Antidepressant Effect of Extracts from Ginkgo biloba Leaves in Behavioral Models

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Extracts of Ginkgo biloba (EGB) are a complex product prepared from green leaves of the Ginkgo biloba tree. In the present study, the antidepressant effect of EGB was examined using two behavioral models, the forced swimming test (FST) in rats and tail suspension test (TST) in mice. EGB significantly reduced immobility time in the FST at a dosage of 10 and 50 mg/kg body weight after repeated oral treatment for 14 d, although no change of motor dysfunction was observed with the same dosage in the open field test. These results indicate that EGB might possess an antidepressant activity. In addition, EGB markedly shortened immobility time in the TST after acute inter-peritoneal treatment at a dosage of 50 and 100 mg/kg body weight. The present study clearly demonstrated that EGB exerts an antidepressant effect in these two behavioral models.

Key words Ginkgo biloba extracts; antidepressant effect; forced swimming test; tail suspension test; flavonoid

In recent years, depression has become recognized as a major public health problem. It is estimated that in the US approximately 20% of the population has some depressive symptoms, and around 2–5% are thought to suffer from severe forms of depression. Understanding how to prevent and treat depression is therefore an urgent subject. Although the mechanism provoking depression has not been clearly elucidated, the main trigger is known to be exposure to chronic stress. Many types of antidepressant drugs such as tricyclic antidepressants, and selective serotonin reuptake inhibitors (SSRI), as well as antidepressant herbal medicines like St. John’s wort are used to treat depression. However, most of the synthetic drugs are not without side effects. Furthermore disturbance of the drug metabolizing enzyme systems were revealed with St. John’s wort; thus, the search for a new antidepressant herb without side effects is deemed important.

The Ginkgo biloba tree has been used as a traditional Chinese herbal medicine for thousands of years. Several research groups have shown that extracts from the green leaves of the G. biloba tree (EGB) have diverse effects on improvement of mood and cognitive performance, protection of memory deficits and central nervous system (CNS) disorders, and alleviation of the symptoms of mild to moderate Alzheimer-type dementia. Until now, no antidepressant effect of EGB has yet been revealed. EGB is thought to possess anti-stress properties, and is safe to use without distinct side effects. EGB might be a useful option for the prevention and treatment for stress-induced disorders such as depression. In the present study, we evaluated the antidepressant effect of EGB using two behavioral models for screening antidepressants, the forced swimming test (FST) in rats and tail suspension test (TST) in mice.

MATERIALS AND METHODS

Chemicals The EGB used in the present study was Ginkgolon-24 from Tokiwa Phytochemical Co., Ltd. (Chiba, Japan), which was 75% ethanol extracts from the leaves of Japanese G. biloba tree. This product contains 25.5% of flavonoid glycosides, including more than 8.2% quercetin glycosides, more than 6.4% kaempferol glycosides, 1.6% methylmyricetin glycosides and 1.3% isorhamnetin glycosides, and 6.5% of terpenoids in the form of 2.98% bilobalide, 1.59% ginkgolide A, 1.16% ginkgolide B, and 0.75% ginkgolide C. The antidepressant drug imipramine (hydrochloride form) was purchased from Sigma-Aldrich Co. (St. Louis, MO, U.S.A.).

Animals Male CD rats (240–260 g; Charles River Japan, Inc., Yokohama, Japan) and male C57BL/6J mice (22–26 g; SLC, Inc., Hamamatsu, Japan) were used in the FST and TST, respectively. All animals were housed in a controlled room (temperature, 25±1 °C; humidity, 45–50%; light–dark cycle, 12 h each) with free access to laboratory chow (MF; Oriental Yeast Co., Ltd., Tokyo, Japan) and tap water. Rodents were divided randomly into control and experimental groups. This study was performed according to the guidelines for the care and use of laboratory animals of The University of Tokushima Graduate School, Institute of Health Biosciences.

