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Short communication

Kamishoyosan reduces conditioned fear-induced freezing behavior in socially isolated ovariectomized rats

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ABSTRACT

In the present study, we investigated the effect of kamishoyosan (KSS) on conditioned fear-induced freezing in ovariectomized (OVX) rats. Socially isolated OVX rats showed the longest freezing time among the following four groups: group-housed sham-operated (Sham), isolated Sham, group-housed OVX, and isolated OVX rats. Repeated oral administration of KSS (30–300 mg/kg) reduced conditioned fear-induced freezing in socially isolated OVX rats. The reduction of freezing by KSS was reversed by flumazenil (3 mg/kg) and bicuculline (3 mg/kg). These findings suggest that the $GABA_A$-benzodiazepine receptor complex is involved in the anxiolytic effect of KSS in socially isolated OVX rats.

Kamishoyosan (KSS), a Kampo medicine, is widely used to treat menopausal psychotic symptoms in women. KSS has been reported to improve anxiety and depression in midlife women with psychological symptoms (1). Moreover, KSS improves sleep disturbance in Japanese peri- and post-menopausal women (2). Although it is likely that KSS improves various psychiatric symptoms in midlife female patients, the mechanisms of these effects remain unclear. KSS has been shown, in social interaction testing, to exert an anxiolytic effect through γ-aminobutyric $acid_A$ ($GABA_A$)-benzodiazepine receptor stimulation in male mice (3). However, the anxiolytic effect of KSS in a menopausal experimental model has not been reported.

During menopause, women are more likely to develop anxiety and/or depressive symptoms (4). Midlife changes in hormonal and psychosocial environments contribute to the risk of peri-menopausal depression (5). Moreover, stress exacerbates somatic symptoms of menopause, increasing the risk of recurrence of mood disorder in peri-menopausal patients (6). Especially, the loneliness during female midlife (45–65) and older (over 65) can function as the serious psycho-social stressor raised by diminishing self-esteem with the launching of children (e.g., empty-nest syndrome), grief caused by the loss of parents/close persons, or limited contacts with acquaintances or friends as the empirical life events (7). These observations indicate that psychosocial environments and stress, as well as hormonal changes, play an essential role in the development and exacerbation of anxiety and/or depressive symptoms in peri-menopausal patients. Ovariectomized (OVX) rats are the most common model used in research on menopausal symptoms. The conditioned fear stress test is an established murine anxiety model. Therefore, we investigated the effect of KSS on conditioned fear-induced freezing in socially isolated OVX rats.

Female Sprague–Dawley rats, aged 8 weeks, were obtained from the Kyudo Co. (Tosu). Animals were housed either in social isolation (one rat per cage) or in social groups (four rats per cage) under standardized lighting conditions (lights on 07:00–19:00) at a constant temperature (23 ± 2 °C) with food and water available *ad libitum*. All procedures regarding animal care and use were carried out based on regulations established by the Experimental Animal Care and Use Committee at Fukuoka University, Japan.

KSS (Lot. No. 2020024010) was a generous gift from Tsumura & Co. (Tokyo) and was a dried extract of the following raw materials:

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E-mail address: n-egashi@pharm.med.kyushu-u.ac.jp (N. Egashira).
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Bupleuri Radix (*Bupleurum falcatum*, 3.0 g), Paeoniae Radix (*Paeonia lactiflora*, 3.0 g), Atractylodis Rhizoma (*Atractylodes ovate*, 3.0 g), Angelicae Radix (*Angelica acutiloba*, 3.0 g), Hoelen (*Poria cocos*, 3.0 g), Gardeniae Fructus (*Gardenia jasminoides*, 2.0 g), Moutan Cortex (*Paeonia suffruticosa*, 2.0 g), Glycyrrhizae Radix (*Glycyrrhizae uralensis*, 1.5 g), Zingiberis Rhizoma (*Zingiber officinale*, 1.0 g), and Menthae Herba (*Menthae arvensis*, 1.0 g). Each plant material was authenticated by identification of external morphology and marker compounds of plants specimens, according to the methods of the Japanese Pharmacopoeia and Tsumura & Co.'s standard. The medical herbs were extracted with purified water at 95 °C for 1 h, and the extraction solution was separated from the insoluble waste and concentrated by removing water under reduced pressure. Spray drying was used to produce a dried extract powder, which was subsequently suspended in distilled water. The yield of the extract was about 17.8%, and extracts were manufactured in compliance with the Japanese Pharmacopoeia (Sixteenth Edition, JP16) under Good Manufacturing Practice (GMP).

Diazepam (Wako Pure Chemical Industries Ltd., Osaka) was suspended in 0.5% sodium carboxymethylcellulose solution. Flumazenil (Wako Pure Chemical Industries, Ltd.) and bicuculline (Sigma–Aldrich, St. Louis, MO, USA) were suspended in 1% Tween 80 solution.

