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NOTE

The antidepressant effect of secoisolariciresinol, a lignan-type phytoestrogen constituent of flaxseed, on ovariectomized mice

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Abstract Secoisolariciresinol (SECO) is a natural lignan-type phytoestrogen constituent mainly found in flaxseed. It can be metabolized in vivo to mammalian lignans of enterodiol and enterolactone, which have been proven to be effective in relieving menopausal syndrome. Depression is one of the most common symptoms of menopausal syndrome, and is currently treated with estrogen replacement and antidepressant therapy. However, due to the serious side-effects of such agents, there are urgent needs for safer and more tolerable treatments. In this paper, using two classical depression models, the forced swimming test and the tail suspension test, we report the antidepressant effect of SECO on ovariectomized (OVX) mice by intragastric administration for 14 consecutive days at doses of 5, 10 and 20 mg/kg. The results showed that SECO (10 mg/kg) treatment could significantly reduce the duration of immobility of OVX mice in these two models compared with the control group (OVX mice + vehicle), which was similar to the positive control imipramine. In addition, SECO treatment could substantially increase brain monoamine (norepinephrine and dopamine) levels in OVX mice. The present studies showed that SECO can reverse depressive-like behavior and exhibit monoamine-enhancing effects.

Keywords Secoisolariciresinol · Ovariectomized mice · Forced swimming · Tail suspension · Monoamine level

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Introduction

Menopause is a process of gradual decline of ovary function and estrogen level, as an inevitable stage every female must face, often leading to menopausal syndrome with manifestations such as hectic fever, night sweats and depression [1]. To date, hormone replacement therapy (HRT) by estrogen, often supplemented with antidepressant agents, has been the treatment of choice for depression syndrome in menopausal populations. However, long-term use of estrogen poses risks of endometrial and breast cancer [2]. Additionally, almost all the antidepressant agents, such as amitriptyline, fluoxetine and venlafaxine, have side-effects of drowsiness, dry mouth, blurred vision, constipation, rapid heartbeat, difficulty in urinating and so on. Moreover, overdose of antidepressant agents can cause acute poisoning and even death [3]. In recent years, natural products possessing phytoestrogen activities (two-way regulation of estrogen and anti-estrogen) have been the focus in treating menopausal depression symptoms [4].

Secoisolariciresinol (SECO) is a lignan-type phytoestrogen, which can be metabolized to enterodiol (END) and enterolactone (ENL) by the colon microflora of mammals in vivo [5]. END and ENL have been generally taken as phytoestrogens based on their weakly estrogenic and anti-

estrogenic properties [6–9]. Similar to END and ENL, SECO was also proposed to have important biological activities such as the prevention of breast and colon cancers, atherosclerosis and diabetes, and as an anti-oxidant [10–13]. In a previous study, we established a large-scale biotransformation method for SECO production from defatted flaxseed by the bacterial consortium of a human subject [14].

Based on the potential phytoestrogen activities of SECO, we proposed that SECO may be effective for the treatment of menopausal depression, which is a common symptom in menopausal populations. Therefore, in this study, the antidepressant effects of SECO on OVX mice were studied by using two classical animal models, the forced swimming test (FST) and the tail suspension test (TST). Subsequently, we explored the effect of SECO on the brain monoamine levels in OVX mice.

Materials and methods

Chemicals and reagents

Norepinephrine (NE), dopamine (DA), 5-hydroxytryptamine (5-HT), 5-hydroxyindoleacetic acid (5-HIAA) and sodium dodecyl sulfate (SDS) were purchased from Sigma Co. (USA). Anhydrous sodium acetate was obtained from Chemical Reagent Co. (Tianjin, China). Citric acid, dibutylamine, EDTA-Na_2 and potassium citrate were purchased from Chemical Reagent Co. (Beijing, China). Anhydrous methanol was obtained from Hongda Yinyu Co. (Beijing, China). Potassium hydrogen phosphate was purchased from Guoyao Chemical Reagent Co. (Beijing, China). Perchloric acid was obtained from Jinlu Chemical Co. (Shanghai, China).

High-performance liquid chromatography (HPLC) conditions

A Shimadzu LC-6A system with BASLC-4B electrochemical detection, DIKMA Diamonsil (R) column, and Da-lian JS-3070 Chemstation were used for quantitative analysis of monoamine neurotransmitters in brains of model animals. The HPLC mobile phase consisted of sodium acetate–citrate buffer (pH 3.7, containing citric acid 85 mM, anhydrous sodium acetate 100 mM, EDTA-Na_2 0.2 mM) and methanol (85:15).

