Anti-hyperglycemic Effect of Fangchinoline Isolated from Stephania Tetrandra Radix in Streptozotocin-Diabetic Mice

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Kampo medicine, Stephania tetrandra Radix (Stephania) in Boi-ogi-to increases the blood insulin level and falls the blood glucose level in streptozotocin (STZ)-diabetic ddY mice. These actions of Stephania are potentiated by Astragalus membranaceus Bunge Radix (Astragali) in Boi-ogi-to (Liu et al., J. Traditional Med., 17, 253—260, 2000). In the present study, actions of bis-benzyisoquinoline alkaloids isolated from Stephania were investigated in the hyperglycemia of STZ-diabetic mice. A main bis-benzyisoquinoline alkaloid, fangchinoline (0.3—3 mg/kg) significantly fell the blood glucose level of the diabetic mice in a dose-dependent manner. The effect of fangchinoline was 3.9-fold greater than that of water extract of Stephania. However, another main compound, tetrandrine (1—100 mg/kg) did not have any effect. The water extract of Astragali did not affect singly but potentiated the anti-hyperglycemic action of fangchinoline (0.3 mg/kg). Out of used compounds (1 mg/kg) isolated from Stephania, fangchinoline, fangchinoline 2'-N-oxide and 2'-N-norfangchinoline, which are substituted with 7-hydroxy side chain for 7-O-methyl side chain, decreased to near 50% of high blood glucose level. In addition, tetrandrine 2'-N-β-oxide, tetrandrine 2'-N-α-oxide, tetrandrine 2-N-β-oxide, fangchinoline 2'-N-α-oxide, which are added to 2- or 2'-N-oxide side chain, also decreased to near 50% of the high blood glucose level. In conclusion, fangchinoline but not tetrandrine from Stephania shows the anti-hyperglycemic action in the STZ-diabetic mice. The demethylation of 7-O-position and addition of 2- or 2'-N-oxide side chain in bis-benzyisoquinoline compounds in Stephania have a role for the induction of the anti-hyperglycemic actions.

Key words fangchinoline; Stephania tetrandra Radix; anti-hyperglycemic action; combined effect; Astragali Radix; streptozotocin-diabetic mouse

A Kampo medicine, Boi-ogi-to (Fang-ji-huang-qi-tang), is one of the traditional prescriptions and consists of Stephania tetrandra Radix (Stephania), Astragali Radix (Astragali), Atractyloides Lancea Rhizoma, Glycyrrhiza Radix, Zingiberis Rhizoma and Zizyphi Fructus. Boi-ogi-to has long been used clinically in the treatment of arthritis and edema in China and Japan. Boi-ogi-to is also suggested to improve indirectly an abnormal metabolism of glucose and lipid in diabetic patients with obesity.1,2 We have previously reported that Boi-ogi-to increases blood insulin level and decreases blood glucose level in streptozotocin (STZ)-diabetic mice. The anti-hyperglycemic action of Boi-ogi-to depends on the action of Stephania but not Sinomeni Caulis et Rhizoma in the prescription.3 Astragali does not have a direct anti-hyperglycemic effect but potentiates the actions of Stephania on blood levels of glucose and insulin.4,5 Stephania contains many bis-benzyisoquinoline-type compounds such as tetrandrine and fangchinoline.4,5) Tetrandrine is a principle compound and shown to be effective in experimental allergic encephalitis, airway micro vascular leakage, subcutaneous air pouch inflammation and adjuvant-induced chronic inflammation.6—9) It also inhibits precapillary formation of vascular endothelial cells and angiogenesis of cultured choroidal explants in STZ-diabetic rats.9,10) Fangchinoline and tetrandrine have similar inhibitory activities on angiotensin I converting enzyme,4,5) and induction of proinflammatory cytokines, interleukin-1 and tumor necrosis factor-alpha, by Staphylococcus aureus Cowan I-stimulated human peripheral blood mononuclear cells.11) In addition, these compounds show different actions on the anti-inflammation containing inhibition of cyclooxygenase and interleukin-5 activities although they are similar in chemical structure.12)

In the present study, effects of bis-benzyisoquinoline compounds containing fangchinoline and tetrandrine isolated from Stephania on the blood glucose level were investigated in the STZ-diabetic mice. An effect of Astragali on the action of fangchinoline was also investigated to confirm the combined effect of Stephania and Astragali. Finally, chemical structure–activity relationship of used Stephania compounds were clarified.

