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Protective effect of iridoid glycosides from *Paederia scandens* (LOUR.) MERRILL (Rubiaceae) on uric acid nephropathy rats induced by yeast and potassium oxonate

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ABSTRACT

Iridoid glycosides of *Paederia scandens* (IGPS) are an active component isolated from Chinese herb *P. scandens* (LOUR.) MERRILL (Rubiaceae). Uric acid nephropathy (UAN) is caused by excessive uric acid, which results in damage of kidney tissue via urate crystals deposition in the kidneys. This study aimed to investigate the protective effects of IGPS on UAN in rats induced by yeast and potassium oxonate. Treatment groups received different doses of IGPS and allopurinol (AP) daily for 35 days respectively. The results showed that treatment with IGPS significantly prevented the increases of uric acid in serum and the elevation of systolic blood pressure (SBP), attenuated renal tissue injury, improved renal function and reserved the biological activity of NOS-1. IGPS also inhibited the biological activity of TNF-α and TGF-β1, and suppressed the mRNA expressions of TNF-α and TGF-β1 in renal tissue. Taken together, the present and our previous findings suggest that IGPS exerts protective effects against kidney damage in UAN rats through its uric acid-lowering, anti-inflammatory and immunomodulatory properties. Furthermore, decreasing SBP by up regulation of NOS-1 expression and down regulation of TNF-α and TGF-β1 expression are involved in the effect of IGPS on high uric acid-induced nephropathy.

1. Introduction

Paederia scandens (LOUR.) *MERRILL* (Rubiaceae) is used in Chinese herbal medicine. It is traditionally used as a vegetable and is also one of the members of GRAS (generally recognized as safe) category of plants used for therapeutic purposes (Chen et al., 2009). People in Hainan of China, make lots of paederia snacks such as paederia dumplings, paederia rice noodles and paederia cakes; they are famous regional folk tonics. It has a long history of medicinal use widely in China, Japan, Korea, and Taiwan for the treatment of inflammatory conditions. Extracts of *P. Scandens* (EPS) was described to have analgesic and anti-inflammatory actions, and it is used to treat bruises and rheumatism through clearing away heat and excreting dampness in terms of traditional Chinese medicine. Iridoid glycosides from *P. scandens* (IGPS) are a standardized extract from *P. scandens*, the main constituents of which include asperuloside, paederoside and scanderoside (Ma et al., 2009). Our previous studies suggested that treatment with IGPS could reduce serum urate levels and relieve symptoms in uric acid nephropathy (UAN) rat models induced by adenine and potassium oxonate (Jin et al., 2011).

A great many epidemiological and experimental sources suggest that serum uric acid level may be a significant risk factor for hypertension and kidney disease (Feig et al., 2008a; Bellomo et al., 2010). Higher serum uric acid (SUA) is independently associated with an increased risk of hypertension by worsening renal arteriolopathy in rats and activating the renin-angiotensin system (Mazzali et al., 2002). The elevated UA levels might contribute to the development and progression of renal dysfunction (Kang et al., 2002). Uric acid nephropathy (UAN) is mainly caused by excessive uric acid generation or decreased excretion of uric acid by the kidney, which results in damage of renal tissue via the deposition of urate crystals in the kidneys (Zhu et al., 2012). Serum urate concentrations above the normal range may favor the formation of urate crystals in the joints, causing gout, and in the kidney, predisposing them to urate nephrolithiasis (Martinon et al., 2006). It is widely accepted that monosodium urate crystals stimulated gouty inflammation (Khan, 2004). Not surprisingly, uric acid plays an essential role in the development of hypertension and renal injury.

Increasing evidence shows that uric acid could trigger an inflammatory response leading to infiltration of inflammatory cells

