Hypouricemic Effects of Phenylpropanoid Glycosides Acteoside of *Scrophularia ningpoensis* on Serum Uric Acid Levels in Potassium Oxonate-Pretreated Mice

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Abstract: Phenylpropanoid glycoside acteoside was extracted from the traditional Chinese medicine *Scrophularia ningpoensis* Hemsl. In the present study, we investigated the effects of acteoside administration on serum uric acid levels in mice rendered hyperuricemic with the uricase inhibitor potassium oxonate. When administered orally for 3 days at doses of 50, 100 and 150 mg/kg, acteoside reduced serum uric acid levels by 15.2, 23.8 and 33.1%, respectively, relative to vehicle-treated hyperuricemic mice. Importantly, in non-hyperuricemic mice, the serum uric acid levels were not affected by acetoside treatment. Acteoside also inhibited mouse liver xanthine dehydrogenase (XDH) and xanthine oxidase (XO) activity at all three doses. These results suggest that the hypouricemic action of acteoside may be attributable to its inhibition of XDH/XO activity.

Keywords: Phenylpropanoid Glycoside Acteoside; Hyperuricemia, Hypouricemic Action; Xanthine Dehydrogenase; Xanthine Oxidase.

Introduction

Gout is a common metabolic disorder in humans and is primarily caused by a deposition of monosodium urate crystals in joints and other tissues as a result of extracellular urate...
supersaturation (Boss and Seegmiller, 1979). This can cause inflammation as well as gouty arthritis and uric acid nephrolithiasis (Tomita et al., 2000; Kramer and Curhan, 2002). Gout is often associated with elevated serum levels of uric acids, which result from the overproduction and/or underexcretion of uric acid, and is highly influenced by a high dietary intake of nucleic acids (Chiang et al., 1994; Owen and Johns, 1999).

Compounds that display the ability to inhibit uric acid biosynthesis have been generally employed for treatment of gout (Ishibuchi et al., 2001). In the process of purine metabolism, xanthine oxidase (XO) catalyzes the xanthine and hypoxathine into uric acid (Oettl and Reibnegger, 1999). XO is therefore a common drug strategy used to treat hyperuricemia and gout. Allopurinol is the most common clinically used XO inhibitor prescribed for the treatment of gout, but use of this drug may result in various side-effects including hepatitis, nephropathy and allergic reactions (Osada et al., 1993; Hammer et al., 2001). Thus, the development of novel hypouricemic agents with greater efficacy and a broader safety profile is greatly needed. In the present study, the effects of the phenylpropanoid glycoside acteoside of *Scrophularia ningpoensis* were evaluated in an animal model of hyperuricemia.

**Materials and Methods**

**Chemical Reagents**

Allopurinol, Xanthine, carboxymethyl-cellulose sodium salt and potassium oxonate were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China).

**Extraction of Acteoside**

*Scrophularia ningpoensis* (Scrophulariaceae) roots were collected from the Lingbo region (Zhejiang province) in China. The plant was identified by vice-professor Xiaoqiang Ma from the Shanghai Institute of Materia Medica, Chinese Academy of Science, where the voucher specimen (NO. 95-10) was deposited.

Twenty kilograms of powdered *Scrophularia ningpoensis* roots was extracted with ethanol and concentrated as previously described (Li et al., 2000). The residue was suspended in water and shaken successively with ester and n-BuOH. The n-BuOH layer residue (420 g) was subjected to a macro-porous resin DA-201 column eluted with water and increasing amounts of EtOH. The 40% EtOH fraction was subjected column chromatography (CC) on a silica gel with CHCl3-MeOH-H2O (9:1:0:1-7:3:0.3) to obtain 4 fractions (I–IV). Fraction IV was subjected to CC on Sephadex LH-20 to yield two fractions (a and b). Fraction b was subjected to CC on a silica gel with EtOAc-MeOH-H2O (9:1:0:1-7:3:0.3) and an ODS column (30% MeOH) to obtain acteoside (295 mg). Its structure was confirmed by 1H NMR spectrum as reported (Calis et al., 1987).
Animals

Male Institute of Cancer Research (ICR) mice (26–30 g) were purchased from the Laboratory Animal Center (Shanghai, China) and maintained on a 12-hour light/dark cycle in a temperature-and humidity-controlled room for 1 week prior to the experiment. All animals were randomly divided into the experimental groups (n = 10/group). Mice were given standard chow and water ad libitum throughout the experiment, except 1 hour prior to drug administration when access to food was restricted. All procedures were carried out in accordance with the Chinese legislation on the use and care of laboratory animals and were approved by the respective university committees for animal experiments.

