Comparison of pharmacological effects of Yanbian Toki, Yamato Toki and Hokkai Toki (Angelicae Radix) on oketsu (blood stagnation) and hie-sho (chilliness) in animal models

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Previous reports suggest that Yanbian Toki (Angelica species of northeast China) may become an alternative resource of Angelicae Radix (Toki) when compared with the Japanese indigenous plants of A. acutiloba Kitagawa (Yamato Toki) and A. acutiloba var. sugiyamae Hikino (Hokkai Toki) in their similar chemical and genetic characteristics. In the present study, the pharmacological effects of Yanbian Toki on oketsu and hie-sho were assessed by using animal models in comparison with Yamato Toki and Hokkai Toki.

Oketsu state (stagnation of blood) was induced in mice by a consecutive injection of betamethasone (1.6 mg/kg/day, i.m.). Changes in peripheral blood flow of oketsu mice treated with each Toki extract at a dose of 200 mg/kg were examined both in single and repeated administrations. Yanbian Toki improved the peripheral blood flows of oketsu mice as well as Yamato Toki and Hokkai Toki, and showed no significant statistically differences.

In hie-sho (chilliness) model, accomplished by submerging the rats in cold water, the restoration of body surface temperature was accelerated by a single administration of each Toki extract (200 mg/kg). Especially at the initial 5 min, it was significant. Furthermore, the time versus recovery effect was statistically significant in the treatment of Yamato Toki. However, there were no significant differences among them in their recovery effects.

In both experiments, among Yanbian Toki, Yamato Toki and Hokkai Toki, there were no significant differences in their effects on oketsu and hie-sho. These results suggested that Yanbian Toki is pharmacologically comparable to Yamato Toki and Hokkai Toki.

Key words Toki (Angelicae Radix), Yanbian Toki (Angelica species of northeast China), Yamato Toki (Angelica acutiloba), Hokkai Toki (A. acutiloba var. sugiyamae), oketsu (blood stasis, blood stagnation), hie-sho (chilliness).

Introduction

In recent years, complementary and alternative medicine (CAM), in particular traditional Chinese medicine (TCM) or Kampo medicine is more and more recognized and applied to improve the well-being of people widely. Many medicinal plants have long been used in these traditional medicine systems, especially some of the medicinal plants belonging to the genus Angelica, of which demand and consumption are increasing year by year.

The genus Angelica comprises more than 60 species of medicinally important biennial or perennial herbs. Various herbal preparations containing Angelica species are available widely in ethical or over-the-counter locations, not only in Asia, but also in western countries. In China and Japan, the roots of several different species and varieties of Angelica are used as natural medicines called Danggui or Toki (Angelicae Radix), which is one of the most common natural medicines applied frequently to treatment of blood stasis syndrome (oketsu). Clinically, many women suffer from various gynecological disorders (e.g., menstrual disorders, amenorrhea) caused by oketsu that occurs at peripheral microcirculation in particular and leads to a chill in limbs called hie-sho (chilliness). Toki and the prescriptions containing Toki are used clinically to treat them in TCM or Kampo medicine. However, in China and Japan, the botanical origins of Angelicae Radix (Toki) is different, namely, the Japanese indigenous species of A. acutiloba Kitagawa (Yamato Toki) and A. acutiloba Kitagawa var. sugiyamae Hikino (Hokkai Toki) are prescribed in Japan; A. sinensis (Oliv.) Diels (Kara Toki or Tang Danggui) in China. On the other hand, Angelica sp. of northeast China (Yanbian Toki) is considered to be a pure line of Yamato Toki, which was introduced in northeast China about seventy years ago. In previous studies, Yanbian Toki has been proved to be close genetically and chemically to Yamato Toki, and suggests that Yanbian Toki may become an alternative resource of Angelicae Radix (Toki) in Japan. In the present study, the pharmacological effects of Yanbian Toki on oketsu and hie-sho were evaluated by using their
animal models, and compared with those of Yamato Toki and Hokkai Toki.

Materials and Methods

Animals. Male ddY mice (5-week-old, 27-30 g on arrival) and female Wistar rats (8-week-old, 200-250 g on arrival) from SLC, Inc. (Shizuoka, Japan) used for making oketsu and hie-sho models, respectively. They were group-housed (3 rats or 5 mice/cage) in a temperature-, humidity-controlled room on a reversed 12-hr light/dark cycle (light off, 8:00 AM) and provided laboratory pellet chow and water ad libitum. Before experimental procedures, they are acclimated to the room for 1 week. These experiments were approved by Experimental Animal Care Committee of Kracie Pharma, Ltd.