MATERIALS AND METHODS

Preparation of Streptozotocin-Diabetic Mice Fed male mice (ddY strain; 5 weeks of age; body weight, 22—25 g; Kiwa Laboratory Animal Science Co. Ltd., Wakayama, Japan) were injected with a single dose (150 mg/kg) of STZ (Sigma, St. Louis, MO, U.S.A.) in saline into the tail vein. STZ-induced diabetic mice (8—9 weeks of age; body weight, 22—46 g) were used for experiments 3—4 weeks after the injection. Age-matched normal male mice (ddY strain; 8—9 weeks of age; body weight, 29—42 g) were used in the control experiments. These mice were housed with PMI Laboratory Diet (Japan SLC, Sizuoka, Japan) and water ad libitum at 25—26 °C with lights on from 7 a.m. to 7 p.m. Drugs were administered into mice fasted from 8 p.m. to 10 a.m. The Ethics Review Committee for Animal Experimentation of Hokuriku University approved the experimental protocol.

Preparation and Administration of Drugs Stephania (Stephania tetrandra S. MOORE) and Astragali (Astragalus membranaceus Bunge) were collected from Anhui shen, China, during autumn and from Neimeng gu, China, during...
autumn, respectively. These crude drugs were extracted in 6 volumes of distilled water at 96—98 °C for 40 min with an automatic extractor “Torobi” (Tochimoto, Osaka Japan). Water extracts of these drugs were filtered through a mesh (No. 42, Sanpo, Tokyo, Japan), lyophilized with a freeze-drier (DF-03G, ULVAC, Tokyo), and stored at 4 °C. Dry weight yields of extracts of Stephania and Astragali are 5.7 and 15.0% (w/w), respectively.\(^3\) Tetrandrine (\(1\beta,6\beta,7\beta,12\)-tetrathemoxy-2,2',-dimethyl berbaman) was purchased from Aldrich Chemicals Co. Ltd. (Milw., WI, U.S.A.). Fangchinoline, tetrandrine-2'-N-\(\beta\)-oxide (TD-7), tetrandrine 2'-N-\(\alpha\)-oxide (TD-11), tetrandrine-2-N-\(\beta\)-oxide (TD-10), fangchinoline 2'-N-\(\alpha\)-oxide (TD-8), 2'-N-norfangchinoline (TD-28), 2'-N-methyltetrandrinium chloride (TD-13), cyclevenorine, cyclocleanine (TD-14), cycloline chloride and Stephaneanthrine were obtained from Tsumura Co. Ltd., Tokyo. These chemical structures were summarized in Chart 1.\(^4\)\(^5\) These extracts and compounds were suspended homogeneously in saline containing 1% Avicel (Asahi Chemical Industry, Tokyo), and administered intraperitoneally (i.p.: 0.1 ml/10 g body weight) into 14 h-fasted mice, respectively.

**Measurement of Blood Glucose and Insulin Levels**

Blood samples were collected from orbital vein plexus of mice and centrifuged at 3000 rpm at 4 °C for 20 min. Blood glucose levels of the supernatant were measured by the glucose oxidase method with a glucose B-test (Wako, Osaka) or Backman glucose analyzer (type II, Backman Coulter, Tokyo). Blood glucose levels were measured in fasted mice before, and 6 h after the administration of drugs, respectively. The fall % of blood glucose (BG) was calculated as \([\text{BG (before drug treatment)}−\text{BG (after drug treatment)}]/\text{BG (before drug treatment)} \times 100\). Eighty-five are average of BG of 14 h-fasted normal mice.\(^2\)\(^3\) Blood insulin level of 3 h-fasted mice was measured by the mouse ELISA kit for insulin (Morinaga, Yokohama, Japan).