Hyperuricemia Model in Mice

The uricase inhibitor potassium oxonate was used to induce hyperuricemia in mice as described previously (Stavric et al., 1975; Hall et al., 1990). Briefly, mice were injected intraperitoneally with potassium oxonate (250 mg/kg) 1 hour before the final tested drug administration to increase serum urate levels. Blood was obtained from the mice via tail tip cuts. The blood was allowed to clot for approximately 1 hour at room temperature and then centrifuged at 2500 × g for 10 min. The sera were stored at −30°C until use.

Drug Administration

Access to food, but not water, was restricted 1 hour prior to drug administration. Acteoside and allopurinol at various concentrations (acetoside (50, 100, and 150 mg/kg and for allopurinol (5 mg/kg)) were dissolved in carboxymethyl-cellulose sodium salt. The volume of the suspension administrated to each mouse was based on the body weight of the animal measured immediately prior to each dose. Acteoside and allopurinol (acetoside (50, 100, and 150 mg/kg and for allopurinol (5 mg/kg)) were administered orally once daily at 14:00 for 3 consecutive days. The control groups received vehicle. Uric acid levels were determined by the phosphotungstic acid method (Carroll et al., 1971).

Enzyme Preparation

Mice were decapitated one hour after the last drug administration and the liver rapidly excised and homogenized in 5 volumes of 80 mM sodium pyrophosphate buffer (PH 7.4) at 4°C. The homogenate was then centrifuged at 3000 × g for 10 min and the resulting supernatant fraction was further centrifuged at 10,000 × g for 60 min at 4°C. The supernatant was used for the subsequent assays.

Xanthine Dehydrogenase (XDH) and Xanthine Oxidase (XO) Activity Assay

XDH and XO activity was assayed by monitoring uric acid formation using a spectrophotometric method described previously (Hall et al., 1990; Kong et al., 2002).
Briefly, 100 µl of the supernatant was added to a reaction mixture containing 50 µM xanthine and 5 mM EDTA for a final reaction volume of 5.0 ml. For the XDH assay, 200 µM NAD$^+$ was also present. After incubation for 15 min at 37°C, the reaction was stopped by the addition of 0.5 ml of 0.58 M HCl. The UV absorbance at 290 nm was measured to determine uric acid production. The XDH/XO activities were expressed as nmole uric acid produced per min per mg protein. Protein concentration was determined by the Lowry method using bovine serum albumin as the standard (Lowry et al., 1951).

Statistical Analysis

Each assay was performed in duplicate, and all data are expressed as mean ± standard error of the mean (SEM) for each group. Statistical analyses were performed using student’s t-tests. Two-tailed values of p < 0.05 were considered statistically significant.

Results

Time-Course Potassium Oxonate Effects on Serum Uric Acid Levels in Mice

The effects of potassium oxonate on mice serum uric acid levels are shown in Fig. 1. Uric acid levels in non-hyperuricemic, vehicle-treated mice were 1.03 ± 0.04 mg/dl. Intraperitoneal injection of potassium oxonate (250 mg/kg) markedly increased the serum uric acid levels, and reached a Cmax of 5.20 ± 0.21 mg/dl after 2 hours, followed by slow decrease in serum uric acid levels until normalization 8 hours post-injection.

Dose-Dependent Effects of Acteoside on Serum Uric Acid Levels in Control and Hyperuricemic Mice

The dose-dependent effects of acteoside on serum uric acid levels in the control and hyperuricemic mice were investigated after 3 days of oral administration. As shown in Table 1, no significant difference was observed in serum uric acid levels between non-hyperuricemic mice that received acteoside at doses of 50, 100 or 150 mg/kg and the vehicle-treated mice. By contrast, allopurinol significantly reduced the serum uric acid levels in non-hyperuricemic mice.

