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Effects of Lowering Uric Acid by Caulis Tinosporae Compound

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Published Date: April 27, 2018

ABSTRACT

Introduction: An excess of uric acid level in the body can lead to the formation of crystals of monosodium urate in the joints, resulting in gouty arthritis. Xanthine oxidoreductase (XOR) is the key enzyme in the uric acid production. Allopurinol and febuxostat were common clinical XOR inhibitors to serve as auric acid-lowering agent. However, there are adverse effects of allopurinol such as hepatitis, nephropathy and serious allergic reactions as well as of febuxostat for cardiovascular events. It would be highly desired to search for new agents as alternatives of XOR inhibitors. Chinese Tinospora cordifolia stem extracts (Caulis tinosporae) have been used to relieve joint pain and swelling of gout in traditional Chinese medicine. However, the anti-hyperuricemic effects of Caulis tinosporae remains poorly documented. Therefore, the effects of lowering the serum uric acid levels for Caulis tinosporae in the hyperuricemic rats were assessed.

Methods: Sprague-Dawley male rats were randomly divided into four groups (6 rats per group). In group 1, the normal group, each animal received only water as vehicle. In group 2, the hyperuricemic group, potassium oxonate (PO, 250 mg/kg) as an uricase inhibitor to induce

hyperuricemia, was administrated intraperitoneally. In groups 3 and 4, each animal received PO 1 h before administration of 625mg/kg test compound (Caulis tinosporae) and 5 mg/kg reference drug (allopurinol), respectively. The serum uric acid levels were analyzed by high performance liquid chromatography (HPLC) method.

Results: Caulis tinosporae significantly lowered the elevated uric acid level from 2.39 ± 0.83 mg/dl to 1.2 ± 0.16 mg/dl ($p < 0.05$).

Conclusion: With fewer adverse effects than western medicine, Chinese herb Caulis tinosporae might be a suitable candidate for long-term control of hyperuricemia.

Key words: Caulis tinosporae, Gout, Hyperuricemia, Tinospora cordifolia

INTRODUCTION

Uric acid is the final oxidation product of purine metabolism in humans. Hyperuricemia, defined as abnormally high levels of uric acid, is a worldwide metabolic disorder caused by a purine metabolic disturbance that leads to excessive production of uric acid in the body [1]. With economic development in a country, people with changes in the daily diet, an increased rate of high-purine diets would specifically increase the prevalence of hyperuricemia. Gout, a common rheumatic disease worldwide, is an inflammatory syndrome with pathogenic basis of hyperuricemia, which usually exceeds 390 μmol/L or 6.5 mg/dL of uric acid level in the serum and thereby leads to crystal formation of monosodium urate (MSU) in various tissues, especially in the joints [2]. MSU crystals are potent proinflammatory stimuli and can initiate an innate immune inflammatory response in the joints with a key component of neutrophils migrating to the crystals' surface to cause acute gouty arthritis [2]. Identification of MSU in the joints is considered the gold standard for diagnosis of gout. In clinical practice, gout has been considered as a single disease with different stages, including acute gout (episodes of acute intensely painful and inflammatory arthritis), inter critical gout (the intervals between attacks of gout), and persistent clinical manifestations of chronic gout [3].

Mechanisms of Hyperuricemia

Compared with women, men have a four- to nine fold increased risk of developing gout [4]. Recent studies show that major urate loci are genetic variants of SLC2A9 and ABCG2, which encode secretory uric acid transporters. The SLC2A9 locus involves in renal and gut excretion of uric acid; whereas ABCG2 locus involves primarily in extra-renal uric acid under-excretion [5]. ABCG2 export dysfunction decreases intestinal urate excretion [6]. Apart from hereditary disorders associated with decreasing uric acid excretion and increased purine metabolism, the main causes of gout are conditions of high-level uric acid production mostly due to purine-rich food, alcohol consumption, and overweight [7]. That is way the control of uric acid production has been widely considered as a key factor in the prevention and treatment of these diseases.

Western Hypouricemic Agents

Uricosuric drugs (i.e., probenecid and benzbromarone) could reduce the serum uric acid concentration by increasing the renal excretion of uric acid. However, they may lead to renal tubular aggregation of urate crystals and induce renal damage. Xanthine oxidoreductase (XOR), a cytoplasmic molybdenum-containing oxidoreductase, is the key enzyme in the catabolism of purines and uric acid production. XOR inhibitor (e. g., allopurinol) could decrease serum uric acid by inhabiting uric acid synthesis. XOR can also act as a source of reactive oxygen species (ROS) which may be involve in the pathogenesis of various degenerative diseases [8-10]. That is why the inhibition of XOR activity may decrease the level of uric acid and ROS production, and can result in anti-hyperuricemic and anti-oxidative effects. Acute gout is traditionally treated with nonsteroidal anti-inflammatory drugs, corticosteroids, and colchicine, which have been demonstrated to be effective [11].

