Soybean Isoflavones Eliminate Nifedipine-Induced Flushing of Tail Skin in Ovariectomized Mice

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Abstract. Hot flushes are one of the most frequent symptoms in menopausal women. We investigated effect of soybean isoflavones (Soyaflavone HG) on nifedipine-induced flushing in ovariectomized mice. Ovariectomy markedly aggravated nifedipine-induced increase in tail skin temperature. Soyaflavone HG (10 mg/kg, p.o., once a day for 5 days) inhibited nifedipine-induced flushing in ovariectomized mice. The inhibitory effect of Soyaflavone HG was significantly reversed by an estrogen-receptor antagonist, ICI 182,780, suggesting that Soyaflavone HG prevents nifedipine-induced flushing partially through estrogen receptors. We presented the experimental evidence suggesting that soybean isoflavones including Soyaflavone HG have the benefits for menopausal hot flushes.

Keywords: soybean isoflavone, menopausal flushing, ovariectomy

Hot flushes, which are characterized by a transient episode of flushing, sweating and a sensation of heat, occur because of estrogen deficiency in menopausal women (1). Hot flushes have been linked to a transient disturbance of the thermoregulatory mechanism that activates a heat-loss response including increased peripheral blood flow (2).

Flushing related to Ca²⁺ channel antagonists in women resembles menopausal hot flushes in symptoms (2). Moreover, earlier studies showed that the incidence of flushing induced by Ca²⁺ channel antagonists is high in middle-aged women (3, 4). We previously demonstrated that ovariectomy aggravated nifedipine-induced flushing of tail skin in mice and that this event was blocked by estradiol replacement (5). From these results, we suggested that estrogen deficiency accelerates the increased local blood flow due to vasodilator effects of nifedipine on the local, rather than systemic, vasculature. Thus we propose that nifedipine-induced flushing in ovariectomized mice may be a candidate for an experimental model of menopausal flushing.

Phytoestrogens are natural nonsteroidal plant-derived compounds. Current interests focus on isoflavones among the major classes of phytoestrogens. Soy products are rich sources of isoflavones including genistein, daidzein, and glycitein. Isoflavones have estrogen-like chemical structures and bind to estrogen receptors (6). Several double-blind, randomized placebo-controlled trials have assessed the role of soybean isoflavone products in alleviating hot flushes. On the basis of 11 clinical studies, Kronenberg and Fugh-Berman reported that soybean products show moderate effects, especially on severe hot flushes (7). However, the evaluation and comparison of these studies are considered to be difficult because of large variations in products, dosage, assessment of hot flushes, and menopausal status of patients (7). Research on the efficacy of these products is also limited to clinical observations. The present study was, therefore, aimed at investigating the effect of soybean isoflavones on nifedipine-induced flushing in ovariectomized mice, as a potential model of menopausal flushing.

Female ICR mice weighing 25 – 30 g were purchased from Kyudo (Kumamoto). The mice were maintained on a 12-h light/dark schedule (lights on, 7:00 a.m.) at a temperature of 24 ± 1°C with free access to food and water. Mice underwent a bilateral ovariectomy or sham-operation under sodium pentobarbital anesthesia.
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(50 mg/kg, i.p.) as previously described (8). All animal experiments were performed in accordance with The Japanese Pharmacological Society “Guiding Principles for the Care and Use of Laboratory Animals” and were approved by the Laboratory Animal Care and Use Committee of Fukuoka University.

Soyaflavones extracted from soybean hypocotyl (Soyaflavone HG; Fuji Oil, Osaka) containing 40.74% isoflavones were used. The composition of isoflavones is shown in Table 1. Soyaflavone HG, nifedipine (Wako, Osaka), and ICI 182,780 (Tocris, Ellisville, MO, USA) were administered in a volume of 0.1 ml/10 g body weight. Saline as vehicle or Soyaflavone HG (5 or 10 mg/kg, p.o.) dissolved in saline was administered once a day for 5 consecutive days starting 24 days after ovariectomy. The estrogen-receptor antagonist ICI 182,780 (0.1 mg/kg, i.p.) was administered for 4 consecutive days from 25 to 28 days after ovariectomy. Estradiol valerate (Pelanin Depot, Mochida Pharmaceutical, Tokyo) (1.0 mg/ml per kg) or vehicle (sesame oil) was injected into the thigh muscle once per week for 3 weeks starting 7 days after ovariectomy. Three hours after the 5th administration of vehicle (sesame oil) was injected into the thigh muscle once per week for 3 weeks starting 7 days after ovariectomy. In sham-operated mice, subchronic administration of 5 or 10 mg/kg of Soyaflavone HG caused no changes in 

Tail skin temperature was measured according to a procedure described previously (5). Mice were restrained in a holder in a conscious state. After a 10-min period of adaptation, vehicle was administered i.p. and tail skin temperature was measured with a thermo tracer (TH5108ME; NEC San-ei, Tokyo) for 30 min. Then, nifedipine (50 µg/kg) was administered i.p. and tail skin temperature was measured for a subsequent 30 min. The data were analyzed with TH51-701 and TH51-723 (NEC San-ei). Changes in tail skin temperature were assessed using ΔTST. ΔTST = (the average tail skin temperature in the period from 10 to 25 min after injection of nifedipine) – (the basal tail skin temperature during the same period after injection of the vehicle). Throughout the recording period, the room temperature was maintained at 24 ± 1°C. Blood pressure was measured by the tail-cuff method (MK-2000; Muromachi Kikai Co., Ltd., Tokyo).