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(Peng et al., 2012). Our previous investigation showed that uric acid could induce renal inflammatory chemokines expression via the activation of nuclear factor-kappa B (NF-κB) (Zhu et al., 2012). Uric acid has been found to induce renal inflammation via activation of NF-κB signaling (Zhou et al., 2012; Wu et al., 2012), with stimulation of TNF-α and other inflammatory cytokines. Tumor necrosis factor-α (TNF-α) is mainly secreted by activated monocytes/macrophages and could mediate the induction of other chemokines and attract neutrophil infiltration, resulting in inflammation (Mazzei et al., 2012). Many lines of evidence demonstrate that uric acid may contribute to renal injury by stimulating TNF-α over-expression (Mazzei et al., 2012; Hu et al., 2012). Transforming growth factor-beta1 (TGF-β1), which is known to have a critical role in stimulating pathological tissue fibrosis and renal inflammation (Mazzei et al., 2012), is involved in the progression of chronic kidney disease and the control of hypertension (Talaat and El-Sheikh, 2007). It is also found that hyperuricemic rats show an increase in juxtaglomerular renin and a decrease in macula densa neuronal nitric oxide synthase (NOS-1) (Mazzali et al., 2002), which is involved in regulating afferent arteriolar tone and tubuloglomerular feed-back, resulting in hypertension (Mazzali et al., 2002; Long et al., 2008).

Accordingly, previous investigation undertaken by our group demonstrated that IGPS exhibited anti-inflammatory and immunomodulatory activity in UAN models induced by adenine and potassium oxonate (Zhu et al., 2012). In the present study, we used an experimental model of UAN induced by yeast and potassium oxonate in rat to further explore the protective effect of IGPS and its mechanism on UAN rats, and we focused mainly on the anti-inflammatory and immunosuppressant mechanisms, which are deeply involved in the etiology of UAN.

2. Materials and methods

2.1. Reagents

Allopurinol (AP) was obtained from Shanghai XinYi WanXiang Pharmaceuticals Ltd. (Shanghai, China). Yeast extract paste was purchased from the Chinese medicine group chemical reagent Co., Ltd. (Shanghai, China). Potassium oxonate (POX) was purchased from Sigma–Aldrich (St. Louis, MO, USA). Serum creatinine (Cre) and blood urea nitrogen (BUN) testing kit were purchased from Nanjing Jiancheng Bioengineering Institute. The antibody (rabbit-anti-rat) of NOS-1, TNF-α and TGF-β1, the streptavidin biotin complex (SABC) kit and diaminobenzidine (DAB) were purchased from Wuhan Boster Biological Engineering Co. (Wuhan, China). DNA primer fragments were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China). RevertAid First Strand cDNA Synthesis Kit and PCR Master Mix Kit were purchased from Thermo Scientific (Lithuania, EU). PCR markers was purchased from TaKaRa Biotechnology Co., Ltd. (Dalian, China).

2.2. Plant material and preparation of extracts

The aerial parts of *P. scandens* were purchased from a market specializing in herbs (BoZhou, Herb Market, China) in December of 2010. The plant was authenticated by Dr. Wenming Cheng of the Department of Pharmacognosy, School of Pharmacy, Anhui Medical University, Hefei, China, and voucher specimens (No. 102) were deposited in our laboratory (Dept. of Pharmacology, Anhui Medical University, Hefei 230032, China). Preparation of IGPS was performed as previously described (Yan et al., 2008). The three main compounds of iridoid glycosides were elucidated as asperuloside, paederoside and scanderoside (Fig. 1).

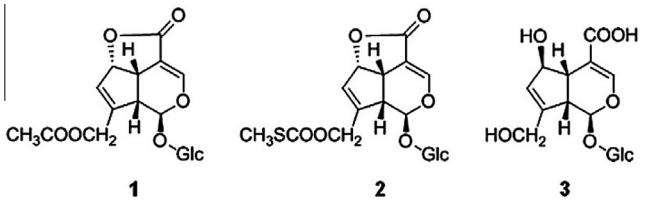

Fig. 1. The chemical structures of asperuloside (1), paederoside (2), and scanderoside (3).

2.3. Animals

Male Sprague–Dawley rats (250 ± 20 g) were purchased from the Laboratory Animal Center of Anhui Medical University, China (permission number, SCXK 2012-001). They were allowed at least 1 week to adapt to their environment before being used for experiments. They were maintained in a room controlled at 22–24 °C with a relative humidity of 60 ± 5% and a 12:12-h light–dark cycle (6:00 a.m.–6:00 p.m.). They were given standard chow and water ad libitum for the duration of the study. All experimental protocols described in this study were approved by the Ethics Review Committee for Animal Experimentation of Anhui Medical University.