Allopurinol

Allopurinol, an analogue of hypoxanthine, is hydrolyzed by XOR to produce oxypurinol, which binds tightly to the reduced molybdenum enzyme, and thus inhibits uric acid synthesis [12]. Allopurinol is the traditional XOR inhibitor under the clinical application and has served as a dominant uric acid-lowering agent in the past three decades [13]. However, there are concerns over some severe adverse effects such as hepatitis, fulminant hepatic failure in rare cases, interstitial nephritis and nephropathy, gastrointestinal reactions, bone marrow depression and serious allergic reactions like Steven-Jonson syndrome and toxic epidermal necrolysis, thus limiting the clinical use of allopurinol [12].

Febuxostat

Febuxostat is a non-purine selective inhibitor of XOR and is structurally distinct from allopurinol. It provides greater effective blockade of uric acid and ROS production and has fewer side effects than allopurinol, and thus can be given as an alternative to allopurinol in patients who require urate lowering but cannot tolerate allopurinol [13]. However, cases of severe febuxostat hypersensitivity skin reactions are reported [14]. Besides, the incidence of cardiovascular events was slightly higher with febuxostat than with allopurinol, reaching statistical significance with hazard ratio for overall mortality, 1.22 (95% CI, 1.01 to 1.47) and hazard ratio for cardiovascular death, 1.34 (95% CI, 1.03 to 1.73). Therefore, febuxostat should be cautiously used in the patients with cardiovascular disease [15, 16].

Colchicine

Colchicine has multiple mechanisms of anti inflammatory effects and has been used as one of the first-line therapies for treating and preventing acute gouty arthritis. Colchicine prevents microtubule assembly and thus disrupts microtubule-based inflammatory cell chemotaxis, generation of leukotrienes and cytokines, inflammasome activation and phagocytosis. Many of

these cellular processes can be found in gout involving acute and chronic inflammation, suggesting clinical efficacy of colchicine in gout-associated conditions [17]. The adverse events of colchicine include diarrhoea, nausea or vomiting, which incidence could be reduced by low-dose colchicine [18].

Chinese Arthromyodynia Medicine

Current XOR-inhibitor drugs such as allopurinol and febuxostat may have significant adverse effects. Therefore, there has been great effort to develop new drugs with less or no toxicity for the long-term treatment or prevention of the hyperuricemia-related diseases. Unlike potentially toxic modern medicines, Chinese medicines and herbs are more natural formulations, which may have little side effects and could be more suitable for long-term control of hyperuricemia. In traditional Chinese medicine, hypouricemic agents belong to the arthromyodynia disease category. Among them, the bulbs of Pseudobulbus Cremastraeseu Pleiones (Shan CiGu) contain colchicine. The Glabrous Greenbrier Rhizome (Tu Fu Ling) contains an active ingredient of astilbin, which has been associated with increased renal blood flow and has shown anti-inflammatory and analgesic actions. The seven-lobed yam rhizome, Jobstears seed, Radix Achyranthis Bidentatae (Ox-Knee Root) and Salvia miltiorrhiza Bge (Danshen) could enhance uric acid excretion, improving microcirculation by reduction of platelet accumulation and anticoagulant function. The actions of these ingredients contribute to reduction in serum uric acid levels [19].

Chuanhu Anti-Gout Mixture

The Chuanhu anti-gout mixture has been used for many years in the treatment of acute gout in Chinese traditional medicine. It is composed of 9 herbs, mainly Caulis Lonicerae (Ren Dong Teng), Rhizoma Polygoni Cuspidati (HuZhang), and Discorea Nipponica Makino (ShanChangShan). In a double-blind and non-inferiority study conducted in China, the Chuanhu anti-gout mixture was non-inferior to colchicine for the treatment of acute gouty arthritis, but was superior to colchicine in lower incidence of adverse events and its protection of kidney and renal function, suggesting an alternative choice for the treatment of acute gouty arthritis [20].

Tinospora cordifolia (Guduchi)

The plant Tinospora cordifolia (also known as Guduchi) has been used for the treatment of fever, jaundice, rheumatism, urinary disorder, skin diseases, diabetes and anemia in Chinese medicine. Guduchi is well known for its immunomodulatory activity. The Guduchi purified protein from dry stem powder extract shows lymphoproliferative properties and displays enhanced phagocytosis of yeast cells by macrophages, demonstrated by lymphocyte proliferation and macrophage activation assays [21]. Furthermore, three bioactive compounds (N-formylannonain, 11-hydroxymustakone and yangambin) are found in the highest quantity in the stem extracts of Tinospora cordifolia by using a simple, normal phase high performance thin-layer chromatography (HPTLC) method. The yangambin is active against KB cells (human

oral squamous carcinoma cell lines), however, N-formylannonain and 11-hydroxymustakone are found active for immunomodulatory activity [22].

