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Research Paper

Anti-hyperuricemia effects of allopurinol are improved by *Smilax riparia*, a traditional Chinese herbal medicine

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ABSTRACT

Ethnopharmacological relevance: The roots and rhizomes of *Smilax riparia* are called "Niu-Wei-Cai" in traditional Chinese medicine (TCM). This botanical has been used in treating the symptoms of gout and other hyperuricemic-related conditions in TCM. Allopurinol is a commonly used medication to treat hyperuricemia and its complications. In this study, we evaluated whether *Smilax riparia* could enhance allopurinol's effects by decreasing the serum uric acid level in a hyperuricemic mouse model induced by potassium oxonate.

Materials and methods: We examined the effects of allopurinol (5 mg/kg) administration alone or in combination with *Smilax riparia* saponins (SRS, 500 mg/kg) on the serum uric acid (S_{UA}), serum creatinine (S_{Cr}) and blood urea nitrogen (BUN) levels in a hyperuricemic mouse model. The effects of allopurinol alone or those of allopurinol plus SRS on the XOD activities were measured. Western blot analysis was used to measure the levels of mURAT1, mGLUT9 and mOTA1 in the mice.

Results: Compared with allopurinol alone, the combination of allopurinol and SRS significantly decreased the serum uric acid level and increased the urine uric acid level (both $P < 0.05$), leading to the normalized serum and urine uric acid concentrations. Data on serum and urine creatinine and BUN supported these observations. The attenuation of hyperuricemia-induced renal dysfunction was linked to the inhibition of both serum and hepatic xanthine oxidase (XOD), the down-regulation of renal mURAT1 and mGLUT9, and the up-regulation of mOAT1.

Conclusion: The anti-hyperuricemia effects of allopurinol are improved by *Smilax riparia* co-administration. The results were supported by the measurement of uric acid, creatinine, BUN, XOD, mURAT1, mGLUT9 and mOAT1. Our data may have a potential value in clinical practice in the treatment of gout and other hyperuricemic conditions.

1. Introduction

Hyperuricemia, a metabolic disease, is characterized by high uric acid levels in the blood that precipitate urate crystals in both the kidneys and joints. Hyperuricemia is a well-known risk factor for gout, hypertension and diabetes (Boffetta et al., 2009).

It has been demonstrated that the under-excretion of urate can result in hyperuricemia (Boffetta et al., 2009; Habu et al., 2003). If urate transporters in the kidney, such as urate transporter 1 (URAT1), glucose transporter 9 (GLUT9) and organic anion transporter 1 (OAT1) are dysfunctional, they can impair the excretion of urate, resulting in hyperuricemia (Enomoto and Endou, 2005; Eraly et al., 2008; Habu et al., 2003; Preitner et al., 2009). These protein transporters thus constitute essential targets for the

Abbreviations: BUN, blood urea nitrogen; BuOH, n-butyl alcohol; CMC-Na, carboxymethyl cellulose sodium; Cr, creatinine; EtOH, ethyl alcohol; FEUA, fraction excretion of uric acid; HPLC, high-performance liquid chromatography; HPLC-ELSD, HPLC-evaporative light scattering detector; HR-ESI-MS, high-resolution electrospray ionization mass spectrometry; IR, infrared spectrophotometer; mGAPDH, glyceraldehyde-3-phosphate dehydrogenase; mGLUT9, mouse glucose transporter 9; mOAT1, mouse organic anion transporter 1; mURAT1, mouse urate transporter 1; MS, mass spectrometer; NMR, nuclear magnetic resonance; RP, reversed phase; SRS, *Smilax riparia* saponins; TCM, traditional Chinese medicine; UA, uric acid; UV, ultraviolet spectrophotometer; XOD, xanthine oxidase

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treatment of hyperuricemia. Allopurinol is one commonly used agent to treat hyperuricemia and its complications, such as chronic gout (Pacher et al., 2006); however, allopurinol has been reported to induce adverse effects, including hypersensitivity (Bardin, 2004; Harris et al., 1999). The most serious adverse effect is a hypersensitivity syndrome characterized by fever, skin rash, eosinophilia, hepatitis, and worsened renal function (Cameron et al., 1993; Halevy et al., 2008; Tsai and Yeh, 2010).

Allopurinol is a xanthine oxidase (XOD) inhibitor that prevents the formation of uric acid and reduces the levels of serum uric acid by decreasing purine synthesis (Cameron et al., 1993). Careful allopurinol dosing is critical in patient treatment, and drug adverse events have been attributed to dosing escalation (Dalbeth and Stamp, 2007; McInnes et al., 1981; Vázquez-Mellado et al., 2001). Thus, it is clinically desirable to combine a compound with allopurinol therapy to potentiate the effects of allopurinol and to reduce the required high doses of the drug for better treatment safety (An et al., 2010; Pacher et al., 2006).

Some natural products have been effective in hyperuricemic control and gout treatment (Meng et al., 2014; Nisar et al., 2014; Wang et al., 2012). *Smilax riparia* A. DC, belonging to the genus *Smilax* in the family Liliaceae, is a botanical widely grown in the southern and central parts of China. The roots and rhizomes of *Smilax riparia* have been used in a traditional Chinese medicine (TCM) or Chinese folk drug, “Niu-Wei-Cai”, to treat the symptoms of gout and hyperuricemia-related conditions, including inflammation and some malignancies (Wang et al., 2013; Zhang and Han, 2012). This herb is an edible plant in some regions of China, indicating its safety (Wang et al., 2000). In modern phytochemical studies some components have been identified from *Smilax riparia*, such as phenylpropanoid glycosides, steroidal saponins, and aromatic compounds (Li et al., 2006; Sashida et al., 1992; Sun et al., 2012). We previously reported that *Smilax riparia* saponins (SRS) reduced serum uric acid levels in a mouse model (Wu et al., 2014). In that preliminary study, however, the underlying mechanisms of action were not explored.