**Statistical Analyses**

All values were expressed as means±S.E.M. Differences between group data were evaluated by one-way analysis of variance followed by the multiple range test of Scheffé, or unpaired \(t\)-test at \(p=0.05\) or 0.01. A value of \(p<0.05\) was considered statistically significant.

**RESULTS**

**Glucose and Insulin Levels in Blood of Streptozotocin-Diabetic Mice**

Blood glucose level in fed STZ-injected mice increased in a time dependent manner and became maximal on 12 d after the STZ-treatment (Fig. 1). The high level of blood glucose was kept up to 28 d after the STZ-treatment. The blood glucose levels in 14 h-fasted diabetic mice on 21 to 28 d after the STZ-treatment decreased to approximately 300 mg/dl but were sustained to the higher level than those of age-matched normal fasted mice (Fig. 1). Blood insulin levels in 3 h-fasted diabetic mice 21 d after STZ treatment were decreased to 22% of those in the age-matched normal mice (Fig. 1). The blood insulin levels in 3 h-fasted diabetic mice were not significantly different from the levels in 14 h-fasted diabetic mice (data not shown). From these results, the diabetic mice 21—28 d after the STZ-treatment were fasted for 14 h and used for the following experiments.

**Effects of Tetrandrine and Fangchinoline on Blood Glucose Level in the Diabetic Mice**

Effects of tetrandrine and fangchinoline on blood glucose level were compared with that of water extract of Stephania in the diabetic mice. Fangchinoline (0.3—3 mg/kg) as well as the water extract of Stephania (0.48—16 mg/kg) significantly fell high level of blood glucose in the diabetic mice in a dose-dependent manner. The anti-hyperglycemic effect of fangchinoline was significantly greater than that of the Stephania extract (Fig. 2).

However, tetrandrine (1—100 mg/kg) did not have a significant action on the blood glucose level compared with that of saline without drug (Fig. 2).

**Combined Effect of Astragali on the Anti-hyperglycemic Action of Fangchinoline**

We have previously reported that Astragali has the combined effect on the anti-hyperglycemic action of Stephania.\(^3\) The present study was investigated effect of Astragali on the anti-hyperglycemic action of fangchinoline. Fangchinoline did not have action at...
0.3 mg/kg in shadowed part of Fig. 3A and in the lowest dose in Fig. 3B and increased anti-hyperglycemic action in a dose-dependent manner. The extract of Astragali (0.48—160 mg/kg) singly did not have a significant anti-hyperglycemic action in the diabetic mice compared with saline group without drugs (Fig. 3B). However Astragali (3—100 mg/kg) potentiated significantly the action of fangchinoline (0.3 mg/kg) in a dose-dependent manner (Fig. 3A). The effect of 30 mg/kg Astragali in the presence of fangchinoline (0.3 mg/kg) was similar to that of fangchinoline (1 mg/kg) without Astragali (Fig. 3).

**Chemical Structure–Activity Relationships of Compounds Isolated from Stephania**

Anti-hyperglycemic effects of tetrandrine 2′-N-β-oxide (TD-7), tetrandrine 2′-N-α-oxide (TD-11), tetrandrine 2-N-β-oxide (TD-10), 2′-N-methyltetrandrinium chloride (TD-13), cycleanorine, fangchinoline 2′-N-α-oxide (TD-8), 2′-N-norfangchinoline (TD-28), cycleanine (TD-14), cyclanoline chloride and stephenanthrine were compared with those of fangchinoline and tetrandrine in the STZ-diabetic mice. Fangchinoline, fangchinoline 2′-N-α-oxide (TD-8) and 2′-N-norfangchinoline (TD-28) (1 mg/kg) decreased significantly to near 50% of the high