Caulis tinosporae

Chinese Tinospora cordifolia stem extracts (Caulis tinosporae) have been used to help relieving joint pain, edema or painful swelling of gout in traditional practice of Chinese medicine. However, the anti-hyperuricemic effectiveness of Caulis tinosporae remains poorly documented. Therefore, the article presents the study to assess the effects of Caulis tinosporae herbal compounds on lowering the serum uric acid levels in the potassium oxonate-induced hyperuricemic rats.

Experimental study

Animals

A total of 30 male Sprague-Dawley rats (or Wistar rats) were obtained from the animal house of Chi Mei Medical Center, Taiwan. They were fed with a commercial laboratory diet and allowed food and water ad libitum for an acclimatization period of 1 week prior to the experiment. All animals were maintained on a 12 h/12 h light/dark cycle and the temperature and humidity were kept at 25 ± 1°C and 50%, respectively. They were handled according to the recommendation of the local and national ethic committees.

Animal model of hyperuricemia in rats

Experimentally-induced hyperuricemia in rats was performed by inhibition of uricase with potassium oxonate (PO), an uricase inhibitor. Briefly, 250 mg/kg PO dissolved in 0.9% saline solution was administrated intraperitonially to each animal 1 h before oral administration of test compounds [23]. Then the anti-hyperuricemic effects of Caulis tinosporae compounds were assessed.

Experimental design

Rats were randomly divided into four equal groups (6 rats per group). In group 1, the normal group, each animal received only water as vehicle. In group 2, the hyperurecemic control group, uricase inhibitor PO (250 mg/kg) was administrated intraperitoneally. In groups 3 and 4 each animal received the same dose of PO 1 h before administration of 625mg/kg test compounds (Caulis tinosporae) and 5 mg/kg allopurinol, respectively. The freshly prepared samples were administrated to corresponding groups by oral gavage once a day for 2 weeks.

Sample preparation

At the end of the experiment, rats were anesthetized between 09.00 and 10.00 am. Blood was taken from the abdominal aorta 1 h after the test compound administration. Serum was obtained by centrifuging blood sample at 6000 g for 10 min. For the high performance liquid chromatography (HPLC) analysis, the serum was filtered using SPARTAN 13/0.45 RC, Watman (USA). The sera were stored at −20°C until use.

Uric acid determination

The serum uric acid levels were analyzed by HPLC method using a system supplied by Waters Associates (Northwich, Cheshire) which consisted of a Waters 515 pump, Waters 717 plus Autosampler, Waters 2487, Dual λ Absorbance Detector. The mobile phase was a mixture of 100 mM KH_2PO_4 (pH 3.5): Methanol (97:3, v/v). Separations were performed on a C-18 column (Perfectsil Target ODS-3, 5 μm particle size, 250 × 4.6 mm) with a C-18 guard column (Perfectsil Target ODS-3, 5 μm particle size, 10 × 4 mm). The effluent was monitored by UV detection at 290 nm at a flow rate of 1.0 ml/min. Standard solutions of uric acid in the range of 10 to 1000 μM/L were prepared in mobile phase. Serum uric acid concentrations were expressed as μM/L. 6- Mercaptopurine (1 mM) was used as the internal standard.

Statistical analysis

All the samples and standards were run in duplicate and the results were expressed as means ± standard deviation (SD). The statistical comparison of each experimental group with control group was performed by Independent-sample t test using SPSS computer program. The probabilities of 5% or less ($p \leq 0.05$) were considered significant.

Results

As shown in Figure 1, the uric acid level in normal rats was only 1.49 ± 0.21(mg/dl). Intraperitoneal injection of PO (250 mg/kg) to normal rats significantly increased the serum uric acid levels, and reached to 2.39± 0.83(mg/dl) at the end of the experiment. Oral administration of test compound (TC) and the reference drug allopurinol lowered the serum uric acid levels in the hyperuricemic rats, reaching statistically significant difference with p values of less than 0.05 and 0.01 respectively.

