Genistein Regulated Serotonergic Activity in the Hippocampus of Ovariectomized Rats under Forced Swimming Stress

Aya KAGEYAMA,1 Hiroyuki SAKABARA,1,2 Wenjun ZHOU,1 Miyuki YOSHIOKA,1 Miho OHSUMI,1 Kayoko SHIMOI,1,2,3 and Hidehiko YOKOGOSHI1,3,4

1Graduate School of Nutritional and Environmental Sciences, University of Shizuoka, Shizuoka 422-8526, Japan
2Institute for Environmental Sciences, University of Shizuoka, Shizuoka 422-8526, Japan
3Global COE Program, University of Shizuoka, Shizuoka 422-8526, Japan

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The mortality of individuals suffering from depression has been increasing, especially post-menopausal women; therefore, their care and treatment are important to maintain a high quality of life. In the present study, we evaluated the antidepressant-like effects of a major isoflavonoid, genistein (4',5,7-trihydroxyisoflavone), using a behavioral model of depression, the forced swimming test (FST), in ovariectomized rats. Daily administration of genistein to ovariectomized rats at a dosage of 10 mg/kg of body weight/d for 14 d significantly reduced the immobility time during the FST without changing motor dysfunction. On the other hand, a higher dosage, 100 mg/kg/d, did not have any effects on the immobility time compared with the vehicle control. Repeated administration of genistein at 10 mg/kg of body weight did not affect serotonergic activities in the hippocampus compared to the vehicle control in ovariectomized rats. A 5-min FST trial stimulated these activities. On the other hand, repeated pretreatment with genistein protected against changes in activity during the FST trial. These results suggest that daily consumption of genistein 10 mg/kg/d might have antidepressant-like effect on ovariectomized rats by regulating changes in serotonergic metabolism in the hippocampus under stressful conditions.

Key words: genistein; depressant; forced swimming test; monoamine; ovariectomized rat

Depression is not an unusual psychological illness. According to the World Health Organization International Consortium of Psychiatric Epidemiology (WHO-ICPE),1,3 6.3–15.7% of the world’s population is estimated to suffer depression once in life. The incidence of depression in women is about twice that in men.4,5 Moreover, older women, especially during menopause, contract the disease more often than young women.5,6 One reason post-menopausal women have increasing incidence of depression compared with men and younger women is reported to be decreasing endogenous estrogen levels in addition to daily physiological and psychological stress.2,6 This suggests that the consumption of estrogenic compounds might prevent and improve stress-evoking diseases, including depression, post-menopause.

Genistein (4',5,7-trihydroxyisoflavone), plentiful in soybeans, has estrogenic activity.3,7 Physiological levels of estrogen are well known to decrease post-menopause and consequently induce hormone-related diseases, including depression.3,7 Therefore, genistein is considered a potential remedy for a wide range of diseases, such as osteoporosis and menopausal symptoms.4,5 Recently, Sapronov and Kasakova reported that genistein reduced the immobility time, a marker of depression, on the forced swimming test, one of the main screening methods for antidepressants.3,7 The same effect was seen in ovariectomized female rats, indicating that genistein may have antidepressant-like activity post-menopause, however it was administered to rats by intramuscular injection. From the viewpoint of nutritional sciences, an intramuscular injection is not acceptable because most compounds, including genistein, are known to be metabolized during absorption. In order to establish the antidepressant effect of genistein, the effects after orally consumption should be investigated. Additionally, the mechanism by which genistein displays antidepressant-like activity has not been elucidated.

In this study, we first studied the antidepressant-like effect of genistein using FST after oral administration of genistein to ovariectomized rats. Subsequently, the effect of genistein on the regulation of serotonergic activities, key mechanisms in the induction of depression, in the hippocampus region were examined with and without FST trial.

