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Effect and mechanism of dioscin from *Dioscorea spongiosa* on uric acid excretion in animal model of hyperuricemia

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ABSTRACT

Ethnopharmacology relevance: Dioscin, a spirostane glycoside, the rhizoma of *Dioscorea septemloba* (Diocoreacea) is used for diuresis, rheumatism, and joints pain. Given the poor solubility and stability of Dioscin, we proposed a hypothesis that Dioscin's metabolite(s) are the active substance(s) *in vivo* to contribute to the reducing effects on serum uric acid levels.

Aim of the study: The aim of this study is to identify the active metabolite(s) of Dioscin *in vivo* and to explore the mechanism of its antihyperuricemic activity.

Materials and methods: After oral administration of Dioscin in potassium oxonate (PO) induced hyperuricemia rats and adenine-PO induced hyperuricemia mice models, serum uric acid and creatinine levels, clearance of uric acid and creatinine, fractional excretion of uric acid, and renal pathological lesions were determined were used to evaluate the antihyperuricemic effects. Renal glucose transporter-9 (GLUT-9) and organic anion transporter-1 (OAT-1) expressions were analyzed by western blotting method. Renal uric acid excretion was evaluated using stably urate transporter-1 (URAT-1) transfected human epithelial kidney cell line. Intestinal uric acid excretion was evaluated by measuring the transcellular transport of uric acid in HCT116 cells.

Results: In hyperuricemia rats, both 25 and 50 mg/kg of oral Dioscin decreased serum uric acid levels over 4 h. In the hyperuricemia mice, two weeks treatment of Dioscin significantly decreased serum uric acid and creatinine levels, increased clearance of uric acid and creatinine, increased fractional excretion of uric acid, and reduced renal pathological lesions caused by hyperuricemia. In addition, renal GLUT -9 was significantly down-regulated and OAT-1 was up-regulated in Dioscin treated hyperuricemia mice. Dioscin's metabolite Tigogenin significantly inhibited uric acid re-absorption *via* URAT1 from 10 to 100 μM. Diosgenin and Tigogenin increased uric acid excretion *via* ATP binding cassette subfamily G member 2 (ABCG2).

Conclusion: Decreasing effect of Dioscin on serum uric acid level and enhancing effect on urate excretion were confirmed in hyperuricemia animal models. Tigogenin, a metabolite of Dioscin, was identified as an active substance with antihyperuricemic activity *in vivo*, through inhibition of URAT1 and promotion of ABCG2.

1. Introduction

Uric acid, a substance with poor solubility, is an end product of purine metabolism in humans. Hyperuricemia, defined as high levels of blood uric acid, is recognized as a risk factor of gout, cardiovascular disease, hypertension, diabetes, and chronic kidney disease (Kumar et al., 2016). The increasing trend of hyperuricemia in urban areas of China has been noted in the past decades. Epidemiological investigation revealed that the prevalence of hyperuricemia were 15.0% in men and 7.3% in women in China (Yu et al., 2016), especially in the Mongolian area, where the prevalence was 17.7% in men (You et al., 2014).

Hyperuricemia in humans can be generally categorized into two pathophysiologic groups, uric acid overproduction and underexcretion. In uric acid formation, xanthine dehydrogenase (XOD) is a key enzyme involved in conversion of xanthine and hypoxanthine to uric acid (Maiuolo et al., 2016). Conditions such as over-consumption of purine-rich foods and genetic enzyme deficiency may contribute to over-production of uric acid and hyperuricemia. Allopurinol, an inhibitor of XOD (Pacher et al., 2006), is commonly used for treatment of uric acid overproduction to reduce uric acid level in clinic. However, only about 40% of patients treated with allopurinol achieved the therapeutic goal of serum uric acid levels less than 6 mg/dl (Tsai et al., 2017). Newer

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medications such as febuxostat were slightly more efficient, but much more expensive. Long-term treatment with XOD inhibitors such as allopurinol increases the risk of adverse effects such as renal and hepatic toxicity, myelosuppression, Stevens Johnson syndrome and toxic epidermal necrolysis (Halevy et al., 2008).

Genome-wide association studies were consistent with the fact that about 90% of hyperuricemic patients suffer from the underexcretion-type (Ichida, 2009). The kidney and the intestine play a major role in uric acid excretion, with more than 70% of urate excretion through the renal pathway (Lipkowitz, 2012). Renal handling of uric acid homeostasis is achieved through a complex interplay of re-absorption and secretion following the classic four-component model of filtration, presecretory re-absorption, secretion and postsecretory re-absorption. Multiple proteins have been identified to be involved in renal transport of uric acid. Among the transport proteins, the best characterized are apical urate transporter 1 (URAT1) and basolateral glucose transporter 9 (GLUT9), both of which were demonstrated with effects on uric acid levels (Bobulescu and Moe, 2012). Uricosuric agents, such as benzbromarone, reduce plasma concentrations of uric acid by blocking renal tubular reabsorption (Iwanaga et al., 2005). Uric acid transporters present attractive targets for new drug research and development of hyperuricemia.

About one-third of uric acid is excreted from the intestines in human (Matsuo et al., 2014). ATP-binding cassette subfamily G member 2 (ABCG2) plays an important role in this process. ABCG2 is a high-capacity urate exporter that mediates renal and intestinal urate excretion, and regulates human serum uric acid levels (Bhatnagar et al., 2016). Over-expression of ABCG2 was observed in the intestines in nephrectomy rats, suggesting a complementary role of intestines in uric acid excretion through ABCG2 in animals with poor kidney function such as in end stage renal disease (Yano et al., 2014). Therefore, ABCG2 could be a potential target for a new generation of urate-lowering medications that could be used in renal failure patients. These new medications could also decrease the risk of adverse effects, such as urolithiasis caused by the traditional uricosuric medications (Ichida et al., 2012). To date, the literatures about ABCG2 enhancers target to treat hyperuricemia are very limited.