**Table 1. Effects of Compounds Isolated from Stephania on the Blood Glucose Levels in the Diabetic Mice**

<table>
<thead>
<tr>
<th>Drugs</th>
<th>n</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>13</td>
<td>10.9±7.7</td>
</tr>
<tr>
<td>Tetrandrine</td>
<td>5</td>
<td>22.2±11.3</td>
</tr>
<tr>
<td>TD-7</td>
<td>4</td>
<td>53.5±15.5*</td>
</tr>
<tr>
<td>TD-11</td>
<td>4</td>
<td>59.0±10.7**</td>
</tr>
<tr>
<td>TD-10</td>
<td>7</td>
<td>48.2±7.2**</td>
</tr>
<tr>
<td>TD-13</td>
<td>4</td>
<td>7.5±10.1</td>
</tr>
<tr>
<td>Cycleanorine</td>
<td>6</td>
<td>20.2±11.8</td>
</tr>
<tr>
<td>Fangchinoline</td>
<td>7</td>
<td>52.7±6.7**</td>
</tr>
<tr>
<td>TD-8</td>
<td>4</td>
<td>50.7±14.5*</td>
</tr>
<tr>
<td>TD-28</td>
<td>5</td>
<td>40.3±11.1*</td>
</tr>
<tr>
<td>Cyclanoline</td>
<td>6</td>
<td>50.1±9.0**</td>
</tr>
<tr>
<td>Cyclanoline chloride</td>
<td>7</td>
<td>24.0±8.8</td>
</tr>
<tr>
<td>Stephenanthrine</td>
<td>7</td>
<td>7.4±15.4</td>
</tr>
</tbody>
</table>

Blood glucose levels were measured before and 6 h after the administration of saline, tetrandrine, tetrandrine-2′-N-β-oxide (TD-7), tetrandrine 2′-N-α-oxide (TD-11), tetrandrine-2-N-β-oxide (TD-10), 2′-N-methyltetrandrinium chloride (TD-13), cycleanorine, fangchinoline, fangchinoline 2′-N-α-oxide (TD-8), 2′-N-norfangchinoline (TD-28), cycleanine, cyclanoline chloride and stephenanthrine (1 mg/kg) in 14-h-fasted diabetic mice. The fall % of blood glucose was calculated as described in the legend of Fig. 2. The values are expressed as means±S.E.M. of 4—13 data. n represents number of data. 

**Fig. 3. Potentiating Effect of Astragali on the Anti-hyperglycemic Action of Fangchinoline in the STZ-Diabetic Mice**

Blood glucose levels were measured before and 6h after the administration of both various doses of Astragali and 0.3 mg/kg fangchinoline (A: ●), and Astragali (B: ○) and fangchinoline (B: ■) singly in 14-h-fasted diabetic mice. The fall % of blood glucose was calculated as described in the legend of Fig. 2. The values are expressed as mean±S.E.M. of 6—15 data. Shadowed column in A represented standard error of mean of 15 data in single administration group of 0.3 mg/kg fangchinoline. **p<0.01: Significantly different from the group of single administration of fangchinoline.

**Chart 1. Chemical Structures of Used Alkaloids Isolated from Stephania**
blood glucose level in the diabetic mice (Table 1). However, tetrandrine, 2'-N-methyltetrandrinium chloride (TD-13) and cycleanorine (1 mg/kg) did not affect the high blood glucose level. In addition, tetrandrine 2'-N-β-oxide (TD-7), tetrandrine 2'-N-α-oxide (TD-11), tetrandrine 2-N-β-oxide (TD-10), fangchinoline 2'-N-α-oxide (TD-8) (1 mg/kg) also significantly decreased to near 50% of the high blood glucose level. Cycleanine (1 mg/kg), which has head to tail ether bonds, had also significant anti-hyperglycemic effect. However, cyclanolone chloride and stephananthenine (1 mg/kg), which do not have a bis-benzylisquinoline structure, did not affect the blood glucose level (Table 1).