Materials and Methods

Animals. Ovariectomized female Wistar rats (110–130 g) were obtained from Japan SLC (Shizuoka, Japan). A surgical certificate of ovariectomy was issued by Japan SLC. We confirmed a marked decrease in ovary weight in the ovariectomized rats during dissection after the experiments (data not shown). The rats were group-housed (6 rats/cage) in a room at constant temperature (23 ± 1°C) and humidity (50 ± 5%) under a 12-h light-dark cycle (light: 7:00–19:00). Controlled purified diets were prepared according to AIN-93G,60 and corn oil was used instead of soybean oil in order to remove unexpected contamination from genistein. The animals consumed the refined diets and improve stress-evoking diseases, including depression, post-menopause.

Abbreviations: FST, forced swimming test; HPLC, high-performance liquid chromatography; DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; 5-HT, 5-hydroxytryptamine; 5-HIAA, 5-hydroxyindoleacetic acid; OFT, open field test

1 To whom correspondence should be addressed. Fax: +81-54-264-7079; E-mail: yokogosi@u-shizuoka-ken.ac.jp
and tap water ad libitum. All experimental procedures were in accordance with the Guidelines of the University of Shizuoka for the Care and Use of the Laboratory Animals, based on those of the American Association for Laboratory Animal Science.

**Chemicals.** Genistein (99%; LC Laboratories, Woburn, MA) and imipramine (99%; Nacalai Tesque, Kyoto, Japan) were suspended in distilled water just before administration to the rats. All other reagents were of the highest grade available, and were purchased from Wako Pure Chemicals (Tokyo).

**FST procedure after acute treatment.** FST was performed following the method of Porsolt et al., with some modifications. Briefly, 42 ovariectomized rats were divided as follows: seven animals were treated with imipramine (30 mg/kg of body weight), 28 with genistein (0.1, 1, 10, or 100 mg/kg, seven animals/group), and the remaining seven animals received vehicle (deionized water). The rats were placed in an acrylic cylinder (64.5 cm × 19.2 cm i.d.) filled with water at 25 ± 1°C to a depth of 17 cm for 15 min (pre-test session) without any treatment (acute test). Twenty-four h after the pre-test session, every rats were exposed to the same conditions for 5 min (test session). Between the pre-test and main test sessions, a drug solution was administered intragastrically 3 times, the first just after the pre-test session, then 5 h before the main test, and finally 1 h before the main test in both tests. The time spent by a rat in active movement and immobility was recorded during the 5-min task (test session) by video camera. The tapes were evaluated by observers not informed of the treatment each animal had received. The water in the tank was changed for each rat. The test solutions were administered 24, 5, and 1 h prior to the FST trial, as described below.

**FST after repeated treatment.** A group of 42 rats was divided as follows: seven animals were treated with imipramine (15 mg/kg) and 28 with genistein (0.1, 1, 10, or 100 mg/kg, seven animals/group), and remaining seven animals received vehicle (deionized water). Test solutions were administered once daily over 14 d between 12:00 and 15:00. Each group was injected with a volume of 0.5 ml solvent/100 g of body weight. After 14 d of administration, the animals were subjected to FST, as described above.

**Dissection and tissue.** After the FST trial, the rats in the chronic administration group were immediately sacrificed by decapitation, and the hippocampus regions in the brain were removed. Trunk blood was collected into a serum tube and centrifuged at 3,000 rpm for 15 min. The serum and hippocampus were stored at −80°C until needed for further analysis.

**Measurement of serum corticosterone.** Serum corticosterone levels were analyzed by high-performance liquid chromatography (HPLC) with a UV detector. Briefly, the serum (100 µl) was mixed with methanol (80 µl) and this was centrifuged at 10,000 rpm for 2 min. A portion of the filtrate (80 µl) was subjected to HPLC on a Unison C18 column (6.0 × 75 mm; Imtakt, Kyoto, Japan). The column was maintained at 45°C with a column oven (ATC-300; Eicom, Tokyo). The mobile phase was acetonitrile:distilled water (25:75) at a flow rate of 1.0 ml/min. Corticosterone was detected using a UV detector. 10) Briefly, the serum (100 µl) was mixed with 990 µl acetonitrile and this was centrifuged at 10,000 rpm for 2 min. The supernatant was filtered through a 0.22 µm Sartorius filter paper and 100 µl was injected into the HPLC system.