In the present study, we investigated whether *Smilax riparia* could potentiate allopurinol's effects on the serum uric acid level in a hyperuricemic mouse model induced by potassium oxonate. We wanted to know whether combining *Smilax riparia* saponins (SRS) with allopurinol would increase the drug's uricosuric activities. We compared the two components alone with a combination treatment on uric acid excretion and on hyperuricemia-induced renal dysfunction. Our results showed that the synergistic effects were linked to the down-regulation of mouse renal URAT1 and GLUT9, the up-regulation of OAT1, and the inhibition of XOD. Our data suggested that using *Smilax riparia* to enhance allopurinol's uricosuric activity have a clinical value in patients suffering from hyperuricemia and its complications.

2. Materials and methods

2.1. Plant material and preparation of Smilax riparia saponins

The roots and rhizomes of *Smilax riparia* were obtained in October 2010 at Tieling (41° 00′ 00″ N; 123° 00′ 00″ E), Liaoning Province, China. There are no protected or endangered animals or plants in this area. The collected plant materials were authenticated as *Smilax riparia* by Prof. Ye Zhou from Tianjin Medical University, Tianjin, China. A voucher specimen (SR-2010-10) was stored at the College of Pharmacy, Tianjin Medical University, Tianjin, China.

The roots and rhizomes of *Smilax riparia* (5.0 kg) were ground and extracted with 90% ethanol (10 × 5 L) at room temperature. The 90% ethanol extract was then concentrated under vacuum to leave a residue, which was suspended in H_2O (3 L) and extracted using petroleum ether, chloroform, EtOAc and BuOH. The BuOH part was processed in a D101 macropore resin and eluted using 30%, 50%, 70% and 90% EtOH. The total saponins of *Smilax riparia* or *Smilax riparia* saponins (SRS) were obtained from the 70% EtOH fraction (SRS, 620 g).

2.2. Isolation and identification of compounds from SRS

SRS was subjected to repeated chromatography on a silica gel column and eluted with $CHCl_3$/MeOH/H_2O or a MeOH–H_2O gradient solvent system. Further purification was performed using reverse phase (RP) preparative HPLC chromatography and other methods, and finally, sixteen saponins were isolated from SRS. On the basis of the analytical methods such as UV, IR, MS and extensive ^1H and ^{13}C NMR spectra analysis and comparison with data in literature (Huang et al., 2006; Kang et al., 2012; Li et al., 2006; Matsuda et al., 2003; Mimaki et al., 2000; Shao et al., 2007; Shen et al., 2012; Zhao et al., 2007), the sixteen saponins were identified as parisyunnanoside A, smilaxchinoside A, protogracillin, parisaponin I, parisyunnanoside C, pseudoproto-pb, riparoside B, parisyunnanoside E, parisyunnanoside D, smilaxchinoside C, paris H, timosaponin J, paris D, timosaponin K, parisvietnaside A and pallidfloside D. The structures of these sixteen saposins are shown in Fig. 1. Their purities were proved to be more than 96% by HPLC analysis.

2.3. HPLC-ELSD analysis of SRS

The multiple-components of the *Smilax riparia* saponins (SRS) were characterized by HPLC-ELSD. The signal from an Alltech ELSD 2000 (Alltech, Deerfield, IL, USA) was transmitted to the Chemstation for processing by an Agilent 35900E A/D interface (Agilent Technologies, Santa Clara, CA, USA). The samples were analyzed using a Zorbax ODS C_{18} column (250 mm × 4.6 mm id, 5 μm) at 35 °C. The binary gradient elution system consisted of water (A) and acetonitrile (B), and separation was achieved using the following gradient program: 0–30 min, 12–15% B; 30–40 min, 15–24% B; 40–50 min, 24–40% B; 50–60 min, 40–100% B. The flow rate was set at 1.4 mL/min and the sample injection volume was 10 μL. The impactor was set to ON mode, the drift tube temperature was 55 °C, and the nebulizer nitrogen gas flow rate was 1.4 L/min.

2.4. Preparation of hyperuricemia mouse model and experimental protocol

Male Kunming mice (20 ± 2.0 g), eight per group, were obtained from China BK Experimental Animal Center (Beijing, China). We carried out all our experiments in compliance with the regulations and guidelines for the care of laboratory animals, and the protocol was approved by the Ethics Committee on Animal Experiments of the Tianjin Medical University. Chloral hydrate anesthesia was used in all surgical procedures to reduce the animals' suffering to the minimum.

Mouse hyperuricemia was induced by potassium oxonate, a uricase inhibitor (Li et al., 2011; Wang et al., 2010). To induce hyperuricemia, each animal was given an intraperitoneal injection of 250 mg/kg potassium oxonate dissolved in 0.9% NaCl solution once daily for 7 consecutive days. Mouse allopurinol and SRS doses were determined based on conversion from human clinical practice and our preliminary studies (Chinese Pharmacopoeia Committee, 2005; Wu et al., 2014). The test agents (allopurinol and SRS), alone or in combination, were dispersed in 0.3% carboxymethyl cellulose sodium (CMC)-Na aqueous solution and were orally administered once daily from day 1 to day 7; the normal control mice were treated with a solvent vehicle. After 7 days of treatment, food was removed from the cages 12 h before the mice were sacrificed. No adverse events were observed in the experimental animals during the 7-day observation period.