Importantly, acteoside was found to remarkably reduce serum uric acid levels in hyperuricemic mice treated with potassium oxonate when compared with mice treated with vehicle for 3 days (Table 2). In non-hyperuricemic mice, serum uric acid levels were 1.12 ± 0.09 mg/dl. Two hours following potassium oxonate administration, serum uric acid levels were elevated to 5.32 ± 0.18 mg/dl. Acteoside treatment significantly and dose-dependently decreased the serum uric acid levels in hyperuricemic mice 1 hour following oral administration (2 hours following potassium oxonate administration). The reduction in serum uric acid levels after 50, 100 and 150 mg/kg acteoside administration was 15.2%, 23.8%, and 33.1%, respectively. The reference drug allopurinol (5 mg/kg, P.O) also significantly reduced serum uric acid levels by 37.6%.
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Figure 1. Time-course effect of potassium oxonate on uric acid levels.

Table 1. Effects of Acteoside on Serum Uric Acid Levels in Mice Untreated with the Uricase Inhibitor, Potassium Oxonate

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>n</th>
<th>Uric Acid (mg/dl) mean ± SEM</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td></td>
<td>10</td>
<td>1.52 ± 0.18</td>
<td></td>
</tr>
<tr>
<td>Acteoside</td>
<td>50</td>
<td>10</td>
<td>1.49 ± 0.11</td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>10</td>
<td>1.45 ± 0.12</td>
<td>4.8</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>10</td>
<td>1.47 ± 0.09</td>
<td>3.3</td>
</tr>
<tr>
<td>Allopurinol</td>
<td>5</td>
<td>10</td>
<td>1.03 ± 0.08**</td>
<td>32.2</td>
</tr>
</tbody>
</table>

***p < 0.01 when compared with vehicle control group.

Table 2. Effects of Acteoside on Serum Uric Levels in Mice Treated with the Uricase Inhibitor, Potassium Oxonate

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>n</th>
<th>Uric Acid (mg/dl) mean ± SEM</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td></td>
<td>10</td>
<td>1.12 ± 0.09</td>
<td></td>
</tr>
<tr>
<td>PO</td>
<td></td>
<td>10</td>
<td>5.32 ± 0.18</td>
<td></td>
</tr>
<tr>
<td>PO + AC</td>
<td>50</td>
<td>10</td>
<td>4.76 ± 0.16*</td>
<td>15.2</td>
</tr>
<tr>
<td>PO + AC</td>
<td>100</td>
<td>10</td>
<td>4.05 ± 0.12**</td>
<td>23.8</td>
</tr>
<tr>
<td>PO + AC</td>
<td>150</td>
<td>10</td>
<td>3.56 ± 0.15**</td>
<td>33.1</td>
</tr>
<tr>
<td>PO + AP</td>
<td>5</td>
<td>10</td>
<td>3.39 ± 0.16**</td>
<td>37.6</td>
</tr>
</tbody>
</table>

PO: potassium oxonate; AC: acteoside; AP: allopurinol. *p < 0.05, **p < 0.01 when compared with PO group.

Table 3. Effects of Acteoside and Allopurinol on Xanthine Dehydrogenase and Xanthine Oxidase Activities in Mice Pretreated with Potassium Oxonate

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>n</th>
<th>XDH (nmole uric acid/mg protein)</th>
<th>XO (nmole uric acid/mg protein)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td></td>
<td>10</td>
<td>5.65 ± 0.37</td>
<td>4.92 ± 0.54</td>
<td></td>
</tr>
<tr>
<td>AC</td>
<td>50</td>
<td>10</td>
<td>4.73 ± 0.35**</td>
<td>4.18 ± 0.33**</td>
<td>16.3</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>10</td>
<td>4.05 ± 0.41**</td>
<td>3.29 ± 0.28**</td>
<td>28.3</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>10</td>
<td>3.62 ± 0.28**</td>
<td>2.88 ± 0.17**</td>
<td>35.9</td>
</tr>
<tr>
<td>AP</td>
<td>5</td>
<td>10</td>
<td>3.44 ± 0.28**</td>
<td>2.79 ± 0.23**</td>
<td>39.1</td>
</tr>
</tbody>
</table>

AC: acteoside; AP: allopurinol. ***p < 0.01 when compared with vehicle control group.
Dose-Dependent Effects of Acteoside on Liver XDH and XO Activity in Hyperuricemic Mice

In order to perform a detailed investigation of the hypouricemic properties of acteoside, we investigated liver XDH and XO activity in the hyperuricemic mice. Relative to the vehicle-treated hyperuricemic group, mice treated with 50, 100, or 150 mg/kg acteoside exhibited liver XDH activity levels that were decreased by 16.3%, 28.3% and 35.9%, respectively. Likewise, liver XO activity was decreased relative to vehicle-treated mice by 15.0%, 33.1% and 41.5%, respectively. Administration of allopurinol at 5 mg/kg reduced the XDH and XO activity in mouse liver by 39.1% and 43.3%, respectively (Table 3).

