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Hypouricemic effect of allopurinol are improved by Pallidifloside D based on the uric acid metabolism enzymes PRPS, HGPRT and PRPPAT

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ABSTRACT

Allopurinol is a commonly used medication to treat hyperuricemia and its complications. Pallidifloside D, a saponin glycoside constituent from the total saponins of *Smilax riparia*, had been proved to enhanced hypouricemic effect of allopurinol based on uric acid metabolism enzyme XOD. In this study, we evaluated whether Pallidifloside D (5 mg/kg) enhanced hypouricemic effect of allopurinol (5 mg/kg) related to others uric acid metabolism enzymes such as PRPS, HGPRT and PRPPAT. We found that, compared with allopurinol alone, the combination of allopurinol and Pallidifloside D significantly up-regulated HGPRT mRNA expression and down-regulated the mRNA expression of PRPS and PRPPAT in PC12 cells (all $P < 0.01$). These results strongly suggest that hypouricemic effect of allopurinol are improved by Pallidifloside D via numerous mechanisms and our data may have a potential value in clinical practice in the treatment of gout and other hyperuricemic conditions.

1. Introduction

Hyperuricemia is one of the most extensive metabolic diseases. It is characterized by high uric acid levels in the blood that precipitate urate crystals in both the kidneys and joints, and is a well-known risk factor for gout, diabetes and hypertension [4]. It has been demonstrated that the under-excretion of urate or increase in the production of urate can result in hyperuricemia [4,10]. There are two pathways for uric acid synthesis or the production of urate. The main pathway is the purine biosynthesis pathway, in which inosine monophosphate is converted into adenine monophosphate or guanine monophosphate. The other pathway is the salvage pathway, in which purine bases from the liver are directly synthesized into purine nucleotides [23]. In this pathway, there are four important uric acid metabolism enzymes, xanthine oxidase (XOD), phosphoribosyl pyrophosphate synthetase (PRPS), hypoxanthine-guanine phosphoribosyl transferase, (HGPRT), phosphoribosyl pyrophosphate amino-transferase (PRPPAT) [18]. If these four important uric acid metabolism enzymes are dysfunctional, they can increase in the production of urate, resulting in hyperuricemia [3].

Allopurinol is one commonly used agent to treat hyperuricemia and its complications, such as chronic gout [21]; however, allopurinol has been reported to induce adverse effects, including hypersensitivity [2,12]. The most serious adverse effect is a hypersensitivity syndrome characterized by fever, eosinophilia, skinrash, hepatitis, and worsened renal function [5,11,24]. Careful allopurinol dosing is critical in patient treatment, and drug adverse events have been attributed to dosing escalation [7,19,25]. 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 [1,21].

Smilax riparia 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 *S. 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 [24,32]. This herb is an edible plant in some regions of China, indicating its safety [19]. We previously reported that Pallidifloside D, a saponin glycoside isolated from the total saponins of *S. riparia* by us, had been proved to enhanced

Abbreviations: PRPS, phosphoribosyl pyrophosphate synthetase; HGPRT, hypoxanthine-guanine phosphoribosyl transferase; PRPPAT, phosphoribosyl pyrophosphate amino-transferase; PRPP, 5-ribose phosphate-1-pyrophosphoric acid; XOD, xanthine oxidase; IMP, inosine monophosphate; AMP, adenine monophosphate; GMP, guanine monophosphate; EtOH, ethyl alcohol; BuOH, n-butyl alcohol; HR-ESI-MS, high-resolution mass spectrometer; NMR, nuclear magnetic resonance; UA, uric acid; NC, normal control.

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hypouricemic effect of allopurinol based on XOD which is one of uric acid metabolism enzyme [14].

In the present study, we investigated whether the synergistic hypouricemic effects of Pallidifloside D and allopurinol were linked to other uric acid metabolism enzymes such as PRPS, HGPRT and PRPPAT. We compared the two components (Pallidifloside D and allopurinol) alone with a combination treatment on mRNA expression of PRPS, HGPRT and PRPPAT in PC12 cells. We found that, compared with allopurinol alone, the combination of allopurinol and Pallidifloside D significantly up-regulated HGPRT mRNA expression and down-regulated the mRNA expression of PRPS and PRPPAT in PC12 cells. These results strongly suggest that hypouricemic effect of allopurinol is improved by Pallidifloside D not only via regulating XOD, but also via regulating other uric acid metabolism enzymes such as PRPS, HGPRT and PRPPAT. Our data may have a potential value in clinical practice in the treatment of gout and other hyperuricemic conditions.