Plant materials and extracts preparation. Yanbian Toki (Angelica sp. of northeast China cultivated in Yanbian region, China), and Yamato Toki (A. acutiloba Kitagawa) and Hokkai Toki (A. acutiloba var. sugiyamae Hinoko) (cultivated in the same field at the Research Center for Medicinal Plant Resources, National Institute of Biomedical Innovation, Hokkaido, Japan) were used in this study. After the plant materials were identified, the voucher specimens were deposited at the herbarium of Kampo Research Laboratories, Kracie Pharma, Ltd.

The materials were subjected to extraction after all the harvested roots were processed with 50-60 °C water for 30 min and dried under natural conditions. Each of the dried roots (1.0 kg) was extracted with 10 L of 80 °C water for 1 hr. The aqueous extract was collected by centrifugation, dried by spray method. The yields of Yanbian Toki, Yamato Toki and Hokkai Toki were 14.3%, 11.0% and 11.5%, respectively. For animal experiments, the extracts were suspended in the 0.5% sodium carboxymethyl cellulose (CMC-Na) just before use.

Betamethasone-induced oketsu mice and drug administration. Oketsu mice were made by intramuscular injection of betamethasone sodium phosphate solution (Rinderon8, Shionogi Co., Ltd., Japan) at a dose of 1.6 mg/kg/day for 7 or 14 days.9 On the day before measuring peripheral blood flow rate (BFR), dorsal hairs of mice were clipped carefully with an electric clipper. BFR (ml/ml/100g) of mouse was measured with Laser Blood Flow Meter (ALF-2100 with φ 8.0 mm of a disk probe, Advance Co., Ltd., Japan) under anesthesia (Nembutal8, Dainippon Pharmaceutical Co., Ltd., Japan, 70 mg/kg, i.p.).

1) Single administration

Mice were divided into two groups on the basis of peripheral BFR values measured before injection of betamethasone. One was given consecutively betamethasone (i.m.) to mice for 7 days and the other one was given 0.9% saline in the same way as normal control. On day 7, the peripheral BFR (pre-value) was measured for checking whether the oketsu model was successful. Thereafter, the betamethasone-treated group was further divided into four subgroups on the basis of the pre-values. Among them, on day 10, Yanbian Toki, Yamato Toki and Hokkai Toki were given at a dose of 200 mg/kg by oral administration to each subgroup. Meanwhile, one subgroup served as control receiving 0.9% saline together with the normal control group. After 1 hour of drug administration, their peripheral BFR (post-value) were measured.

2) Repeated administration

Experimental method of repeated administration is basically the same as single administration except for the administration schedule. In brief, betametasone was continuously given for 6 days after pre-value measurement of peripheral BFR. From day 8, betametasone-treated mice were given three Toki extracts (200 mg/ml) respectively by consecutive oral administration until day 14.

In addition to the above experiments, the effect of three kinds of Toki on the peripheral BFR of normal mice were also examined with intramuscular injection of 0.9% saline instead of betamethasone.

Hie-sho rats model and drug administration. Hie-sho model was made in the terms of cold water-immersion method reported by Hirasawa et al.9 Briefly, rats were placed a quiet, air-controlled room (temperature of 23 ± 2 °C and humidity of about 50%) for 30 min after an overnight fasting, and then orally given each of the Toki extracts at a dose of 200 mg/kg respectively. After 30 min of drug administration, they were treated with cold immersion which was accomplished by submerging the rats in shoulder-deep cold water maintained at approximately 15 °C for 15 min. The body surface temperature (BST) at tail was measured successively for 120 min with Infrared Thermal Imaging Camera (TH9100MW, NEC San-ei Instruments, Ltd., Japan) when wiped immediately off the water attached to body of the rats released from the treatment of cold water immersion. Recovery temperature (Δ °C) was calculated by the following equation:

\[ Δ °C = T₀(°C) - T₁(°C) \]

(\(T₀\): BST at time point 0; \(T₁\): BST at t time point)

Statistical analysis. The experimental data are expressed as mean ± S.E.M. Statistical significance was determined by Student’s t-test when two groups were compared. The time versus recovery effect of body surface temperature was analyzed by two-factor repeated measure ANOVA with statistical package (Statcel, OMS Publishing Inc., Japan). Values of \(P\) less than 0.05 were accepted as statistically significant.

