Original paper

Contribution of Volatile Components in Winter Savory (*Satureja montana* L.) to Changes in Body Temperature in Humans Who Experience Cold Sensitivity

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We have reported that ingestion of the volatile fraction of winter savory (WSV) increased core body temperature (CBT), inhibited the decrease in body surface temperatures (BSTs) of the wrist, finger and ankle, and increased BSTs of the forehead and neck in humans who experience cold sensitivity. We also showed that carvacrol, a main component of WSV, contributed to the CBT-increase, but not to the inhibition of BSTs-decreases. Therefore, to elucidate the components in WSV affecting BST, we studied the effects of thymol, the second major component of WSV, on body temperature. Thymol ingestion inhibited BSTs-decreases of the wrist and finger. Moreover, changes in BST and CBT by a mixture of thymol and carvacrol were similar to those by WSV. These results suggest that thymol might facilitate heat transfer from the body's core to the surface, and that the combination of thymol and carvacrol greatly contributed to BST- and CBT-changes by WSV.

Keywords: thymol, carvacrol, winter savory, body surface, core, body temperature, human

Introduction

‘Cold sensitivity’ or a ‘feeling of cold’, known as hie-sho in Japanese, is a symptom characterized by feeling a sensation of coldness, particularly in the lower torso and the limbs (Terasawa, 1987). This symptom is thought to be caused by disturbances in the peripheral circulation (Shibahara and Itoh, 1999; Ushiroyama, 2005), and typically accompanies a variety of symptoms including shoulder stiffness, headache, swelling, sleeplessness, frequent urination, peripheral numbness, limb pain, chilblains, and purple fingernails (Miura et al., 2001; Yamato and Aomine, 2002). Taking into account that many females experience cold sensitivity (Takao et al., 2005; Imai et al., 2007), a study investigating the alleviation of cold sensitivity is considered timely.

We reported that a hot water extract of dried leaves of winter savory (*Satureja montana* L.), a Lamiaceae herb, alleviated the decrease in body surface temperature (BST) in people who experience cold sensitivity (Masuda et al., 2011). The volatile fraction of winter savory (WSV) was found to be one of the effective fractions contributing to the following changes in body temperature: an increase in the core body temperature (CBT), an inhibition of decreases in BSTs of the wrist, finger and ankle, and an increase in BSTs of the forehead and neck (Masuda et al., 2013). In addition, the ingestion of carvacrol, a main component of WSV, is reported to increase CBT (tympanic membrane temperature) in people with cold sensitivity (Masuda et al., 2013). However, it did not affect BSTs of the wrist, finger, ankle, forehead and neck. Therefore, we proposed that active components of WSV besides carvacrol contribute to the changes in BSTs.

Thymol, one of the components of WSV, has been used as a spicy flavoring ingredient (Burdock, 2005). Thymol possesses a
variety of pharmacological activities such as antimicrobial, antioxidant, spasmyloptic, antitussive and anti-inflammatory effects (Wagner et al., 1986; Basch et al., 2004; Bozin et al., 2006; Prieto et al., 2007; Anthony et al., 2012; Nikolić et al., 2014). Peixoto-Neves et al. (2010), as well as Kawasaki and Matsubara (2007), reported that thymol induced vasodilation in isolated rat aorta. In addition, Aftab et al. (1995) reported that administration of thymol lowered the blood pressure of anaesthetized rats. These effects might be caused by blocking calcium influx through the cell membrane and/or the regulation of calcium sensitivity in the cell. These reports suggest that thymol participates in the circulatory system; however, the effects of thymol on the blood flow in skin vessels, which plays an important role in the regulation of BST (Kanosue, 2009), remain unknown. Furthermore, taking into account that blood flow is reported to be separately controlled in each body part (Iriki, 1983; Franchili and Cowley, 2004; Goldstein, 2004), studies determining whether thymol could affect skin blood flow, followed by changes in BST, are considered useful.

The aim of the present study was to elucidate the components in WSV that affect BSTs (wrist, finger, ankle, forehead and neck). First, we studied the effects of thymol, the second major component of WSV, on BST and CBT in subjects experiencing cold sensitivity (in Exp. 1). Next, to elucidate the contribution of a mixture of thymol and carvacrol to changes in BST and CBT by WSV, we compared the changes in body temperature after ingestion of the mixture to those following ingestion of WSV (in Exp. 2). Then, to investigate the relationship between the mixture dose and changes in BST and CBT, we carried out Exp. 3 using half the mixture dose used in Exp. 2.