At 8 weeks of age, rats were anesthetized with intra-peritoneal (i.p.) sodium pentobarbital (50 mg/kg; Tokyo Kasei, Tokyo) and underwent bilateral ovariectomy. In sham-operated (Sham) groups, rats underwent the same incisions and the ovaries and fallopian tubes were exposed and then replaced in the abdominal cavity before the muscle and skin were closed. For recovery, animals were housed for 3 weeks prior to testing.

On the first test day, each rat was placed in a shock chamber (30 × 30 × 30 cm) with a grid floor and allowed to habituate for 5 min. The next day, rats were individually subjected to inescapable electric foot shock (1 mA of scrambled shock, duration of 4 × 5 s and interval of 15 s) in the same chamber, and were removed from the shock chamber after receiving the electric shock. The electric shock was provided by a shock generator (Model SGS-002; Muromachi Kikai Co., Ltd., Tokyo). Twenty-four hours after the shock, rats were again placed in the shock chamber and the duration of freezing behavior within a 5-min period was recorded. Freezing was defined as the absence of all observable movements of the skeleton and the vibrissae, except for those related to respiration.

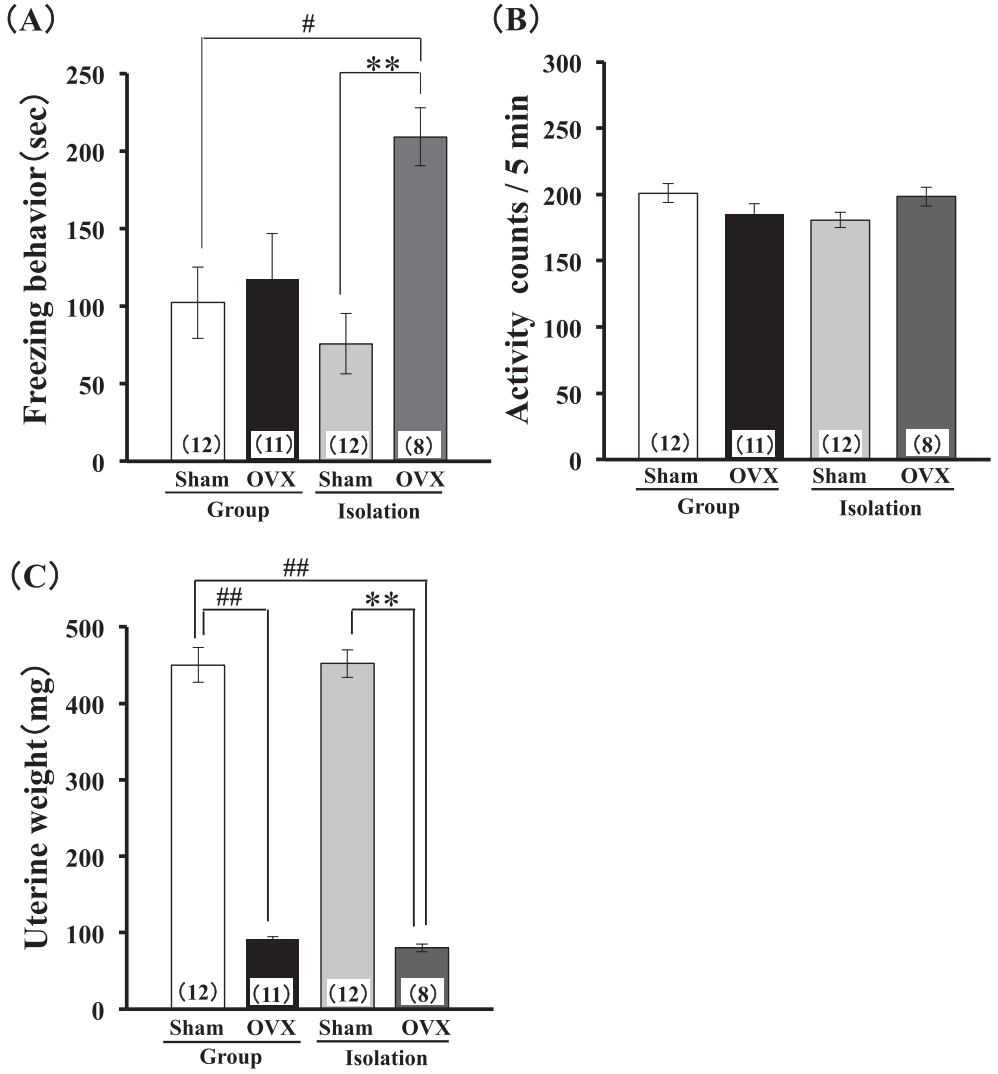

Fig. 1. Effect of social isolation on freezing behavior (A), locomotor activity (B), and uterine weight (C) in sham-operated (Sham) and ovariectomized (OVX) rats. Values are expressed as the mean ± SEM. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ compared with group-housed Sham rats, $^{**}P < 0.01$ compared with isolated Sham rats. The number of rats per group is shown at the base of each column.