Results

Effect on the peripheral BFR in oketsu mice. As shown in Fig.1, consecutive administration of betamethasone (1.6 mg/kg/day) to mice for 7 or 14 days induced a significant decrease in peripheral BFR. The results showed the oketsu model was made successfully.

Each of the Toki extracts at a single dose of 200 mg/kg was administered orally to oketsu mice. Fig.1A shows that Yanbian Toki increased the peripheral BFR as well as Yamato Toki and Hokkai Toki compared with that of control, but, only Hokkai Toki was significant. Furthermore, at repeated doses of 200 mg/kg of 7 days administration, as
shown in Fig. 1B, they all increased significantly the peripheral BFR in the same order of Hokkai Toki > Yamato Toki = Yanbian Toki as single administration.

However, in both single and repeated administration (Fig. 1A and B), there were no significant differences among them in increasing the peripheral BFR of oketsu mice.

**Effect on the peripheral BFR in normal mice.** In addition, the effect of three kinds of Toki on the peripheral BFR in normal mice was also examined in the same way. The results showed they did not alter significantly the peripheral BFR both single and repeated administration with the same dose (Fig. 2).

**Effect on body surface temperature (BST) in hie-sho rats.** With or without treatment of each Toki extract at a single dose of 200 mg/kg, the recovery temperature of rats exposed to cold water are tabulated in Table 1. After being released from the treatment of cold water immersion, their BST recovered slowly with time, whereas the restoration of BST was accelerated by oral administration of each of the Toki extracts. Especially at the initial 5 min they were significant (p<0.05, Student’s t-test).

Furthermore, the time (120 min) versus the recovery effect was statistically significant in treatment of Yamato Toki administration (p<0.05, ANOVA analysis). However, there was no significant difference between Yamato Toki and Yanbian Toki or Hokkai Toki in their recovery effect.

**Discussion**

In order to compare the pharmacological effect of Yanbian Toki (*Angelica* sp. in the northeast of China) with Yamato Toki and Hokkai Toki, the effects on oketsu and hie-sho were examined and compared by use of animal models.

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![Fig. 1](image1.png)

**Fig. 1** Effect of three Toki extracts on peripheral blood flow rate measured in oketsu mice
Values represent the mean ± S.E.M (n=10). **A**: P<0.05, **B**: P<0.01 vs control group.

![Fig. 2](image2.png)

**Fig. 2** Effect of three Toki extracts on peripheral blood flow rate measured in normal mice
Values represent the mean ± S.E.M (n=10). N.S: no significant; vs normal.
Pharmacological evaluation of Yanbian Toki

Table 1  Recovery of Body Surface Temperature (ΔR, °C) of each point against Temperature at time point 0.

<table>
<thead>
<tr>
<th></th>
<th>5 min</th>
<th>10 min</th>
<th>20 min</th>
<th>30 min</th>
<th>40 min</th>
<th>50 min</th>
<th>60 min</th>
<th>80 min</th>
<th>100 min</th>
<th>120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.71 ± 0.26</td>
<td>1.63 ± 0.19</td>
<td>2.59 ± 0.26</td>
<td>3.38 ± 0.36</td>
<td>3.85 ± 0.30</td>
<td>4.27 ± 0.25</td>
<td>4.66 ± 0.20</td>
<td>4.96 ± 0.29</td>
<td>5.37 ± 0.33</td>
<td>5.54 ± 0.28</td>
</tr>
<tr>
<td>Yanbian Toki</td>
<td>1.55 ± 0.16</td>
<td>2.24 ± 0.15</td>
<td>3.11 ± 0.26</td>
<td>3.69 ± 0.28</td>
<td>4.10 ± 0.20</td>
<td>4.63 ± 0.12</td>
<td>5.09 ± 0.19</td>
<td>5.69 ± 0.23</td>
<td>6.09 ± 0.27</td>
<td>6.14 ± 0.24</td>
</tr>
<tr>
<td>Yamato Toki</td>
<td>1.60 ± 0.20</td>
<td>2.31 ± 0.16</td>
<td>3.12 ± 0.25</td>
<td>3.74 ± 0.23</td>
<td>4.43 ± 0.22</td>
<td>4.86 ± 0.16</td>
<td>5.53 ± 0.20</td>
<td>5.54 ± 0.21</td>
<td>6.10 ± 0.26</td>
<td>6.83 ± 0.26</td>
</tr>
<tr>
<td>Hokkai Toki</td>
<td>1.38 ± 0.12</td>
<td>2.11 ± 0.14</td>
<td>2.83 ± 0.22</td>
<td>3.58 ± 0.19</td>
<td>4.22 ± 0.17</td>
<td>4.73 ± 0.12</td>
<td>5.09 ± 0.18</td>
<td>5.38 ± 0.24</td>
<td>5.86 ± 0.23</td>
<td>6.13 ± 0.26</td>
</tr>
</tbody>
</table>