Drug Treatment Imipramine and EGB were dissolved homogeneously in deionized water by sonication. Both solutions were prepared daily just before administration. Control animals received the same volume of deionized water only.

FST Procedure The FST was performed according to the method of Porsolt et al. with some modifications. Briefly, a group of 35 rats was divided at random into five groups and treated as follows: one group was orally administered imipramine (15 mg/kg body weight), three groups were orally administered EGB (5, 10, and 50 mg/kg body weight, respectively), and one group was administered vehicle only.
RESULTS

FST Using Rats EGB (5, 10, 50 mg/kg body weight) or the synthetic antidepressant, imipramine (15 mg/kg), were orally administered to the rats once daily for 14 d. There were no differences in body weight gains after 14 d among all treatment groups (Table 1). Figure 1 shows the activities of EGB in the FST using rats. EGB (10, 50 mg/kg) significantly reduced the immobility time 14 d after daily treatment. The decrease in immobility for EGB was comparable to that of the imipramine treatment group. No effect was observed after administration of 5 mg EGB/kg. Neither administration of imipramine (15 mg/kg) nor EGB (5, 10, 50 mg/kg) resulted in any overt behavioral changes or motor dysfunction in the open field test (Table 2).

TST Using Mice As shown in Fig. 2, immobility time in the TST using mice was markedly shortened after acute inter-peritoneal treatment with imipramine (30 mg/kg). Administration of EGB at dosages of 50 and 100 mg/kg significantly reduced the immobility time, however, there was no effect with the lower treatment dosage (10 mg/kg).

Table 1. Body Weight Gain

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day 1</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>135 ± 8</td>
<td>244 ± 9</td>
</tr>
<tr>
<td>Imipramine</td>
<td>133 ± 17</td>
<td>240 ± 17</td>
</tr>
<tr>
<td>EGB 5 mg/kg</td>
<td>131 ± 15</td>
<td>253 ± 11</td>
</tr>
<tr>
<td>EGB 10 mg/kg</td>
<td>132 ± 15</td>
<td>242 ± 17</td>
</tr>
<tr>
<td>EGB 50 mg/kg</td>
<td>138 ± 6</td>
<td>258 ± 13</td>
</tr>
</tbody>
</table>

Table 2. Locomotor Activity in the Open Field Test

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Locomotion</th>
<th>Rearing</th>
<th>Defecation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>83 ± 6</td>
<td>14 ± 4</td>
<td>1.6 ± 0.6</td>
</tr>
<tr>
<td>Imipramine</td>
<td>70 ± 14</td>
<td>18 ± 3</td>
<td>2.4 ± 0.5</td>
</tr>
<tr>
<td>EGB 5 mg/kg</td>
<td>84 ± 15</td>
<td>14 ± 5</td>
<td>2.0 ± 0.4</td>
</tr>
<tr>
<td>EGB 10 mg/kg</td>
<td>84 ± 2</td>
<td>16 ± 3</td>
<td>1.8 ± 0.5</td>
</tr>
<tr>
<td>EGB 50 mg/kg</td>
<td>92 ± 8</td>
<td>14 ± 3</td>
<td>1.2 ± 0.7</td>
</tr>
</tbody>
</table>

The behavioral parameters were recorded for 5 min. Locomotion: number of line crossings, Rearing: number of times seen standing on hind legs, Defecation: number of defecations within 5 min. The behavioral parameters were considered significant if the probability of error was 5% or less.

Rats were placed in an acrylic cylinder (450 × 192 mm i.d.) filled with water at 25 ± 1 °C to a depth of 17 cm for 15 min (pre-test session) after 14 d treatment. Twenty-four hours after the pre-test session, the animals were once again exposed to the same conditions for 5 min (test session). Between the pre-test session and main session drug solutions were administered orally three times as follows: just after the pre-test session, 5 h before the main test, and 1 h before the main test. A rat was judged immobile if it remained floating in the water, except for small movements to keep its head above the water. The FST was performed between 1—3 p.m. and recorded using a video camera. The tapes were evaluated by observers not informed about the kind of treatment each animal had received.