Animals

Female ICR mice (18–22 g) were purchased from Weitonglihua Biological Co. [Beijing, China, License: SCXK (Jing) 2007-0001]. On arrival, the animals were housed eight per cage and acclimatized to a colony room with controlled ambient temperature (22 ± 1 °C), humidity (50 ± 10 %) and a 12 h natural light/dark cycle. They were fed with standard diet and water ad libitum and were allowed to acclimatize 7 days before they were used. All experiments were conducted in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985), and approved by the Peking University Committee on Animal Care and Use.

Ovariectomy

Experimental mice were ovariectomized (OVX) under nembutal [65 mg/kg, intraperitoneal (i.p.)] anesthesia. The ovariectomy operation was conducted as described in the literature [15]. Sham surgery subjects received the same incisions as the OVX animals but the ovaries were palpated instead of being removed.

Drugs and drug administration

SECO was isolated from defatted flaxseeds using the method of biotransformation set up by our research group [14]; the purity was above 98 % based on HPLC analysis (Fig. 1). Imipramine hydrochloride (Lot: SL04325) was purchased from Sigma Co. (USA). SECO was dissolved in 0.5 % Tween 80 solution for intragastric administration (i.g.), imipramine was dissolved in redistilled water for i.p. injection, and they were diluted to the desired concentration on the day of testing. For the sham operation group and OVX control group, equal volumes of 0.5 % Tween 80 solution without SECO were administered.

The first administration started immediately after surgery. All chemicals were administered once a day (9:00 a.m.–11:00 a.m.) and continued for 2 weeks. The tests were conducted 1 h after the last drug administration.

Locomotor activity test

The assessment of locomotor activity was carried out before forced swimming and TST by using the previous method [16]. Briefly, the locomotor activity of the mice was measured by an ambulometer with five activity

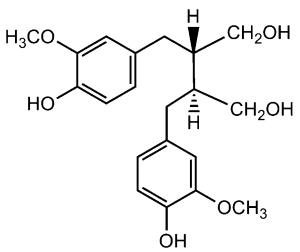

Fig. 1 The structure of SECO isolated from defatted flaxseeds

chambers. Mice were placed in the chambers and their paws contacted or disconnected to active bars producing random configurations that were converted into pulses. The pulses, which were proportional to the locomotor activity of the mice, were automatically recorded as the cumulative total counts of motor activity. The mice were placed in test chambers for acclimatization for 15 min prior to the evaluation and then locomotion counts were recorded for a period of 10 min.

Forced swimming test

The forced swimming test (FST) was similar to that described elsewhere [17]. Briefly, the mice were subjected to a swimming-stress session for 15 min (pre-test), 24 h before being individually placed in glass cylinders (height 25 cm, diameter 10 cm, containing 10 cm of water at 24 ± 1 °C) for 6 min (test). A mouse was judged to be immobile when it ceased struggling and remained floating motionless in the water, making only small necessary movements to keep its head above water. The duration of immobility was recorded during the last 4 min of the 6-min testing period.

Tail suspension test

The tail suspension test (TST) was based on the reported method [18]. The mice were individually suspended 50 cm above the surface of a table, using an adhesive tape placed 1 cm away from the tip of the tail. The mice were considered immobile only when they hung passively and were completely motionless. The duration of immobility was recorded during a test period of 6 min.

Determination of brain monoamine levels and uterus weight

The mice were quickly decapitated after the TST, and their brains and uterus were removed and weighed.

As described in the previous method [15], the brains were placed into A solution (0.6 M perchloric acid containing quantitative internal standard, 4 °C preservation) at 1:5 (w:v) ratio. Brain tissues were dissected and homogenized by an ultrasonic cell disruption apparatus and centrifuged (at 20,000*g*, 4 °C) for 20 min. An aliquot (0.3 ml) of the supernatant was incubated in 0.5 ml of B solution (potassium citrate 20 mmol/l, potassium hydrogen phosphate 300 mM, and EDTA–Na_2 2 mM, 4 °C preservation) for 5 min, and centrifuged (at 20,000*g*, 4 °C) for 20 min. The supernatant was then centrifuged (at 20,000*g*, 4 °C) again for 20 min, and preserved at 4 °C. The content of monoamine neurotransmitters, including NE, DA, 5-HT and 5-HIAA were measured by HPLC.

The index of the uterus [uterine weight (mg)/body weight (g)] was also calculated.

Statistical analysis

The data were expressed as mean ± standard error (SE). Statistical significance of differences was assessed by one-way analysis of variance (ANOVA) followed by Tukey's HSD test with $\alpha = 0.05$. This treatment was carried out using SPSS 13.0 for Windows software.

