beijing, China). Rabbit anti-mouse OCTN1 and OCTN2 antibodies were purchased from Alpha Diagnostic International, Inc. (San Antonio, TX, USA). Rabbit anti-mouse OAT1 antibody was also from Cellchip Biotech. Rabbit anti-mouse ABCG2 antibody was purchased from Cell Signaling Technology, Inc. (Boston, MA, USA). Rabbit anti-mouse NLRP3, ASC, and IL-1β antibodies were purchased from Abcam (Cambridge, MA, USA). Mouse GAPDH monoclonal antibody was from Kangcheng Biotech (Shanghai, China). All other chemicals used in the present study were commercial products of the highest purity available.

UPLC-MS analysis of Siwu decoction

Qualitative and quantitative analyses of ferulic acid, paeoniflorin, and ligustrazine in Siwu decoction were accomplished using ultra performance liquid chromatography (UPLC)-Mass spectrometric (MS) analysis. UPLC was performed on a Water ACQUITY UPLC™ system (Waters Corporation, Milford, MA, USA) equipped with a binary solvent delivery system and autosampler. The chromatography was performed on a Water ACQUITY UPLC BEH C18 (2.1 mm × 100 mm, 1.7 μm). The mobile phase consisted of the following 22 min sequence of linear gradients and an isocratic flow of 0.1% formic acid-water (solvent A) balanced with solvent B (0.1% acetonitrile) at a flow rate of 0.4 $mL \cdot min^{-1}$ under the condition of column temperature 30 °C: 0–2 min, 95% A, 5% B; 2–13 min, 95%–60% A, 5%–40% B; 13–17 min, 60%–20% A, 40%–80% B; 17–19 min, 20%–5% A , 80%–95% B; 19–20 min, 5% A, 95% B; 20–21 min, 5%–95% A, 95%–5% B; and 21–22 min, 95% A, 5% B. The injection volume for all analyses was 10 μL.

MS analysis was carried out with a Waters ACQUITY Synapt Q-TOF mass spectrometer (Waters MS Technologies, Manchester, UK) connected to the Waters ACQUITY UPLC system via electrospray ionization (ESI) interface. High purity nitrogen was used as the nebulizer and auxiliary gas, and argon was the collision gas. The Q-TOF MS was operated in negative ion mode with a capillary voltage of 3 kV, a sampling cone voltage of 45 V, a desolvation temperature of 500 °C, a source block temperature of 150 °C, a collision energy of 6–40 V, and a ion energy of 1 V.

Animals

Male Kun-Ming mice (weighing 20 ± 2 g) were purchased from the Laboratory Animal Center of Academy of Military Medical Sciences (Beijing, China, Certificate NO. SCXK- (Military) 2012-0004) and housed in a same room in which the temperature was 25 ± 1 °C with relative humidity (55 ± 5)% and 12 h light/12 h dark cycles were maintained with the lights on at 7 : 00 a.m. They are given a standard chow and water ad libitum for the duration of the study. The mice were allowed to adapt to the environment for a week before being used for the experiment. All studies were performed with the standards established by the Institutional Animal Care Committee at the Nanjing University and the China Council on Animal Care at the Nanjing University (The Ministry of Science and Technology of the People's Republic of China, 2006), and experiment number approved by Institutional Animal Care Committee is 201305620.

Hyperuricemic mice and drug administration

Hyperuricemia was induced with uricase inhibitor potassium oxonate (250 $mg \cdot kg^{-1}$) in mice as described in our previous report [22]. According to the Tai-ping-hui-min-he-ji-ju-fang in Song dynasty and Traditional Chinese Medicine Lexicon Attachment, the dosage of Siwu decoction for adults is 11.19 $g \cdot d^{-1}$ (the total raw materials), and the equivalent mouse dosage is 1 455 $mg \cdot kg^{-1} \cdot d^{-1}$ as calculated by the