Materials and Methods

This study was conducted with the approval of the Ethics Committee at The University of Shiga Prefecture in accordance with the principles of the Declaration of Helsinki. Informed consent was obtained from all subjects after a full explanation of the content of the consent form.

Subjects for measurement of BST, CBT and blood flow The time-course of changes in human body temperature after sample ingestion was measured as follows: the ingestion of thymol or placebo (in Exp. 1), a mixture of thymol and carvacrol, WSV or placebo (in Exp. 2), and the mixture of thymol and carvacrol (each dose is half of that used in Exp. 2) or placebo (in Exp. 3). Subjects were selected using a questionnaire focusing on the symptoms of cold sensitivity as reported by Takumi et al. (2010). The test was carried out in nine, nine, and seven Japanese female volunteers for Exps. 1, 2 and 3, respectively. Subject characteristics (values are means ± SD) in Exp. 1 were as follows: age, 20 – 22 years; height, 155.3 ± 4.1 cm; body weight, 49.9 ± 5.1 kg; and body mass index (BMI), 20.6 ± 1.6. In Exp. 2, values were as follows: age, 19 – 21 years; height, 155.2 ± 3.2 cm; body weight, 47.3 ± 3.1 kg; and BMI, 19.6 ± 1.2. In Exp. 3, values were as follows: age, 19 – 22 years; height, 155.1 ± 5.7 cm; body weight, 47.6 ± 4.0 kg; and BMI, 19.8 ± 1.2. In order to avoid fluctuations by menstrual cycle, we carried out Exps. 1, 2 and 3 during the luteal phase.

Test protocols for measurement of BST, CBT and blood flow Experiments 1, 2 and 3 were carried out in December, in early June to late July and in October, respectively. To reduce the influence of seasonal changes in body temperature and to accentuate the differences in body temperature between each sample, room temperatures were set as follows: in Exp. 1, 23 ± 0.5°C, and in Exp. 2 and 3, 24 ± 0.5°C. Room humidity was maintained at approximately 50% in all experiments. Subjects sat for > 30 min in the same temperature-controlled room with approximately 50% humidity before sample ingestion.

This study was conducted using a randomized, double-blind, placebo-controlled, single-ingestion crossover design. Each measurement was taken between 09:00 and 13:00 to avoid the influence of diurnal variations in body temperature. Subjects were forbidden from eating and drinking anything other than water after waking on the day of the experiment. Ingestion of alcohol or irritant foods such as spices on the day before testing was prohibited. Each subject wore the same clothing (sweatsuit and socks) for each measurement to exclude the influence of clothing on body temperature.

The time-course of changes in BSTs of the wrist, middle finger, ankle, forehead and neck was measured using two thermometers (BTH-601, Bio Research Center Co., Ltd., Nagoya, Japan; AM-8000 K, Anritsu Meter Co., Ltd., Tokyo, Japan). Thermoprobes were fixed on each skin surface using surgical tape. The time-course of changes in temperature of the tympanic membrane was measured using an earplug with a thermistor thermometer (ITP010-27, Nikkiso-Therm Co., Ltd., Tokyo, Japan). The time-course of changes in blood flow on the tip of the ring finger was measured using an ALF 21D Laser Doppler Flowmeter (Advance Co., Ltd., Tokyo, Japan). Each temperature and blood flow was measured every minute from 10 min before to 60 min after sample ingestion.

One sample was ingested on the first day and the other sample was ingested on the second day. The order of ingestion was randomized. To exclude the effects of the first sample, we arranged a washout period of > 1 day. The washout period was determined after consideration of the absorption, metabolism and urinary excretion of thymol and carvacrol in rats (Austgulen et al., 1987). All subjects were in good health throughout the study, and there were no complaints of discomfort after ingestion of any sample.