Results

The effects of SECO on mice locomotor activity

As seen in Fig. 2, the locomotor activity did not show a significant difference between sham-operated group and OVX control group, and neither SECO administration (5, 10 and 20 mg/kg) nor imipramine administration (10 mg/kg, i.p.) showed an influence on the locomotor activity of OVX mice.

The effects of SECO on the duration of immobility of OVX mice in FST and TST

The effects of SECO on the behavior of OVX mice in FST are shown in Fig. 3. It was observed that ovariectomy (control group) could significantly increase the duration of immobility of model mice, resulting in a 23.8 % ($P < 0.01$) increase compared to the sham-operated group. SECO showed significant ability to reduce the immobility duration of OVX mice after administration for 14 consecutive days, resulting in 22.9 % (5 mg/kg, $P < 0.05$), 32.7 % (10 mg/kg, $P < 0.001$) and 20.1 % (20 mg/kg, $P < 0.01$) immobility reductions, respectively, compared

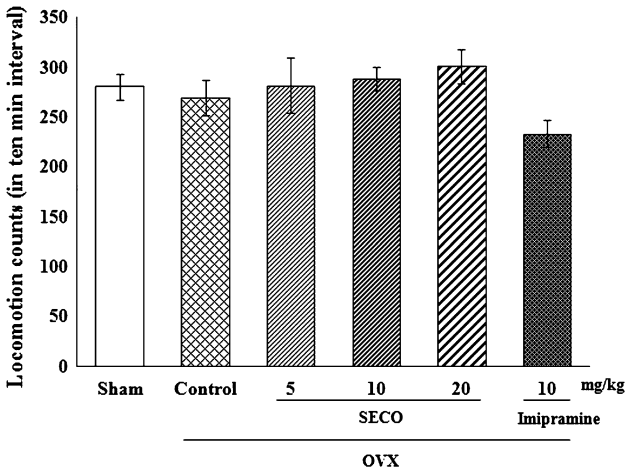

Fig. 2 The effects of SECO on the locomotor activity of OVX mice. Values are mean ± SEM of 12 mice in each group

with the control group. In TST, similar results were observed (Fig. 4): ovariectomy could significantly result in a 32.6 % ($P < 0.01$) increase in the duration of immobility

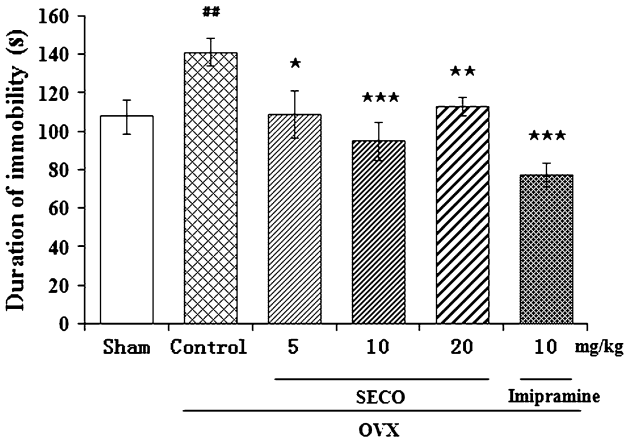

Fig. 3 The effects of SECO on the duration of immobility of OVX mice in FST. Values are mean ± SEM of 12 mice in each group. $^{\#\#}P < 0.01$ versus sham-operated group; $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ versus OVX control group

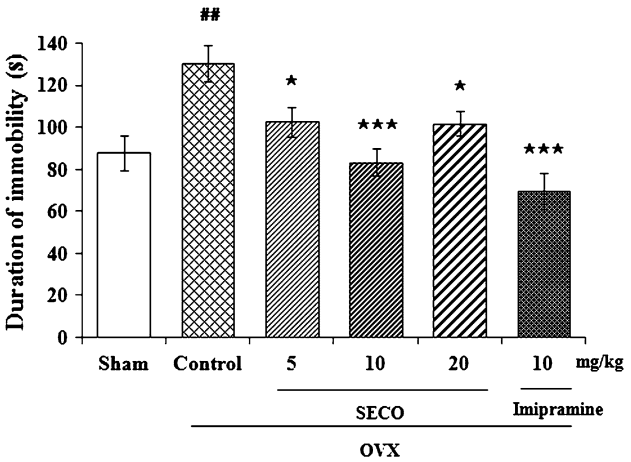

Fig. 4 The effects of SECO on the duration of immobility of OVX mice in TST. Values are mean ± SEM of 12 mice in each group. $^{\#\#}P < 0.01$ versus sham-operated group; $^{*}P < 0.05$ and $^{***}P < 0.001$ versus OVX control group

compared to the sham-operated group. The administration of SECO also significantly inhibited immobility time, with respective reductions of 21.3 % (5 mg/kg, $P < 0.05$), 36.0 % (10 mg/kg, $P < 0.001$) and 21.9 % (20 mg/kg, $P < 0.05$).