**Neurochemical analysis in the hippocampus.** The concentrations of neurotransmitters in the hippocampus region were analyzed as reported previously. Briefly, brain tissue was homogenized with a 20-fold volume of 0.2 M perchloric acid buffer and kept on ice for 1 h. Hippocampus homogenate was centrifuged at 15,000 rpm for 15 min, and then the supernatant was filtered through a 0.45 µm cellulose acetate membrane filter (Millipore, Bedford, MA). The HPLC system, which was equipped with a reversed-phase column (MA-SODS, 4.6 × 150 mm; Eicom) and an electrochemical detector (ED623; GL Science, Tokyo) was used under the following conditions: the mobile phase was 0.1M sodium acetate citric acid buffer and 15% methanol containing 5 mg/l of EDTA-2Na and 160 mg/l of 1-octanesulfonic acid sodium salt. The column was maintained at 25°C. Recording of chromatograms and all calculations were performed using an integrator (Ezchrom Elite; GL Science, Tokyo). The amounts of serotonin (5-Hydroxytryptamine, 5HT), dopamine (DA), and their metabolites, 5-hydroxyindole-3-methoxyphenylacetic acid (5-HIAA) and 3,4-dihydroxyphenylacetic acid (DOPAC), were quantitatively analyzed.

**Fig. 1.** Effect of Genistein on Immobility Time in FST on Ovariectomized Rats.

A, A test solution was administrated orally 3 times at 24, 5, and 1h before the FST trial. Imp indicates the antidepressant drug imipramine (30 mg/kg of body weight). B, A test solution was administrated orally once daily for 14 d. Imp (15 mg/kg of body weight). All values are mean ± SE (n = 7). The FST trial effect was analyzed by one-way ANOVA. Different alphabetical superscripts indicate significant difference at p < 0.05 by the Fisher-PLSD test.

**Statistical analysis.** All data were analyzed using Stat View 5.0 (SAS Institute, Tokyo). Data on body weight gain, locomotor activity, and immobility time in FST were analyzed by one-way ANOVA with the Fisher-PLSD test. Data on corticosterone levels and monoamine metabolism were analyzed by two-way ANOVA with the Fisher-PLSD test.

**Results**

**FST and OFT with rats**

As shown in Fig. 1A, acute administration of genistein did not change immobility in FST, although pretreatment with an antidepressant, imipramine, significantly decreased immobility times as compared with the control rats. When genistein (0.1, 1, 10, or 100 mg/kg of body weight) was orally administered to rats once daily for 14 d, there were no differences in body weight gain among the groups after 14 d of genistein administration (Table 1). Figure 1B shows the effect of genistein during the FST trial. Daily treatment of rats with genistein 10 mg/kg for 14 d significantly reduced the immobility time in the FST. The decrease in immobility time in the rats treated with genistein was comparable to that in the rats treated with imipramine. On the other hand, no effects on the FST were observed after the administration of 0.1, 1, and 100 mg of genistein/kg of body weight. Administration of genistein at all dosages resulted in no overt behavioral changes in the open field test (Table 2).
**Table 1.** Body Weight Gain during Genistein Administration to Rats for 14 d

<table>
<thead>
<tr>
<th>Administration</th>
<th>Day 1</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (deionized water)</td>
<td>123.2 ± 4.5a</td>
<td>177.7 ± 13.5a</td>
</tr>
<tr>
<td>Imipramine 15 mg/kg</td>
<td>123.5 ± 4.0a</td>
<td>166.0 ± 9.7a</td>
</tr>
<tr>
<td>Genistein 0.1 mg/kg</td>
<td>123.2 ± 4.9ab</td>
<td>180.2 ± 10.9b</td>
</tr>
<tr>
<td>1 mg/kg</td>
<td>123.5 ± 6.5a</td>
<td>178.7 ± 9.1a</td>
</tr>
<tr>
<td>10 mg/kg</td>
<td>122.1 ± 5.2a</td>
<td>176.7 ± 12.0b</td>
</tr>
<tr>
<td>100 mg/kg</td>
<td>123.6 ± 3.8a</td>
<td>173.3 ± 8.2a</td>
</tr>
</tbody>
</table>

All values are the mean ± SD (n = 14). The treatment effect was analyzed by one-way ANOVA (treatment effect, p < 0.01). Different superscripts indicate significant differences at p < 0.05 as evaluated by the Fisher-PLSD test.