Statistical analysis was performed using the unpaired Student’s t-test or one-factor analysis of variance (ANOVA) followed by Scheffe’s F test. A value of P<0.05 was considered significant. The intraobserver or interobserver variation was <5% in each experiment.

In saline-treated mice, 50 µg/kg of nifedipine significantly increased ΔTST to 0.39 ± 0.09°C in ovariectomized mice, but this elevation was not observed in sham-operated mice (−0.05 ± 0.08°C) (Fig. 1). Nifedipine (50 µg/kg) had no effect on blood pressure in either sham-operated or ovariectomized mice at any time in the period lasting 10 to 25 min after injection (127.8 ± 3.6 and 117.7 ± 6.3 mmHg in sham-operated and ovariectomized mice, respectively). In sham-operated mice, subchronic administration of 5 or 10 mg/kg of Soyaflavone HG caused no changes in ΔTST increased by nifedipine. In ovariectomized mice, nifedipine-induced increase in ΔTST was dose-dependently attenuated by Soyaflavone HG and completely blocked at 10 mg/kg (Fig. 1). Neither 5 nor 10 mg/kg of Soyaflavone HG influenced blood pressure in sham-operated and ovariectomized mice with or without nifedipine (data not shown). A slight decrease in blood pressure was observed at doses of more than 10 mg/kg. Therefore, we employed here 10 mg/kg of Soyaflavone HG contains a total of 40.74% isoflavones.

<table>
<thead>
<tr>
<th>Isoflavone form</th>
<th>g/kg</th>
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<tbody>
<tr>
<td>Daidzin</td>
<td>239.4</td>
</tr>
<tr>
<td>Genistin</td>
<td>73.4</td>
</tr>
<tr>
<td>Glycitin</td>
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</tr>
<tr>
<td>Acetyl glycitin</td>
<td>9.3</td>
</tr>
<tr>
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<tr>
<td>Glycitin</td>
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</table>

Soyaflavone HG contains a total of 40.74% isoflavones.

Fig. 1. Dose-dependent effect of Soyaflavone HG on the change in tail skin temperature (ΔTST) induced by nifedipine in sham-operated (sham, open bar) and ovariectomized (ovx, closed bar) mice. Values represent the mean ± S.E.M. for 6 to 11 animals. ΔTST was calculated as follows: ΔTST = (the average TST from 10 min to 25 min after injection of nifedipine) – (the basal TST during the same period after injection of the vehicle). *P<0.01, significant difference from each corresponding vehicle-treated group.
As shown in Fig. 2, pretreatment with estradiol (1.0 mg/kg, i.m., once per week for 3 weeks) abolished nifedipine-induced increase of TST in ovariectomized mice. This inhibitory effect was comparable to that observed with 10 mg/kg of Soyaflavone HG. An estrogen-receptor antagonist, ICI 182,780 (0.1 mg/kg, i.p., once a day for 4 days), significantly reversed the inhibitory effect of Soyaflavone HG on nifedipine flushing (Fig. 2).

Recently, we have shown that nifedipine-induced flushing of tail skin is aggravated in ovariectomized mice. It is conceivable that the loading of nifedipine unmasks a pre-existing destabilized thermoregulatory system under conditions where estrogen is lacking. This phenomenon may be available for a mouse model of menopausal hot flushes. In the present study, nifedipine-induced increase in tail skin temperature was dose-dependently attenuated by a subchronic administration of Soyaflavone HG in ovariectomized mice. The observed effects appear to be unrelated to a decrease in blood pressure, because nifedipine and Soyaflavone HG did not affect blood pressure in either sham-operated or ovariectomized mice. The basal vascular tonus increased by ovariectomy is considered to induce the larger response to vasodilatory action of nifedipine in ovariectomized mice than that in sham-operated mice. This leads to the differences in nifedipine-increased peripheral blood flow between two groups. The protective effect of Soyaflavone HG may be related to an improvement of the abnormal local vascular tonus and autoregulation. Soybean isoflavones are heterocyclic phenols with structural similarity to estrogen-17β, and they can mimic the hormonal properties of estrogen by binding to estrogen receptors (6). Estrogen replacement almost reversed the aggravation of nifedipine-induced flushing due to ovariectomy. ICI 182,780 employed here has been reported to completely prevent uterotrophic activity induced by estradiol (9). The inhibitory effect of Soyaflavone HG on nifedipine-induced flushing was significantly reversed by pretreatment with ICI 182,780, indicating that an activation of estrogen receptors may, at least in part, contribute to the anti-flushing action of Soyaflavone HG.

Soybean isoflavones have received much attention as an alternative to conventional estrogen replacement therapy, largely because supplementing the diet with soybeans is a benign intervention. The present study provided experimental evidence supporting that soybean isoflavone extracts including Soyaflavone HG have benefits for the treatment of menopausal hot flushes.

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References