In both depression models, the effects of SECO accorded with those observed for the classical antidepressant imipramine (10 mg/kg, i.p., $P < 0.001$). The percentages of inhibition for imipramine were 44.9 % ($P < 0.001$) and 46.6 % ($P < 0.001$) in FST and TST, respectively.

The influence of SECO on brain monoamine levels

The contents of four monoamine neurotransmitters, NE, DA, 5-HT and 5-HIAA, in brains of the model animals were determined as listed in Table 1. While the content of NE in the OVX control group was decreased by 37.8 % ($P < 0.01$) over that in the sham-operated group, no significant differences were observed for the other three indexes between the OVX and sham-operated groups. The NE content of OVX mice was increased after administration of SECO for 14 consecutive days, resulting in 23.7 % (5 mg/kg, $P < 0.01$), 39.8 % (10 mg/kg, $P < 0.001$) and 29.0 % (20 mg/kg, $P < 0.001$) increases, respectively, compared to the OVX control group. The antidepressant agent imipramine exhibited the best effect on the NE content in brain of OVX mice, resulting in a 50.5 % increase ($P < 0.001$).

We did not observe significant differences between the OVX control group and the sham-operated group in the levels of DA, 5-HT and 5-HIAA; however, compared to the content of 1508.5 ± 37.2 ng/g for DA in the OVX control group, they were increased by 14.1 % ($P < 0.05$) and 11.5 % ($P < 0.05$) after administration of SECO at doses of 10 and 20 mg/kg, respectively. Imipramine could also increase the levels of DA and 5-HT in brains of the OVX group, resulting in a 18.8 % increase for DA ($P < 0.05$) and a 21.5 % increase for 5-HT ($P < 0.01$), respectively.

The influence of SECO on uterine weight of OVX mice

As shown in Fig. 5, the uterus index in the OVX control group (1.16 ± 0.08) was decreased after operation for

Table 1 The effects of SECO (i.g.) on brain monoamine levels of OVX mice (mean ± SE, $n = 8$)

Group	Dose (mg/kg)	NE (ng/g)	DA (ng/g)	5-HT (ng/g)	5-HIAA (ng/g)
Sham	–	839.5 ± 99.1	1559.9 ± 106.2	473.7 ± 32.7	588.9 ± 38.9
OVX control	–	522.1 ± 8.8$^{\#\#}$	1508.5 ± 37.2	459.9 ± 25.5	613.1 ± 29.1
SECO	5	645.7 ± 32.6**	1545.1 ± 47.9	455.7 ± 21.6	643.8 ± 34.0
SECO	10	729.8 ± 34.2***	1721.2 ± 67.9*	549.3 ± 55.7	707.8 ± 41.8
SECO	20	673.3 ± 22.8***	1682.2 ± 46.2*	465.5 ± 31.8	662.8 ± 27.9
Imipramine	10	786.0 ± 42.5***	1792.1 ± 106.8*	558.8 ± 18.2**	657.4 ± 50.1

$^{\#\#}$ $P < 0.01$ versus sham-operated group; $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ versus OVX control group

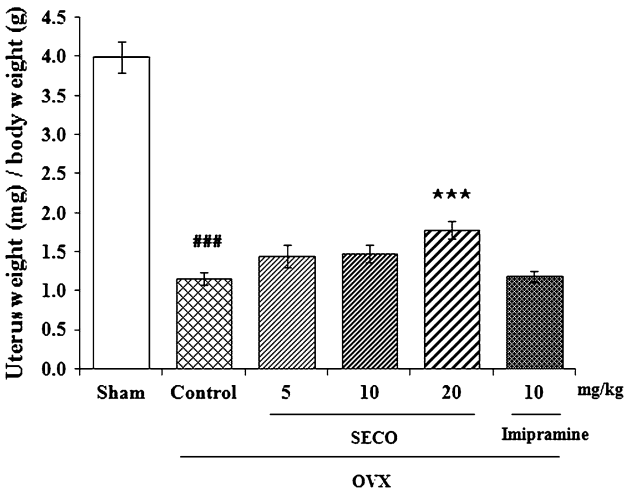

Fig. 5 The effects of SECO on the uterus index of OVX mice. Values are mean ± SEM of 12 mice in each group. $^{###}P < 0.001$ versus sham-operated group; $***P < 0.001$ versus OVX control group