**DISCUSSION**

We have previously reported that Kampo medicine, Boi-ogi-to, increases blood insulin level and falls high level of blood glucose in the STZ-diabetic mice. The water extract of Stephania, but not Sinomeni Caulis et Rhizoma in Boi-ogi-to shows increase in blood insulin release and anti-hyperglycemic action.23 The present study demonstrates that fangchinoline, a main bis-benzylisquinoline compound in Stephania had a potent anti-hyperglycemic action in the STZ-diabetic mice. Fifty% inhibitory dose of fangchinoline with 95% confident limit was 1.23 mg/kg (0.81—1.89) and significantly smaller than the dose of Stephania extract [4.77 mg/kg (2.59—8.80)]. The potency of fangchinoline was 3.9-fold greater than that of Stephania extract. However, tetrandrine, another main bis-benzylisquinoline compound, did not have such an anti-hyperglycemic action in the diabetic mice. These results demonstrate that fangchinoline but not tetrandrine has a role for these actions of Stephania in Boi-ogi-to.

Fangchinoline and tetrandrine are reported to have similar inhibitory effects on the experimental thrombosis induced by collagen and epinephrine,13 human polymorphonuclear leukocyte function,14 human platelet aggregation and thromboxane B2 formation,15 the activity on angiotensin I converting enzyme,4,5 and the induction of proinflammatory cytokines, interleukin-1 and tumor necrosis factor-alpha.11 These results indicate that the active moiety of fangchinoline is similar to that of tetrandrine for these actions. On the other hand, fangchinoline and tetrandrine have different effects on the activities of cyclooxygenase and interleukin-5. Fangchinoline inhibits the activity of cyclooxygenase but tetrandrine does not show any inhibition.23 The present results also demonstrate that fangchinoline but not tetrandrine fell the high level of blood glucose in the diabetic mice, indicating that the active moiety of fangchinoline differs from that of tetrandrine in the falling action on blood glucose level. Pre-treated tetrandrine has been reported to protect beta cells from injury induced by 5 h-exposed alloxan.16 But, tetrandrine did not influence the higher blood glucose of STZ-diabetic mice 21d after STZ injection. On the other hand, tetrandrine inhibits the angiogenesis of cultured choroidal capillary in the STZ-diabetic rat10 and activation of NF-kappa B in rat alveolar macrophages.17 The diabetic state-induced choroidal angiogenesis may be associated with the angiogenic action of N^2-(carboxy methyl)lysine (CML), a major advanced glycation end product (AGE) in the diabetic rat.18 These results suggest that Stephania may have several actions during the course of diabetes such as anti-hyperglycemic action of fangchinoline and improving actions of tetrandrine on the mechanisms linking hyperglycemia with tissue damage such as the mechanism of AGEs in the diabetic complication.

We have previously reported that Astragali does not have direct effect but potentiates both insulin-releasing action and anti-hyperglycemic action of Stephania in the STZ-diabetic mice.23 The present study confirms that Astragali significantly potentiated the anti-hyperglycemic action of fangchinoline in Stephania. Effect of 30 mg/kg Astragali in the presence of 0.3 mg/kg fangchinoline was similar to the effect of 1 mg/kg fangchinoline alone, indicating that Astragali potentiates the action of fangchinoline to a 3-fold greater extent. Some components in Astragali extract may potentiate insulin-releasing action and anti-hyperglycemic action of fangchinoline, although we do not have a direct evidence for actions of fangchinoline on insulin release yet. Mechanisms of combined action of Astragali components and fangchinoline are unknown at the present time. The details need to be further investigated on their combined effects on insulin release in the diabetic mice.