**Table 2.** Locomotor Activity in the Open Field Test in Ovariectomized Rats

<table>
<thead>
<tr>
<th>Genistein (mg/kg)</th>
<th>Locomotion (cm)</th>
<th>Defecation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (deionized water)</td>
<td>2082 ± 237a</td>
<td>2.3 ± 0.7a</td>
</tr>
<tr>
<td>Imipramine 15 mg/kg</td>
<td>1661 ± 225b</td>
<td>3.4 ± 1.2a</td>
</tr>
<tr>
<td>Genistein 0.1 mg/kg</td>
<td>2092 ± 187a</td>
<td>0.7 ± 0.4a</td>
</tr>
<tr>
<td>1 mg/kg</td>
<td>2333 ± 173b</td>
<td>0.7 ± 0.5a</td>
</tr>
<tr>
<td>10 mg/kg</td>
<td>2599 ± 192a</td>
<td>0.4 ± 0.4a</td>
</tr>
<tr>
<td>100 mg/kg</td>
<td>2098 ± 204a</td>
<td>0.4 ± 0.4a</td>
</tr>
</tbody>
</table>

Behavioral parameters were recorded for 5 min. Locomotion is the distance moved. All values are the mean ± SE (n = 7). The treatment effect was analyzed by one-way ANOVA (treatment effect, p < 0.01). Different superscripts indicate significant differences at p < 0.05 as evaluated by the Fisher-PLSD test.

**Fig. 2.** Effect of Genistein on Serum Corticosterone Level in Rats after the FST Trial.

Genistein (0.1, 1, 10, and 100 mg/kg of body weight) was administered orally once daily for 14 d. The FST trial was performed for 5 min, as described in “Materials and Methods.” All values are mean ± SE (n = 7). Group and treatment effects were analyzed by two-way ANOVA. Different letters indicate statistically significant differences at p < 0.05 as evaluated by the Fisher-PLSD test. N.S., not significant. *Treatment effect: p = 0.069

**Corticosterone levels in the serum**

The basic levels of corticosterone in the rat serum were not affected by genistein administration, as shown in Fig. 2. On the other hand, it was significantly increased during the 5-min FST trial compared with the control. Genistein administration at all dosages (0.1, 1, 10, or 100 mg/kg) did not prevent this elevation in the FST trial.

**Neurotransmitter turnover in the hippocampus**

Both the dopaminergic ratio (DOPAC/DA) and serotonergic ratio (5-HIAA/5-HT) in the hippocampus significantly increased after the 5-min FST trial (Fig. 3). Pretreatment of genistein at 10 mg/kg for 14 d indicated a decreasing tendency on the serotonergic ratio stimulated by the FST trial (p = 0.059), and the ratio remained at the levels of the control without FST. On the other hand, the dopaminergic ratio was not inhibited by treatment with the same amount of genistein (p = 0.428). Genistein treatment without the FST trial did not affect neurotransmitter turnover in the hippocampus.