Samples Both thymol and carvacrol were obtained as commercially available ingredients (food-additive grade; Sigma-Aldrich, St. Louis, MO, USA). WSV, essential oil of winter savory, was prepared as follows. Briefly, steam was introduced to dried winter savory leaves (200 g, production area: Albania) in a steam distillation apparatus. The condensate was obtained by cooling of the evaporated steam. The oil of WSV (1.6 g, yield against winter savory leaves: 0.8%) was then separated from the condensate.
The clinical study was conducted by ingestion of a hard capsule made of gelatin (Matsuya Corporation, Osaka, Japan) containing the sample. In Exp. 1, the capsule contained thymol (0.095 mg) in corn oil (100 mg, 0.9 kcal, J-Oil Millus, Tokyo, Japan). In Exp. 2, the capsule contained a mixture of thymol (0.095 mg) and carvacrol (0.44 mg) in corn oil (100 mg), or WSV (1.06 mg) in corn oil (100 mg). In Exp. 3, the capsule contained a mixture of thymol (0.047 mg) and carvacrol (0.22 mg) in corn oil (100 mg). In Exps. 1, 2 and 3, the capsule contained corn oil (100 mg) as a placebo. Each amount of thymol (0.095 mg) and carvacrol (0.44 mg) was the same as the amounts contained in WSV (1.06 mg). Similarly, each amount of thymol (0.047 mg) and carvacrol (0.22 mg) corresponded to the amounts contained in WSV (0.53 mg). No odor was detected outside each capsule. Each capsule was ingested with 37°C water (50 mL).

**Measurement of thymol and carvacrol in WSV**  Quantitative analyses of thymol and carvacrol were carried out using a high-performance liquid chromatography system as described previously (Masuda et al., 2011).

**Statistical analyses**  Data are means ± SEM. In Exps. 1 and 3, the time-course data were evaluated by a two-way repeated measures analysis of variance (ANOVA) using GraphPad Prism version 5 (GraphPad Software, Inc., CA, USA). Comparisons between treatment groups at predetermined times were evaluated by a paired t-test. In Exp. 2, the time-course data were evaluated by contrast analysis. For comparison between three groups at predetermined times, one-way ANOVA with Tukey’s multiple-comparison post-hoc test was conducted. Contrast analysis was carried out with the StatView software package (Macintosh Version J 5.0; Abacus Concepts, Berkeley, CA, USA) and the Super ANOVA software package (Macintosh Version 1.11; Abacus Concepts, Berkeley, CA, USA). Statistics of the one-way ANOVA and post-hoc test were calculated with GraphPad Prism version 5. A probability level of < 0.05 was considered to indicate significance.

**Results**

**Changes in BST, CBT and blood flow after the ingestion of WSV**

Changes in BST, CBT and blood flow after the ingestion of a mixture of thymol and carvacrol, WSV or placebo in Exp. 2 The ingestion of a mixture or WSV significantly inhibited the decrease in BSTs of the wrist and finger (Fig. 3A and B).

Ingestion of WSV significantly inhibited the decrease in BST of the ankle; however, ingestion of the mixture did not (Fig. 3C).

As shown in Fig. 3D and E, there was a significant difference between the WSV- and the placebo-ingestion groups in the time-course of changes in BSTs of the forehead and neck, but not between the mixture- and the placebo-ingestion groups. On the other hand, the mean of changes in BSTs of the forehead and neck after ingestion of the mixture or WSV was significantly higher than that after ingestion of the placebo.

In the time-course of changes in tympanic membrane temperature (CBT), there were no significant differences between the three groups (Fig. 3F). However, the mean of changes in the tympanic membrane temperature after ingestion of WSV was significantly lower than that after ingestion of the mixture or placebo.

As for the mean of changes in BSTs of the wrist, the inhibition intensity (against the temperature decrease) increased in the following order: the placebo-ingestion group < the mixture-ingestion group < the WSV-ingestion group (Fig. 3A). In addition, as for the mean of changes in BST of the forehead, the temperature increased in the following order: the placebo-ingestion group < the mixture-ingestion group < the WSV-ingestion group (Fig. 3D).

Blood flow of the finger after the ingestion of the mixture or WSV was significantly higher than that after ingestion of placebo (Fig. 4). As for the mean of changes in blood flow of the finger, the intensity of inhibition (against the blood-flow decrease) increased in the order: the placebo-ingestion group < the mixture-ingestion group < the WSV-ingestion group.

**Changes in BST, CBT and blood flow after the ingestion of a mixture of thymol and carvacrol (each dose half) that used in Exp. 2 or placebo in Exp. 3** Comparing the time-course of changes in BSTs of the wrist, finger, ankle, forehead and neck, and CBT (tympanic membrane temperature) after ingestion of WSV in Exp. 2 with that from a previous study (Masuda et al., 2013), CBT showed a different tendency as follows: in the previous study, CBT after ingestion of WSV was significantly higher than that after ingestion of the placebo; whereas, in Exp. 2, CBT after ingestion of WSV showed a lower tendency compared to that after ingestion of the placebo. In the previous study, the dose of WSV was half that used in Exp. 2. Taking into account that the difference in dose might lead to different results, we carried out Exp. 3 with half the dose of mixture used in Exp. 2. That is, each amount of thymol and carvacrol was the same as the amounts contained in WSV in the previous study.