Discussion

Gout is a common metabolic disorder in humans, reportedly afflicting more than 2 million men and women in the US alone (Kramer and Curhan, 2002). The disease is also rapidly increasing in prevalence in China (Li et al., 1997), most likely due to recent changes in dietary habits.

Chronic disorders of purine metabolism will lead to hyperuricemia and gout (Chen, 2003). Primary hyperuricemia is primarily caused by a decrease in uric acid excretion by the kidneys or by excessive uric acid generation (Gurwitz et al., 1997). Xanthine oxidase (XO) and xanthine dehydrogenase (XDH) catabolize the conversion of hypoxanthine into xanthine and xanthine into uric acid, the last step of purine metabolism. In most mammalian species, uric acid is further converted by urate oxidase into allantoin, a highly polar and water soluble product, which is ultimately excreted with urea (Nguyen et al., 2005). However, humans lack urate oxidase, thus uric acid is the final product of purine degradation (Osada et al., 1993). Gout is often associated with elevated serum levels of uric acids, resulting in the deposition of urate crystals in joints and kidneys causing uric acid nephrolithiasis and gouty arthritis. Because of this, XDH and XO are attractive targets for the development of drugs to treat hyperuricemia and gout. Allopurinol is the most common clinically used xanthine oxidase inhibitor prescribed for the treatment of gout. However, its use is often limited by hypersensitivity reactions (Hammer et al., 2001), Stevens- Johnson syndrome (Fritsch and Sidoroff, 2000), renal toxicity (Horiuchi et al., 2000), and even fatal liver necrosis (Pereira et al., 1998). Therefore, attempts to identify novel XDH and XO inhibitors with greater efficacy and a broader safety profile are critical (Zhou et al., 2001; Kong et al., 2004).

Administration of potassium oxonate, a well-known inhibitor of urate oxidase, is widely used to create an animal model of hyperuricemia. In the present study, potassium oxonate administration successfully induced hyperuricemia in mice, with a peak effect 2 hours following administration.

Scrophalaria ningpoensis Hemsl roots are widely used in Chinese medicine (named “Xuanshen”) for the treatment of various inflammatory diseases (Duck and Agensu, 1985). In the present study, we extracted acetoside from scrophularia ningpoensis, and administered the extract to mice for 3 days. We found that acetoside administration...
significantly and in a dose-dependent manner, prevented the increase in serum uric acid levels induced by potassium oxonate. The reference compound, allopurinol, was slightly more efficacious than acetoside (Table 2).

Importantly, acetoside only lowered the serum uric acid levels in hyperuricemic mice, and had no effect in normouricemic mice. By contrast, allopurinol exhibited a hypouricemic action in both hyperuricemic and normal mice. Similar results were also observed in previous studies with cassia oil (Zhao et al., 2006) and procyanidins (Wang et al., 2004). The difference in the mechanism of action between these compounds and acetoside remains to be determined, however, this characteristic of acetoside may be seen as an advantage over other treatment options (Wang et al., 2004). Although hyperuricemia induces gout and possibly other pathological conditions (Liese et al., 1999; Fang and Alderman, 2000; Tomita et al., 2000; Puddu et al., 2001), uric acid can also prevent DNA damage due to its antioxidant properties (Singh et al., 1998; Burkhardt et al., 2001). Therefore, a decrease in the basal levels of uric acid in non-hyperuricemic states may be counterproductive.

Acetoside administration was able to not only decrease serum uric acid levels but also inhibit XDH and XO activity in hyperuricemic mice. Taken together, the hypouricemic effect of acetoside may therefore be explained, at least in part, by a lowering of XDH/XO activity in drug-treated animals.

The present results clearly demonstrate a beneficial hypouricemic effect of acetoside. This effect may be mediated, in part, by inhibiting XDH/XO activity in the liver. Acteoside may therefore represent a safe and effective (Tomita et al., 2000; Kramer and Curhan, 2002) therapeutic strategy in the treatment of hyperuricemia and gout in clinical settings.

Acknowledgments

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