**Discussion**

Many reports have indicated the possibility that exogenous estrogen administration can cure and/or treat depression using animal models, but clinical findings suggest that hormone replacement therapy causes side effects, for example, a high risk of stroke, venous thromboembolism, and breast cancer. Hence it is necessary to develop a treatment method for depression that takes the place of estrogen without any side effects, for example, ingredients of daily food. In this study, we focused on genistein, a major estrogenic food ingredient present mainly in leguminous plants, and evaluated its antidepressant-like effect using FST. The immobility displayed by rodents, including mice, when subjected to an unavoidable stress such as forced swimming is thought to reflect depressive illness in humans. Additionally, immobility time has been shown to be reduced by treatment with antidepressant drugs. Moreover, a strong correlation has been reported between the clinical efficacy of antidepressant drugs and their potency in the FST. Therefore, the FST is
thought to be a good animal model for the screening of antidepressant-like foods and drugs. We found that repeated treatment of genistein to ovariectomized rats at 10 mg/kg of body weight/d significantly reduced the immobility time in the FST compared with vehicle control rats (Fig. 1B) without any changes in motor dysfunction in the open field test (OFT) (Table 2). Other dosages (0.1, 1, and 100 mg/kg) did not affect the duration of immobility. Such a U-shaped activity curve has been reported for some herbal medicines.16–18) Although the reasons have not been clarified, we considered that higher dosage of genistein, such as 100 mg/kg, might exert detrimental effects19) and thus diminish the antidepressant-like effect.

On the other hand, acute treatment with genistein at 0.1–100 mg/kg of body weight did not affect the immobility time (Fig. 1A). Although most antidepressants increase the concentrations of monoamine several h or d after administration, they have a delayed onset of effects relieving the symptoms of depression. Our results clearly indicate that repeated oral administration of genistein might have antidepressant-like effect on ovariectomized rats. Recently, Sapronov reported similar results for intact and ovariectomized rats after repeated treatment for 14 d, but their treatment was administered via intramuscular injection. In this study, repeated treatment with genistein at 10 mg/kg of body weight/d for 14 d had typical antidepressant-like effect in the rodent model. This amount (10 mg/kg of body weight/d for rats) is corresponds with 600 mg of genistein or about 500 g of fresh soybean/d for a human,20) although this is difficult to calculate simply.

### Table 2

<table>
<thead>
<tr>
<th>Effect</th>
<th>p-Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>p &lt; 0.01</td>
</tr>
<tr>
<td>Treatment</td>
<td>p &lt; N.S.*</td>
</tr>
<tr>
<td>Group × Treatment</td>
<td>p &lt; N.S.</td>
</tr>
</tbody>
</table>

### Figure 3

**Effect of Genistein on Dopaminergic and Serotonergic Activities in the Rat Hippocampus after the FST.**

A, DOPAC/DA ratio; B, 5-HIAA/5-HT ratio. Genistein was administered orally once daily over 14 d. The FST trial was performed for 5 min, as described in “Materials and Methods.” DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; 5-HT, 5-hydroxytryptamine; 5-HIAA, 5-hydroxyindoleacetic acid. Each value is the mean ± SE (n = 7). Group and treatment effects were analyzed by two-way ANOVA. All values are the mean ± SE (n = 7), and different letters indicate significant differences at p < 0.05 as evaluated by the Fisher-PLSD test. N.S., not significant.
because the metabolic patterns of isoflavones including genistein are known to be quite different between humans and rats.\textsuperscript{21–23} Genistein is widely present in plants, including leguminous plants, mainly as many types of glucosides, and some as aglycone.\textsuperscript{24} When genistein glucosides are consumed, they are first deglucosidated in the alimentary tract, and become genistein aglycone. Subsequently, genistein is absorbed by the body via the intestines. Here, genistein undergoes some modification, including glucuronide and sulfate, and genistein metabolites circulate in the blood flow.\textsuperscript{21,23} On the other hand, only 6–7\% of administered genistein is absorbed, and then excreted in the urine within 24 h of administration in a rat experiment.\textsuperscript{23} That is, plasma genistein have been reported to remain at higher levels, because genistein is still present a little in the body after 24 h, when genistein is repeatedly administered. This indicates that daily consumption of genistein might increase plasma levels of genistein, and hence have antidepressant-like effects.