Measurement of Locomotor Activity Another set of rats who received the same drugs using the same treatment regimen as in the FST underwent an open field test according to Carlini et al. Rats were placed in an open field apparatus composed of an arena 70 cm in diameter divided into 18 approximately equal areas. For open field observations, each rat was individually placed in the center of the arena 15 h after the last treatment. Hand-operated counters were employed to score the following behavioral parameters: locomotion (number of line crossings), rearing frequencies (number of times seen standing on hind legs), and number of defecations within 5 min. Open field observations were made between 8—10 a.m.

TST Procedure The TST was performed according to Steru et al. with slight modifications. In brief, a group of 50 mice was divided at random into five groups and treated as follows: one group was inter-peritoneally administered imipramine (30 mg/kg body weight), three groups were inter-peritoneally administered EGB (10, 50, and 100 mg/kg body weight, respectively), and the final group received vehicle (deionized water). Test solutions were administered intraperitoneally 30 min prior to the test. Values represent the mean ± S.E. (n = 7). *p < 0.05 vs. control group.

Fig. 1. Effect of EGB on Immobility Time in the FST Using Rats

Test solutions were administered orally once daily over a period of 14 d. Values represent the mean ± S.E. (n = 7). *p < 0.05 vs. control group.

Fig. 2. Effect of EGB on Immobility Time in the TST Using Mice

Test solutions were administered intraperitoneally 30 min prior to the test. Values represent the mean ± S.E. (n = 10). *p < 0.05 vs. control group.
DISCUSSION

The purpose of this study was to evaluate the antidepressant effect of EGB using behavioral animal models. A major problem in the screening for new antidepressants is the establishment of a valid animal model able to sufficiently and accurately identify diverse antidepressant treatments. In this study, we used two animal models, the forced swimming test (FST) in rats developed by Porsolt et al. and tail suspension test (TST) in mice developed by Steru et al. The immobility displayed by rodents when subjected to unavoidable stress such as forced swimming is thought to reflect a state of despair or lowered mood, which are thought to reflect depressive disorders in humans. In addition, the immobility time has been shown to be reduced by treatment with antidepressant drugs. Moreover, a significant correlation was found between the clinical efficacy of antidepressant drugs and their potency in both models.

In the present study, EGB significantly reduced immobility time in the FST after repeated administration of 10 and 50 mg/kg to rats for 14 days. The intensity of immobility reduction was stronger at a dosage of 10 mg/kg than 50 mg/kg. Such U-shaped activity has been seen in St. Johns wort and Apocynum extract. The efficacy of the decreasing immobility in the FST was also shown previously with psychostimulants, which exert an indiscriminate motor stimulating activity. In order to exclude this false positive result, we employed an additional open field test to check the motor stimulating activity of EGB. Both administration of EGB at a dosage of 10 and 50 mg/kg for 14 days, active dosages in the FST, resulted in no behavioral changes or motor dysfunction in the open field test, indicating that the reduction in immobility time after 14 days administration of EGB was attributed to an inherent antidepressant effect. On the other hand, Porsolt et al. reported that EGB administration at a dosage of 50 and 100 mg/kg was devoid of activity in the FST. They used EGB761, which was the acetone extracts from G. biloba. However we employed the ethanol extracts, Ginkgolon-24, indicating that active compounds for antidepressant action existed in both extracts might be not completely same. It might be desirable experiments to compare the active compounds in both extracts. In addition, in their study, the administration period was only 5 days compared to 14 days in the present study. Montgomery proposed that a treatment period of 14 days is a valid interval for demonstrating antidepressant efficacy.