BSTs of the wrist, ankle, forehead and neck after ingestion of the mixture were significantly higher than those after ingestion of the placebo (Fig. 5A and C – E). As for CBT, the value after
Fig. 1. Time-course of changes in body temperature before and after the ingestion of thymol: THY or placebo in Exp. 1. Values are differences from the mean temperature at 0 min, given as means ± SEM (n = 9). Time × treatment effects with different superscript letters, $p < 0.05$ for −10 to 60 min by a two-way repeated measures ANOVA. * indicates $p < 0.05$ by a paired $t$-test.

Fig. 2. Time-course of changes in blood flow of the finger before and after the ingestion of thymol: THY or placebo in Exp. 1. Values are differences from the mean temperature at 0 min, given as means ± SEM (n = 9). Time × treatment effects with different superscript letters, $p < 0.05$ for −10 to 50 min by a two-way repeated measures ANOVA. * indicates $p < 0.05$ by a paired $t$-test.
ingestion of the mixture was significantly higher than that after ingestion of the placebo (Fig. 5F).

As for the BST of the finger and blood flow of the finger, there were no significant differences between the mixture- and the placebo-ingestion groups (Figs. 5B and 6).

**Discussion**

We observed that the ingestion of thymol, the second major
component of WSV, induced the inhibition of the decrease in BSTs of the wrist and finger, and in the blood flow of the finger in Exp. 1. In addition, the mixture of thymol and carvacrol also inhibited the decrease in BSTs of the wrist and/or finger (in Exps. 2 and 3). These results suggest that thymol is involved in the changes in peripheral BSTs.

Skin blood flow, which plays an important role in the maintenance of body temperature, is innervated mainly by the sympathetic nervous system in skin blood vessels (Berne and Levy, 2000; Shen et al., 2009). Skin blood flow is controlled by two types of sympathetic nervous systems in skin blood vessels: the sympathetic noradrenergic vasoconstrictor nerves and sympathetic active vasodilator nerves (Iriki, 1989 and 2003; Kellogg, 2006; Hodges and Johnson, 2009). In addition, it has been reported that each skin area is regulated by different components of the sympathetic nervous system (Blair et al., 1960; Roddie, 1983 and 2003; Iriki, 1989; Kihara et al., 2004). For example, the blood flow in skin vessels is regulated separately from that in the coronary and intestinal vessels. Thus, although quantitative studies between thymol and carvacrol are needed to evaluate the effects on the sympathetic nerve fibers in skin blood vessels, it is assumed that the actions of thymol and carvacrol on the skin vessels might be different from each other.

Heat, which is generated in the body core, is transferred to the body surface through blood flow to maintain the balance between heat production and heat loss, and to control the body temperature (Charkoudian, 2003; Guyton and Hall, 2006). According to the data in Exp. 1, the ingestion of thymol inhibited the decrease in BSTs of the wrist and finger. In addition, in Exp. 2 and 3, the ingestion of a mixture of thymol and carvacrol increased the body areas in which BST-changes occurred compared to the BST after the ingestion of thymol (in Exp. 1). On the other hand, CBT (tympanic membrane temperature) after the ingestion of thymol tended to be lower than that after the ingestion of placebo (in Exp. 1). However, CBTs after the ingestion of the mixture were similar (in Exp. 2) or significantly higher (in Exp. 3) compared to those after the ingestion of placebo. We previously reported that the ingestion of carvacrol contributed to the increase in CBT but not to the changes in BST (Masuda et al., 2013). Therefore, comparing CBTs in Exp. 2 and 3 with CBT in Exp. 1, the differences in CBTs could be attributed to the action of carvacrol (by generating heat in the body core). Moreover, as for changes in BSTs in Exp. 2 and 3, taking into account that carvacrol was not involved in BST-changes, heat generated in the body core could be transferred to the body surface by the action of thymol.

The body area, in which body-temperature changes occurred, varied with the dose of the mixture (thymol and carvacrol). As for the limbs, in Exp. 2, the ingestion of the mixture significantly inhibited the decrease in BSTs of the wrist and finger, but did not
Contribution of Volatile Components in Winter Savory to Changes in Body Temperature in Humans

Fig. 5. Time-course of changes in body temperature before and after the ingestion of a mixture (thymol: THY and carvacrol: CAR, each dose is half that used in Exp. 2) or placebo in Exp. 3. Values are differences from the mean temperature at 0 min, given as means ± SEM (n = 7). Time × treatment effects with different superscript letters, p < 0.05 for -10 to 60 min by a two-way repeated measures ANOVA. * indicates p < 0.05 by a paired t-test.