2. Materials and methods

2.1. Reagents and drugs

All chemicals were of analytical grade. Petroleum ether, chloroform, ethyl acetate, and n-butanol were purchased from Tianjin Hengxing Chemical Reagent Company (Tianjin, PR China). Allopurinol and potassium oxonate were purchased from MP Biomedicals China (Shanghai, PR China). Agarose, ethidium bromide and DMSO were obtained from Sigma Chemical Co. (St. Louis, MO, USA). TRIzol reagent was obtained from Invitrogen Life Technologies. Revert AidTM, First Strand cDNA Synthesis Kit (K1621) and PCR Master Mix Kit (K0171) were obtained from Fermentas.

2.2. Preparation of Pallidifloside D

The method of isolating the compound Pallidifloside D (Fig. 1) from *S. riparia* was according to the previous study we reported with a little modification [25]. Briefly, dried roots and rhizomes of *S. riparia* powder were ground and extracted with 95% EtOH. The 95% EtOH extract was suspended in water and extracted with petroleum ether, chloroform, EtOAc and BuOH, respectively. The BuOH layer was concentrated under reduced pressure to give the brown extract. The BuOH fraction was passed through a D101 macropore resin (0.25–0.84 mm) and eluted successively with 35% EtOH, 55% EtOH, 75% EtOH and 95% EtOH, respectively, and the total saponins of *S. riparia* were obtained from the 75% EtOH fraction. Then the total saponin fraction was passed through a Sephadex LH-20 column with MeOH, the fraction eluted with MeOH was passed through silica gel column chromatography with a $CHCl_3$/MeOH/H_2O solvent system (10:1:0.5–5:1:0.5). Finally, the fractions eluted by $CHCl_3$/MeOH/H_2O (9:1:0.5) were subjected to semi-preparative HPLC (10–100 mm) to afford Pallidifloside D. HPLC showed that the purity of this compound was 98.8%.

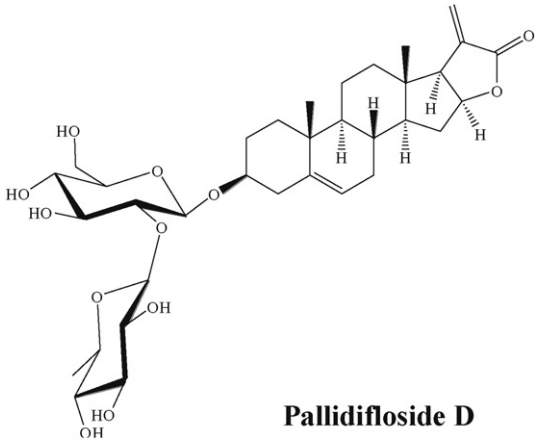

Fig. 1. Structure of pallidifloside D.

2.3. 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 [17,27]. 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 Pallidifloside D doses were determined based on conversion from human clinical practice and our preliminary studies [6,29]. The test agents (allopurinol and Pallidifloside D), 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. The blood was kept clotted for 1 h at 25 °C and was centrifuged at 3000 rpm for 10 min to collect the serum. The serum and urine were kept at −20 °C before testing [8]. The levels of uric acid (UA) in the liver and kidneys were determined by the colorimetric method using commercial kits (Beijing Aoboseng Bioengineering Institute, China), based on the instructions of the manufacturers.

2.4. Cell culture

A stable rat adrenal gland pheochromocytoma cell lines (PC12) was used in our experiments (Tianjin Key Laboratory on Technologies Enabling Development of Clinical, Therapeutics and Diagnostics, Tianjin Medical University, Tianjin, China). The cells were cultured in RPMI-1640 medium (Gibco, Invitrogen Ltd., Paisley, UK) with 10% solcoseryl (Gibco, Invitrogen Ltd., Paisley, UK), 80 U/ml penicillin and 100 U/ml streptomycin. PC12 cells in the exponential growth phase were adjusted to $1 * 10^6$/ml, and 1 ml of cells was plated into 24-well plates with 3 replicates. After adherence, 10 μl of allopurinol or Riparsaponin was added to a final concentration and 10 μl of DMSO was added to the control cells. Each test was repeated 3 times.

2.5. RT-PCR analysis

Semi-quantitative RT-PCR was performed on 4 groups: Control group; Allopurinol group (allopurinol, 5 mg/kg); Pallidifloside D group (Pallidifloside D, 5 mg/kg); Combined group (allopurinol 5 mg/kg plus PallidiflosideD 5 mg/kg). RT-PCR was performed as described previously [16]. The gene-specific PCR primer sequences, annealing temperatures and product lengths were shown in Table 1. PCR products were electrophoresed on 1.2% agarose gels, visualized with the Bio-Rad ChemiDoc XRS Gel Documentation system, and then quantified using Bio-Rad Quantity One 1-D analysis software. The relative quantitation of the PCR products was performed after normalization to β-actin mRNA levels.

2.6. Statistical analysis

Datawere presented as mean ± standard error (S.E.). One-way analysis of variance (ANOVA) was applied to estimate the significance level. A value of $P < 0.05$ was considered statistically significant. Statistical