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Lithospermic acid as a novel xanthine oxidase inhibitor has anti-inflammatory and hypouricemic effects in rats

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ABSTRACT

Lithospermic acid (LSA) was originally isolated from the roots of *Salvia mitiorrhiza*, a common herb of oriental medicine. Previous studies demonstrated that LSA has antioxidant effects. In this study, we investigated the in vitro xanthine oxidase (XO) inhibitory activity, and in vivo hypouricemic and anti-inflammatory effects of rats. XO activity was detected by measuring the formation of uric acid or superoxide radicals in the xanthine/xanthine oxidase system. The results showed that LSA inhibited the formation of uric acid and superoxide radicals significantly with an IC_{50} 5.2 and 1.08 μg/ml, respectively, and exhibited competitive inhibition. It was also found that LSA scavenged superoxide radicals directly in the system β-NADH/PMS and inhibited the production of superoxide in human neutrophils stimulated by PMA and fMLP. LSA was also found to have hypouricemic activity on oxonate-pretreated rats in vivo and have anti-inflammatory effects in a model of gouty arthritis. These results suggested that LSA is a competitive inhibitor of XO, able to directly scavenge superoxide and inhibit superoxide production in vitro, and presents hypouricemic and anti-inflammatory actions in vivo.

1. Introduction

Gout is a clinical syndrome of tissue damage induced by a chronic metabolism disorder, which is associated with increased uric acid level in blood. The incidence of hyperuricemia and gout increased gradually with the improvement of living standards in recent years [1]. A study in Shanghai China showed that the incidence of hyperuricemia in males and females was 14.2 and 7.1%, respectively [2]. In purine metabolism, xanthine and hypoxanthine are oxidized into uric acid by activity of xanthine oxidase. It is demonstrated that overproduction and/or under excretion of uric acid lead to hyperuricemia. Excessive alcohol intake and a number of medications that decreased excretion of urate can also cause hyperuricemia [3]. Deposition of urate monohydrate crystals in joints and kidneys results in gouty arthritis and uric acid nephrotithiasis [4]. The increased risk of hyperuricemia has been also linked with the development of hypertension, hyperlipidaemia, cancer, diabetes, and obesity [5].

Compounds that enhance the excretion of uric acid or inhibit uric acid biosynthesis, or have anti-inflammatory actions are generally used for the treatment of gout [6]. Allopurinol, the most common and perhaps only inhibitor of XO, is used in clinical practices. But allopurinol has severe adverse effects in some patients including nepatilis, nephopathy, and allergic reactions [7]. Thus, the development of new, safer and more effective hypouricemic agents is highly warranted. Previous studies have demonstrated that lithospermic acid (LSA), a major component in 'Danshen' (*S. miltiorrhiza*) (Fig. 1), can elicit endothelium-

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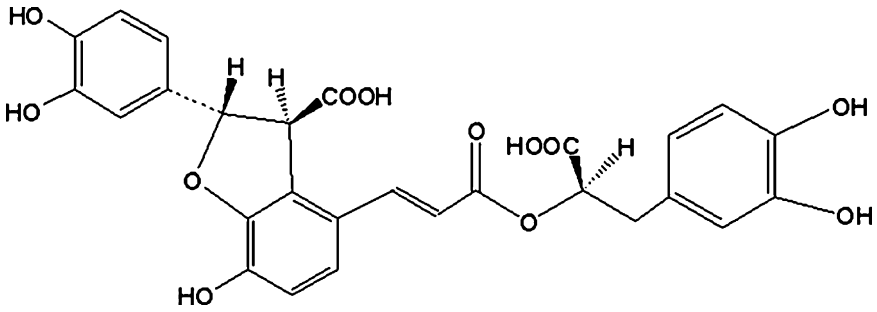

Fig. 1. Chemical structure of LSA.

dependent vasodilatation [8], lower blood pressure [9], and produce antioxidant effects [10]. However, no study has been reported on the inhibitory effects of LSA on XO. Based on our previous study about xanthine oxidase (XO), we considered that the antioxidant effect of LSA may be associated with the inhibitory effect of XO. So, in this study, we reported the inhibitory effect on xanthine oxidase and hypouricemic effect as well as on anti-inflammatory effect in a model of gouty arthritis by LSA.