There are some hypotheses as to the depressive mechanism, but the antidepressant mechanisms of genistein are unclear. In this study, we investigated the mechanisms of the antidepressant-like effect of genistein. Physiological stress, such as forced swimming, has been reported to activate the hypothalamic-pituitary-adrenal (HPA) axis, and consequently to secrete corticosterone into the blood.\textsuperscript{18} Our results suggested that a 5-min FST trial was associated with significant elevation of serum corticosterone (Fig. 2), but, repeated administration of genistein, including an active dosage during FST (10 mg/kg), did not prevent this elevation, indicating that genistein did not regulate the HPA axis stimulated under stress conditions. Bu et al. reported that cortisol, a human glucocorticoid, did not change when food containing many isoflavones, including genistein, was consumed.\textsuperscript{25} When stressors such as abdominal injection are introduced to rodents, their plasma corticosterone levels increase chronologically at least until 30 min.\textsuperscript{20} In this study, we collected serum samples at only one time point, just after the FST trial. Therefore, there is a possibility that repeated treatment with genistein regulates the HPA axis stimulated by exposure to stress after a period of time. However, the mechanism underlying the antidepressant-like effect of genistein observed in our result, at least, does not result from regulation of HPA axis stimulation under the 5-min of forced swimming stress. The other pathway related to the development of depression is neurotransmitter levels and ratios in the brain.\textsuperscript{27–30} The FST trial significantly increased the metabolite/transmitter ratio (DOPAC/DA and 5-HIAA/5-HT) (Fig. 3). This indicates that forced swimming stress activates serotonergic pathways and consequently decreases transmitter levels in the hippocampus. When we administered genistein for 14 d at 10 mg/kg/d, the serotonergic ratio indicated a decrease ($p = 0.059$), and remained at the control level even after the FST trial, indicating that genistein regulates the serotonergic pathway under stressful conditions. Other doses (0.1, 1, and 100 mg/kg) did not affect these changes, but such U-shaped activities conform to the results of FST, as shown in Fig. 1B. Many flavonoids, such as kaempferol and apigenin, have been reported to regulate transmitter turnover by acting as inhibitors of monoamine oxidase-A (MAO-A), a mitochondrial enzyme that catalyzes the oxidation of monoamines, including 5-HT and DA, in the brain.\textsuperscript{31,32} Hence genistein might regulate MAO-A activity.\textsuperscript{33} It is necessary to determine whether genistein acts as an MAO-A inhibitor in future study. On the other hand, monoamine levels, such as DA and 5-HT, have been reported to be affected by estrogenic ingredients. For example, exogenous treatment with estrogen of ovariectomized animals changes monoamine metabolism in the brain.\textsuperscript{11–14} Walf et al. reported that estrogen receptor (ER) $\beta$-specific selective ER modulator (SERM) showed an antidepressant-like effect, but not ER$\alpha$-specific SERM, and concluded that the antidepressant-like effect of estradiol may involve ER$\beta$ in the hippocampus.\textsuperscript{14} ER$\beta$, rather than ER$\alpha$, is dominantly expressed in the hippocampus.\textsuperscript{14} The binding capability of genistein to ER$\beta$ is stronger than ER$\alpha$. In this study, administration of 10 mg/kg of body weight/d had typical antidepressant-like effect. After the administration of this amount of genistein, the plasma level is considered to have reached to 6–7\%.\textsuperscript{31} Tsai reported that genistein was detected in the brain, when he/she administered 10 mg/kg of genistein (intravenous injection) into rats, indicating that genistein can penetrate the blood-brain barrier and reach the brain.\textsuperscript{30} This suggests that the antidepressant-like mechanism of genistein is restricted by regulation of the brain neurotransmitter via ER$\beta$ in the hippocampus.

In conclusion, the present study indicates for the first time that oral administration of genistein at 10 mg/kg for 14 d had an antidepressant-like effect on ovariectomized rats with an animal model of depression. After reaching the hippocampus region, genistein might regulate monoamine metabolism, especially serotonergic activity, and consequently have antidepressant-like effect, but it was not involved in the prevention of HPA axis stimulation under stress conditions.

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**References**

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