Anti-hyperglycemic actions of used compounds isolated from Stephania were compared. Tetrandrine 2'-N-β-oxide (TD-7), tetrandrine 2'-N-α-oxide (TD-11), tetrandrine 2-N-β-oxide (TD-10), fangchinoline 2'-N-α-oxide (TD-8), 2'-N-norfangchinoline (TD-28) and cycleanine had similar anti-hyperglycemic actions as fangchinoline did in the diabetic mice. The yields of these compounds in the water extract of Stephania are estimated to be 0.22% (fangchinoline), 0.0062% (tetrandrine 2'-N-β-oxide; TD-7), 0.0065% (tetrandrine 2'-N-α-oxide; TD-11), 0.0014% (tetrandrine 2-N-β-oxide; TD-10), 0.00059% (fangchinoline 2'-N-α-oxide; TD-8) and 0.00066% (cycleanine), respectively.4,5 Since the effects of these compounds did not recover completely the effect of Stephania extract, these compounds and other compounds might interact synergistically in the anti-hyperglycemic action of Stephania extract.

Relation of chemical structures with anti-hyperglycemic activity was investigated using these anti-hyperglycemic alkaloids in Stephania. Fangchinoline, fangchinoline 2'-N-α-oxide (TD-8) and 2'-N-norfangchinoline (TD-28), which substituted with a 7-hydroxy side chain, had similar anti-hyperglycemic actions to each other. But tetrandrine, 2'-N-methyl tetrandrinium chloride (TD-13) and cycleanorine, which have a structure of 7-O-methyl, did not affect the blood glucose level. These results demonstrate that the demethylation at the 7-O position in these compounds is essential for their anti-hyperglycemic actions. In addition, tetrandrine 2'-N-β-oxide (TD-7), tetrandrine 2'-N-α-oxide (TD-11), tetrandrine 2-N-β-oxide (TD-10), fangchinoline 2'-N-α-oxide (TD-8), which are added to side chain of 2- or 2'-N-oxide, showed significant anti-hyperglycemic actions. But tetrandrine, 2'-N-methyltetrandrinium chloride (TD-13) and cycleanorine, which have 2- and/or 2'-N-methyl side chain, had no effect. From these results, the 2- or 2'-N-oxide added to tetrandrine as well as the demethylation at the 7-O position have an important role for their anti-hyperglycemic actions. Fangchinoline 2'-N-α-oxide (TD-8), which has both 7-hydroxy and 2'-N-oxide side chains, did not increase the anti-hyperglycemic action of fangchinoline. The result suggests...
that one of demethylation of 7-O position and addition of 2- or 2′-N-oxide has a role for the induction of anti-hyperglycemic action.

It is reported that anti-hypertensive effect of tetrandrine is attenuated by the demethylation at the 7-O position and the substitution of N-methyl groups at the 2- and 2′-position with N-oxide, indicating that the anti-hypertensive moieties of tetrandrine are 7-O-methyl group and N-methyl group at the 2- and 2′-positions. The active moieties of tetrandrine-derivatives for the anti-angiogenic activity in the diabetic choroidal capillary are reported to be the bis[tetrahydroisoquinoline] moiety connected by oxy-bis[phenylenemethylene] and 2,2′-N-methyl group. These different active moieties of bis-benzylisoquinoline compounds in Stephania interact with different sites of action for the anti-hyperglycemic and anti-angiogenic effects during the course of diabetes.

In conclusion, fangchinoline, but not tetrandrine in Stephania had a potent anti-hyperglycemic action in STZ-diabetic mice. From the chemical structure–activity relation of Stephania compounds, the demethylation at the 7-O position and/or addition of 2- or 2′-N-oxide side chains in these bis-benzyl-isoquinoline compounds have a role for the induction of anti-hyperglycemic action in the diabetic mice.

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