14 days, resulting in a 70.9 % ($P < 0.001$) reduction over that in the sham-operated group. After administration of SECO for 14 consecutive days, the uterus indexes of OVX mice were increased by 24.1 % (no significant difference, 5 mg/kg), 27.6 % (no significant difference, 10 mg/kg) and 53.4 % ($P < 0.001$, 20 mg/kg). In contrast, the antidepressant imipramine (10 mg/kg, i.p.) did not show any effect on the uterus index of OVX mice.

Discussion

In the present study, the antidepressant-like effects of SECO on OVX mice were evaluated in two behavioral models, FST and TST. The immobility behavior displayed in mice when subjected to an unavoidable and inescapable stress has been hypothesized to reflect behavioral despair, which in turn may reflect depressive disorders in humans. Animal models are widely used in pre-clinical antidepressant evaluation and to provide insights into the neuropathology of depression [19]. FST and TST are the most widely used tools for inducing behavioral deficits which can subsequently be reversed by antidepressant treatments. There is a significant correlation between clinical potency and effectiveness of antidepressants in both models [20].

In this study, an ovariectomy operation could increase the depressive degree of the control group compared to the sham-operated group, which is consistent with previous reports [21]. SECO at doses of 5, 10 and 20 mg/kg (i.g.) showed a tendency to reduce the immobility duration after chronic administration (2 weeks) to OVX mice in both the FST and TST. Chronic treatment with the classical antidepressant imipramine (10 mg/kg, i.p.) reduced the immobility duration more significantly. As changes in the duration of immobility in FST and TST could also result from the effects of locomotor activity caused by central nervous system stimulants, the OVX mice were first tested in a locomotor activity chamber. The results showed that SECO, at doses producing an antidepressant-like effect, did not significantly change locomotor activity. Therefore, SECO appeared to produce selective antidepressant-like behavioral effects.

Monoamine neurotransmitters play important roles in depression and in mediating behavioral effects of antidepressant drugs [22]. Most of the antidepressants currently exert their primary neurochemical effects by regulating synaptic concentrations of serotonin, noradrenaline (NE) and dopamine (DA). The levels of monoamine neurotransmitters in the brain and cerebrospinal fluid of patients with depression are generally shown to be lower than normal levels [23]. The present study demonstrated that ovariectomy could lead to a decrease in NE level in mouse brain compared to the sham-operated group, but had no influence on the levels of DA, 5-HT and 5-HIAA. After chronic administration of SECO, the levels of NE and DA in the OVX group were increased, but influence on the levels of 5-HT and 5-HIAA was not observed. The antidepressant imipramine showed activity increasing the levels of NE, DA and 5-HT in brains of the OVX group.

Due to the hypofunction of the ovaries during menopause, the estrogen level declines, which in turn inevitably influences the state of the reproductive organs, such as the uterus. As SECO is the precursor of the phytoestrogens END and ENL, its effect on increasing the uterus weight in OVX mice was predicted and observed. Based on the data of the uterus index [uterus weight (mg)/body weight (g)], it was found that SECO could significantly increase the uterus index of OVX mice by 53 % after administration at a dose of 20 mg/kg for 14 consecutive days, while the antidepressant imipramine did not show any effect on the uterus of OVX mice. Whether there exists any relationship between the phytoestrogen-like activity and antidepressant effect of SECO is worth close attention.

Although the activities of antidepressant drugs are widely accepted, most of them are restricted for long-term use due to their widespread side-effects. In contrast, SECO exists widely as a natural constituent in various whole-grain cereals (such as barley, rye and wheat), seeds (such as flaxseed), nuts, legumes and vegetables [24]. To our knowledge, there have not been any reports on side-effects of SECO. Therefore, the antidepressant activity of SECO on OVX mice by chronic administration is of great value.

Conclusions

Chronic administration of SECO shows antidepressant-like effects on OVX mice in behavioral despair tests, perhaps

by increasing NE and DA levels in brains of OVX mice. The active mechanism of SECO may also be related to its phytoestrogen activity to some degree, as it was able to increase the uterus index of OVX mice. However, the mechanisms of the antidepressant-like effects of SECO on OVX mice need to be investigated further.

This finding suggests that SECO may be useful as a natural antidepressant agent for menopausal female patients.

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