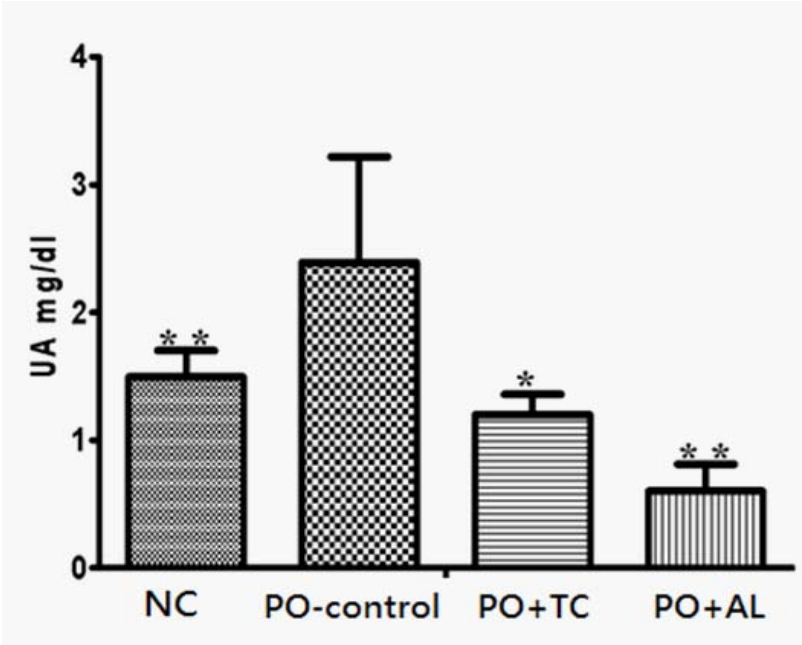

Figure 1: Effects of lowering serum uric acid level for test compound and allopurinol. All values are expressed as mean ± SD. Conversion factor for unit for uric acid in mg/dL to μmol/L: ×59.48. Independent-sample t test was used for statistical significance assessment in comparison to PO-control group.

* indicates p≤0.05;

** indicates p≤0.01. UA: uric acid; NC: normal control animal with H2O ingestion (UA of NC: 1.49±0.21); PO: potassium oxonate-administered control animal (UA of PO-control: 2.39±0.82); TC: test compound of Caulis tinosporae administered with 625mg/kg (UA of PO+TC: 1.2±0.16); AL: allopurinol administered with 5 mg/kg (UA of PO+AL: 0.6±0.21).

DISCUSSION

Hyperuricemia is a metabolic disorder which may play an important role in the development of gout and oxidative stress related disease such as cancer and cardiovascular diseases [24, 25]. At the present, allopurinol (a purine analogue) and febuxostat (not a purine analogue), which cause inhibition on XOR, are the preferred drugs with clinical application to lower uric acid production [12, 15]. Due to some serious side effects of allopurinol and some concern of skin and cardiovascular events for febuxostat, many attempts are made to find safer alternatives particularly from natural sources [25-27].

Natural products with medicinal value are gradually gaining importance in clinical research due to their well-known property of low incidence of adverse reactions. Tinospora cordifolia commonly named “Guduchi” is known for its application in the treatment of various diseases

in the traditional medicine as described in ayurvedic literature. The water and ethanol extracts of stems of Tinospora cordifolia and T. sinensis could inhibit immunosuppression produced by cyclophosphamide [28]. Different ayurvedic formulations containing Guduchi have been found to be active in immunomodulation and anti-inflammatory activities [29]. Furthermore, aqueous extract of Tinospora cordifolia stem parts could directly act on peritoneal macrophages to boost the non-specific host defenses of the immune system, which could ameliorate the intoxicated effect of carbon tetrachloride on the bacterial killing capacity of peritoneal macrophages of mice [30]. Recently, the discovery of active components from the plant and their biological function in disease control has led to active interest in the plant across the globe [31]. In the present study, the orally administered herb compound (Caulis tinosporae) demonstrated significant effects of lowering the uric acid levels compared to hyperuricemic control rats. The mechanism of uric acid-lowering effects for Caulis tinosporae needs to be furthered investigated.

CONCLUSION

Hyperuricemia, characterized by abnormally high levels of uric acid, is the main pathogenic basis of gout. Control of uric acid production is a key factor in the prevention and treatment of gout. XOR-inhibitor drugs such as allopurinol and febuxostat are currently available uric acid-lowering agents with clinical application. However, they may have significant adverse effects. Due to some serious side effects of allopurinol and potential concerns of cardiovascular events for febuxostat, many attempts are made to find a safer alternative particularly from natural sources. Chinese Tinospora cordifolia stem extracts (Caulis tinosporae) have been used to help relieving joint pain including painful swelling of gout in traditional practice of Chinese medicine. In the present study, orally administered Caulis tinosporae prepared in herbal test compound was used to treat experiment-induced hyperuricemic rats and showed that Caulis tinosporae and allopurinol both could significantly lower the uric acid levels. Caulis tinosporae with little side effect might be a potential candidate to attenuate and or to reverse hyperuricemia for long-term maintenance therapy.

ACKNOLEDEMENT

The study was supported by a research grant (CMFHR10107) from Chi Mei Medical Center in Tainan City, Taiwan. No potential conflicts of interest were disclosed.

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