Fig. 6. Time-course of changes in blood flow of the finger before and after the ingestion of a mixture (thymol: THY and carvacrol: CAR, each dose is half that used in Exp. 2) or placebo in Exp. 3. Values are differences from the mean temperature at 0 min, given as means ± SEM (n = 7). Time × treatment effects with different superscript letters, p < 0.05 for -10 to 60 min by a two-way repeated measures ANOVA. * indicates p < 0.05 by a paired t-test.
inhibit the decrease in BST of the ankle. On the other hand, in Exp. 3, the ingestion of the mixture (half the dose used in Exp. 2) significantly inhibited the decrease in BSTs of the wrist and ankle, but did not inhibit the decrease in BST of the finger. BST is regulated through changes in skin blood flow. As for the regional differences in the limb, the blood flow of the hand was reported to be higher than that of foot (Tsuchida, 1987). In addition, Ogawa and Low (1997) reported that the blood flow of the finger began to increase before the blood flow of the toe at a lower ambient temperature. Accordingly, BSTs of the wrist and finger are considered to be more easily affected by blood flow than BSTs of the ankle and toe. On the other hand, according to the data of Takumi et al. (2010), the ingestion of α-glucosylhesperidin significantly inhibited the decrease in BSTs of the finger and toe, but did not inhibit that of the wrist and ankle. The cause of regional differences between the wrist and finger, or the ankle and toe has not been reported. In our study, the dose of mixture used in Exp. 2 differs from that in Exp. 3. Therefore, the difference in the balance between heat production and heat transfer (which might be induced by ingesting carvacrol and thymol, respectively) could induce the regional difference in BSTs between the wrist, finger and ankle. However, further studies are needed to assess the quantitative relationships between the sample dose and the affected body areas in which the body-temperature changes occur.

In Exp. 2, comparing the effects of a mixture of thymol and carvacrol on BSTs of the wrist, finger, forehead and neck with those of WSV, the former approached the latter. In addition, we compared the changes in body temperature after ingestion of the mixture of thymol and carvacrol in Exp. 3 (half the dose used in Exp. 2) with those after ingestion of WSV (the amounts of thymol and carvacrol in WSV are the same as those in the mixture used in Exp. 3) reported by Masuda et al. (2013). As a result, the ingestion of the mixture (in Exp. 3) and that of WSV (Masuda et al., 2013) similarly inhibited the decreases in BSTs of the wrist and ankle. Moreover, the ingestion of mixture (in Exp. 3) and that of WSV (Masuda et al., 2013) similarly elevated the BSTs of the forehead and neck, and CBT (tympanic membrane temperature). On the other hand, the ingestion of the mixture did not inhibit the decrease in BST of the finger (in Exp. 3), whereas the ingestion of WSV did (Masuda et al., 2013). These results suggest that both thymol and carvacrol greatly contribute to the changes in body temperature by WSV. However, the effects of ingestion of the mixture on BST and CBT were not the same as those by WSV ingestion; therefore, further study is needed to elucidate the other components of WSV.

As for seasonal changes in metabolic and body temperature responses, van Ooijen et al. (2004) reported that the magnitude of the metabolic response differed between summer and winter. However, the decrease in BST was not significantly different between seasons because of the regulation of the environmental conditions such as room temperature. In our study, Exps. 1, 2 and 3 were performed in December, in early June to late July and in October, respectively. We measured the body temperature under the conditions that BST decreased gradually to the room temperature. Therefore, taking into account the outside temperature, we changed the room temperature. Thus, the regulation of room temperature corresponding to season might reduce the seasonal differences.

In conclusion, we clarified that the ingestion of thymol, the second major component of WSV, induced the changes in peripheral BSTs. In addition, we demonstrated that the combination of thymol with carvacrol, the main component of WSV, increased the body areas in which the changes in body temperature are observed. The influenced body areas varied with the dose of thymol and carvacrol. These data suggest that both thymol and carvacrol greatly contributed to changes in body temperature by WSV. Additionally, the balance between heat production and heat transfer, which might be induced by the ingestion of carvacrol and thymol, respectively, could produce the regional differences in the temperature changes in the human body.

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References


Contribution of Volatile Components in Winter Savory to Changes in Body Temperature in Humans

Satureja montana