2. Materials and methods

2.1. Materials

Phenzazine methosulfate (PMS), β-nicotinamide adenine dinucleotide (NADH), nitroblue tetrazolium chloride (NBT), monosodium urate crystal, phorbol-12-myristate-13-acetate (PMA), *N*-formyl-methionyl-leucyl-phenylalanine (fMLP), superoxide dismutase (SOD), potassium oxonate and allopurinol were purchased from Sigma and Aldrich Chemical Co. (St. Louis, MO, USA). XO was purchased from Roche Ltd. (Shanghai, China) and other analytical reagents were produced in Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Lithospermic acid was provided by professor Zhu (State key Laboratory of Drug Research, Institute of Materia Medica, Shanghai Institute for Biological Science, China).

2.2. Animals

Adult male albino rats of Wistar strain weighing about 220 ± 60 g were purchased from the Laboratory Animal Center (Shanghai, China) and maintained on a 12-h light/dark cycle in a temperature- and humidity-controlled room for 1 week prior to the experiment. All procedures were carried out in accordance with Chinese legislation on the use and care of laboratory animals and approved by the respective university committees for animal experiments.

2.2.1. Detection of inhibitory activity of XO by measuring the formation of uric acid

The in vitro inhibitory activity of XO was assayed by spectrophotometry at 295 nm under aerobic conditions as described previously [11,12,13]. The reaction mixture contained 80 mM sodium pyrophosphate buffer (pH 7.5), 120 mM xanthine and 0.1 U XO, with or without LSA (100 μl, in 2.0, 4.0, 8.0 and 16 μg/ml). Allopurinol was used as a positive control. The reaction was started by addition of XO. The formation of uric acid was detected by UV absorption increments at 295 nm.

2.2.2. Detection of inhibitory activity of XO by measuring the formation of superoxide radicals

The reaction mixtures contained the same proportion of components as those in the measurement of uric acid. Superoxide radicals (SOR) were detected by the reduction of nitroblue tetrazolium (NBT) at 100 μM, and then by spectrophotometry at 560 nm [14].

To evaluate the type of inhibition, this procedure was repeated with different concentrations of xanthine (60, 120, 180, 240 and 300 μM). For making the Lineweaver-Burk plot, LSA was assayed for measuring the inhibitory activity of XO at the concentrations of 1.0, 2.0, 4.0, and 8.0 μg/ml. IC_{50} values were calculated by linear regression analysis.

2.2.3. Direct SOR scavenging effect of LSA

SOR can be produced by enzymatic system and non-enzymatic system. The non-enzymatic system include the β-NADH/PMS, the FRAP [15] and DPPH, etc. [16]. In this study, the anti-radical activity of LSA was determined according to previous reports [17,18] in which SOR were generated in the system β-NADH/PMS (NBT 25 μM, PMS 10 μM, and β-NADH 156 μM). The reaction was conducted at room temperature for 2 min with or without LSA, and initiated by the addition of PMS (SOD as positive control). The anti-radical activity was evaluated from the decreased value of absorption at 560 nm, and calculated by the following equation: % scavenging effect = $[(A_0 - A_1)/A_0] \times 100\%$., where A_0 is the absorption of the control (without LSA) and A_1 is the absorption of the mixture containing LSA.

2.2.4. Hypouricemic activity in rats pretreated with uricase inhibitor potassium oxonate

Sixty rats were equally divided into six groups: group 1 served as the control, which received normal saline; group 2 served as hyperuricemic control, which received potassium oxonate (300 mg/kg body weight, i.p.) [19,20]; groups 3–5 received oral LSA (10, 20, and 30 mg/kg body weight, respectively); and group 6 received the reference drug allopurinol orally (10 mg/kg body weight). Group 3–6 animals were injected i.p. with potassium oxonate