Values represent the mean ± S.E.M (n=11–12), *: P<0.05; **: P<0.01, vs control by student’s t-test

Oketsu and hie-sho are important pathological concepts in TCM or Kampo medicine. The former is defined as a pathological state resulting from the reverse or impeded flow of blood in the body or the stagnation of blood flow in local parts. The latter is a consequence of oketsu occurring in the peripheral microcirculation which ensues abnormal cold feeling. Clinically, many women suffer from oketsu-induced various gynecological disorders such as premenstrual syndrome, dysmenorrhea, especially a chill in limbs called hie-sho. Hie-sho is hard to treat in Western medicines, whereas Angelicae Radix (Toki) and the prescriptions containing Toki are considered to be effective.

Up to now, although the mechanism of oketsu and hie-sho are not yet clarified, several animal models have been established, for instance, betamethasone-induced hemorrhological disorders, and cold water immersion-induced the microcirculation disorders, which indicates similar symptoms like oketsu or hie-sho state described in TCM or Kampo medicine.

In betamethasone-induced oketsu mice, the peripheral blood flows were improved by single and repeated administration of Yanbian Toki, as well as Yamato Toki and Hokkai Toki. In addition, no change in peripheral blood flow was observed when each Toki extract was given to normal mice in the same way. These results are consistent with the traditional medical uses of Toki described in ancient literature. In cold water immersion-induced hie-sho rats, the body surface temperature was restored quickly by oral administration of Yanbian Toki, Yamato Toki and Hokkai Toki, respectively. Also, only Yamato Toki was statistically significant in the time versus recovery effects.

However, in both experiments, Yanbian Toki showed no significant difference in comparison with Yamato Toki or Hokkai Toki in their effects on oketsu and hie-sho. These results suggested that Yanbian Toki is pharmacologically comparable to Yamato Toki and Hokkai Toki.

Moreover, it is also noticed that the potencies of improving the peripheral blood flow in the order of Hokkai Toki > Yamato Toki = Yanbian Toki, and the recovery effects of body surface temperature in the order of Yamato Toki > Yanbian Toki = Hokkai Toki were observed in the experiments. Similarly, Tanaka et al. also reported that there was a significant difference between Yamato Toki and Hokkai Toki in anti-nociceptive activity. Such difference between Yamato Toki and Hokkai Toki existed not only in chemical and genetics, but also appeared clearly in their morphological characteristics (Picture 1). In fact, it has been made clear that Yamato Toki and Hokkai Toki derive from a different origin, namely, A. acutiloba Kitagawa and A. acutiloba var. sugiymae Hikino, respectively. It appears that the different origin could cause the differences both in chemical constituents and pharmacological effects.

The original plants of Yanbian Toki are very close genetically to that of Yamato Toki, and it is not easy to distinguish by morphological characteristics (Picture 1). In this study, Yanbian Toki showed an almost equal pharmacological effect to Yamato Toki. These results, when taken together, show that Yanbian Toki could be an alternative resource of Toki (Angelicae Radix) in Japan. However, recently, xanthotoxin (methoxsalen) was considered to be an active constituent of enhancing peripheral blood flow, of which content in Yamato Toki was about 3-fold higher than that of Yanbian Toki. It suggests that the other constituents may contribute to an enhancing effect of Yanbian Toki on peripheral blood flow. With respect to this point, detailed

Picture 1. Morphological characteristics of leaves observed in the original plants of three kinds of Toki

Yanbian Toki (Angelica sp. of northeast China) Yamato Toki (A. acutiloba Kitagawa) Hokkai Toki (A. acutiloba Kitagawa var. sugiymae Hikino)
analysis of active constituents and further pharmacological study are now under investigation.

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References