2.4. Animal model of uric acid nephropathy rats and drug administration

The uricopoiesis promoter yeast extract paste and uricase inhibitor POX were used to induce hyperuricemia in rats (Yan et al., 2007; Chen et al., 2006; Eräranta et al., 2008). The experimental animal model of UAN was made by orally administering yeast extract paste (15 g/kg, bid × 42 days) and intraperitoneal injection of POX at the end of every week (250 mg/kg, qw × 6 weeks). The modeling process lasted for 6 weeks. All drugs were dissolved in distilled water except for POX; it is dissolved in 0.9% saline solution. The volume of the suspension administered was based on body weight measured immediately prior to each dose, respectively.

Seventy rats were randomly divided into six groups (except model group containing 20 rats), each group containing 10 rats, control group received corresponding vehicles; model group receiving yeast extract paste, POX and vehicle. Three IGPS groups received yeast extract paste, POX and different amounts of IGPS (280, 140, 70 mg/kg, qd × 5 weeks), respectively. AP group received yeast extract paste, POX and AP (50 mg/kg, qd × 5 weeks). IGPS, AP and vehicle (model group) received intragastric administration from the eighth day of the whole modeling process.

2.5. Histopathology of renal tissues

Rats were sacrificed after final administration and kidney tissues were dissected quickly on ice, parts of them were immediately fixed for HE stain and immunohistochemical assays, respectively, while others were recovered by scalpel and snap-frozen in liquid nitrogen and stored at −80 °C for further mRNA analysis. Rats were anesthetized by chloral hydrate (10% 350 mg/kg, i.p.) before execution.

Rat kidneys were fixed in 4% phosphate-buffered formalin (pH 7.1) for 24–48 h before being dehydrated, embedded in paraffin and serially sectioned (4 μm) for histological analysis. The paraffin sections were stained with hematoxylin (HE) and eosin for conventional morphological evaluation.

2.6. Systolic blood pressure, serum uric acid, serum creatinine and blood urea nitrogen

Systolic blood pressure (SBP) was measured by a tail-cuff sphygmomanometer with an automated system photoelectric sensor (ALC-Non-Invasive Blood Pressure System, Shanghai Alcott Biotech Co., Ltd.) that has been shown to closely correlate with intra-arterial measurement of SBP (Mazzali et al., 2002; Kang et al., 2002). All rats were preconditioned for SBP measurements at least two times before the actual experiment. Serum uric acid concentration was measured by a carbonate phosphotungstate method. Serum creatinine (Cre) and blood urea nitrogen (BUN) were measured using the UV-light photometric measurement according to Kit instructions.

2.7. Immunohistochemistry for NOS-1, TNF-α and TGF-β1

The paraffin sections from rats' renal tissues were prepared and were used for immunohistochemical assays following the protocol: The sections were deparaffinized in xylene, and dehydrated through graded. The sections were incubated with 3% H_2O_2 for 20 min. After rinsing for 5 min × 3 with PBS, the sections were subjected to microwave irradiation in citrate buffer twice to enhance antigen retrieval and preincubated with 5% BSA for 30 min after rinsing for 5 min × 3 with PBS, followed by incubation with a polyclonal antibody to NOS-1, (diluted 1:50) over-night at 4 °C in a humidified chamber. The sections were rinsed for 5 min × 3 with PBS before incubation with biotinylated goat anti-rabbit IgG antibody for NOS-1, for 30 min at 37 °C, and incubated with streptavidin–biotin complex (SABC) for 30 min, and then rinsed for another 5 min × 3 with PBS before reaction with DAB solution for 10 min. The sections were counterstained with hematoxylin and then enveloped with gelatin and observed under an Olympus microscope. All analyses were performed with the observer blind to the experimental protocol. The optical density of the expression of NOS-1 was analyzed by Image J. The mean optical density (MOD) was calculated with 5 high power field optical density for each sections of 4 rats in each group. Immunohistochemistry for TNF-α and TGF-β1 was according to NOS-1 method.

2.8. Analysis of TNF-α and TGF-β1 mRNA by RT-PCR

The levels of TNF-α and TGF-β1 transcripts were evaluated by reverse transcriptase polymerase chain reaction (RT-PCR). Total RNA in renal tissues was extracted by using Trizol (Sigma, USA) according to the manufacturer's instructions. cDNA