Our results also indicate that the activity of EGB in the FST was more effective at a lower dosage (10 mg/kg). Thus, the effective dosage of EGB in the FST seems to be around 10 mg/kg body weight, while treatment with more than 50 mg/kg will probably cause a decline in activity. The antidepressant effect of EGB was also revealed using the TST according to the reduction in immobility of mice after acute inter-peritoneal treatment with 50 and 100 mg EGB/kg, respectively. Consequently, the present results clearly demonstrate that EGB possesses antidepressant-like activity in the behavioral models, FST and TST. The results in both FST and TST indicated U-shaped activity, indicating to be necessary to decide an effective dose in clinical use of EGB for antidepressant. The Extract of Apocynum was reported to have antidepressant-like activity in the FST as dosage of 30 mg/kg body weight/day for 14 days. The pharmacopoeia of the People’s Republic of China recommends that a clinical dose for beneficial effects of Apocynum is 6—12 g of the dried leaves, which dose corresponds about 600 mg of Apocynum extract. These suggest that effective clinical dose of EGB for antidepressant action might be calculated as around 200 mg per day, similarly to the recommended dose of EGB for cognitive impairment and dementia. Administration of EGB should be repeated, because Cryan et al. concluded that the effects of antidepressants were augmented following chronic treatment. The length of treatment is recommended 4- and 6-week period for the demonstration of acute efficacy in the guidelines of the European Union. However, the onset of an antidepressant’s clinical effect can be demonstrated early in the treatment period. In addition, the significant differences seen at 2 weeks treatment do not appear to be lost at 4 weeks. Hence, administration of EGB for antidepressant action is repeatedly and probably at least for 2 weeks.

EGB is a complex product prepared from green leaves of the Ginkgo biloba tree. Major ingredients are polyphenols, especially flavonoids including quercetin glycosides and kaempferol glycosides. Recently, several studies have suggested the antidepressant effect of quercetin glycosides such as hyperoside, isoquercitrin and rutin using the positive results of FST. Flavonoid glycosides are mostly hydrolyzed into their aglycons by mucosal and bacterial enzymes in the intestines, and then converted to conjugated metabolites during the absorption process. This perhaps indicates that the active form for the antidepressant effect of quercetin glycosides is the conjugated form, not the glycoside form. The EGB used in the present study, Ginkgolon-24, contains more than 8.2% of quercetin glycosides, including rutin and isoquercitrin. Quercetin glycosides are, hence, thought to be one of the major ingredients contributing to the antidepressant effect of EGB. Additionally, the present EGB contained more than 6.4% kaempferol glycosides, 1.6% methylmyricetin glycosides, and 1.3% isorhamnetin glycosides. These flavonoid glycosides seem to appear as conjugated forms in the blood stream as with quercetin glycosides. Transportation of these metabolites into the brain tissues via the blood brain barrier and their effect on the CNS system have been recently argued. Moreover, quercetin metabolites were previously found in the brain tissues of rodents after oral administration. Therefore, one of the antidepressant mechanisms of EGB is thought to involve flavonoid glycosides, which reach the brain tissues through the metabolizing process, protecting brain function from CNS disturbance, and consequently, exerting an antidepressant effect.

Other candidates of EGB that exert an antidepressant effect are the 6.5% of terpenoids, known as bilobalide, and ginkgolides A, B and C, which are known to have efficacy in the CNS. Some of the antidepressant activity of EGB might therefore be derived from these compounds. The present study warrants further investigation into identification of the active compounds in herbal medicines, in particular EGB, with antidepressant effects. Porsolt indicated that central levels of monoamine, such as dopamine and norepinephrine, were important factors for reduction of immobility in the FST. Additionally, recent research reported that swimming stress markedly enhanced a phosphorylation of mito-
gen-activated protein kinases (MAPKs) in the brain. It is likely that changes of monoamine levels and/or activation of MAPKs in the brain are involved in the antidepressant mechanism of EGB.

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REFERENCES