In Traditional Chinese Medicine (TCM), the rhizoma of *Dioscorea spongiosa* (Diocoreacea) is used for diuresis, rheumatism, and joints pain (Committee, 2015). Frequency analysis of TCM indicated rhizoma of *Dioscorea spongiosa* was the second generic herb medicine in clinic hyperuricemia prescription (Lu et al., 2016). Dioscin, a spirostane glycoside, is the major constituent in rhizoma of *Dioscorea spongiosa*. Dioscin has been reported to have anti-fungal, anti-viral, hepatoprotective, anti-cancer (Zhao et al., 2016) and anti-hyperuricemic pharmacological activities. *D. spongiosa* extract and Dioscin showed serum uric acid decreasing effects in potassium oxonate (PO) induced hyperuricemic mice (Su et al., 2014). However, the mechanism remains unclear. From a drug metabolism perspective, as a three-glucose substituted glycoside, Dioscin is difficult to pass through intestinal blockage and keep original structure after liver metabolism, which indicated that Dioscin is not a direct active compound for hyperuricemia.

The study of this paper consists of two parts. First, to evaluate the urate reducing effect and urate excretion enhancing effect of Dioscin in hyperuricemia animal models. Second, to test our hypothesis that metabolites of Dioscin, instead of Dioscin itself, are the active constituents that increase the uric acid excretion. We are the first group to identify Tigogenin, a derivative from Dioscin, as an active substance with uric acid reducing effect, through inhibition of URAT1 and promotion of ABCG2.

2. Materials and methods

2.1. Materials

The dried rhizome of *Dioscorea spongiosa* J.Q. Xi, M. Mizuno *et* W.L. Zhao were bought from Anguo medicinal market, Hebei province, China and identified by one of authors Dr. Yi Zhang. The voucher specimen was deposited at the Institute of Traditional Chinese Medicine, Tianjin University of TCM (No. 20151015). Dioscin was obtained from 70% EtOH extract of *D. spongiosa* by open column and preparative high performance liquid chromatography as described before and the structure was identified by chemical and spectroscopic methods (Zhang et al., 2016). Diosgenin (150911), Sarsasapogenin (151009) and Tigogenin (150211) were purchased from Yifang Science and Technology Co. Ltd, China, and all of the contents were more than 98%.

2.2. Animals

All of animal experiments plan were approved by Science and Technological Committee and the Animal Use and Care Committee of TUTCM. Experiment was carried out in Sprague-Dawley (SD) rats (weight 280–300 g, Vital River Laboratory Animal Technology Co. Ltd., Beijing China) and Kunming mice (weight 25–30 g, Vital River Laboratory Animal Technology Co. Ltd., Beijing China), both of which were acclimated for 1 week before the experiments. All animals were allowed to eat a standard diet and drink *ad libitum*, and adapted to the experimental conditions at 22 ± 2 °C, humidity 60 ± 5% with a fixed 12 h artificial light period.

2.3. PO induced hyperuricemia rats

After 1 week adaption, male SD rats were randomized into six groups (n = 8). Except normal group, rats of other groups were induced by PO (Sigma Chemical Co., Santa Cruz, USA), a urate oxidase inhibitor, to get acute hyperuricemia. Briefly, rats were intragastrically administrated with PO (300 mg/kg body weight) 1 h after the treatment of sample and positive control (Probenecid, Sigma-Aldrich Co., MO, USA). PO and tested sample were suspended in 5% acacia solution, and oral administration volumes were 10 ml/kg bodyweight, while the control group received 5% acacia water solution with same volume. After administration of PO, blood samples (*ca.* 0.6 ml) were collected from infraorbital venous plexus under ether anesthesia at 1 h, 2 h and 4 h and then were centrifuged at 3500 g for 10 min. Supernatant serum was collected and stored at −20 °C until assayed. Perchloric acid (0.3 M) 180 μl was added to 20 μl serum sample, vortex for 10 s and placed in ice-water bath for 30 min, then, centrifuged for 10 min at 10,000 g under 4 °C. Supernatant was neutralized with 0.8 M Na_2HPO_4 solution, and added equal volume acetonitril. After centrifuged for 10 min at 10,000 g under 4 °C, supernatant was stocked for Ultra Performance Liquid Chromatography (UPLC) analysis of uric acid level.

Waters ACQUITY UPLC system H Class (Waters Co. Ltd. USA) with a quaternary solvent manager was used to determine rat serum uric acid (SUA) levels. Column: ACQUITY UPLC BEH Amide (1.7 μm, 2.1 × 50 mm), detect wavelength: 285 nm, mobile phase: 0.1% acetic acid water solution/ acetonitril = 10/90, v/v, flow rate: 0.3 ml/min. The method was validated in terms of specificity, linearity and reproducibility.

2.4. Adenine and PO induced hyperuricemia mice

Hyperuricemia mice were induced by oral administration of uricopoiesis promoter adenine and uricase inhibitor potassium oxonate. Briefly, male Kunming mice were intragastrically administrated with adenine (75 mg/kg/day, volume 20 ml/kg) and PO (200 mg/kg/day, volume 20 ml/kg), and one hour later, samples fixed in 5% acacia were orally administrated with a volume of 5 ml/kg. The same treatments were conducted every a day for 2 consecutive weeks. Blood sample collection and serum uric acid level analysis were as same as "PO induced hyperuricemia rats". Urine sample collection, clearance of uric acid (Cur) and creatinine (Ccr), fractional excretion of uric acid (FEUA)