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Keum-Ryung-Ja-San, an Traditional Herbal Prescription, Ameliorates Depressive Behaviors in Mice

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Abstract – Depression is a very difficult disease to be cured because several nervous systems are involved. In the present study, we evaluated the effects of Keum-Ryung-Ja-San (KRJS), a traditional herbal prescription, on depressive behaviors in mice using the forced swimming test. KRJS was given 1 h prior to the forced swimming test (50, 100, 200, and 400 mg/kg, p.o.). The duration of immobility time in the forced swimming test was significantly reduced by KRJS treatment (200 mg/kg, $P < 0.05$) and similar effects were observed with a classical antidepressant, imipramine (15 mg/kg, i.p.). With subchronic administrations of KRJS and its constituents at several doses for 1 week, a decreased duration of immobility time was observed with KRJS and *Corydalis ternata* (200 mg/kg, p.o. $P < 0.05$). These results suggest that KRJS may have antidepressive activities and CT may contribute to the antidepressive activity of KRJS.

Key words □ Keum-Ryung-Ja-San, antidepressant, *Corydalis ternata, Melia toosendan,* forced swimming test

INTRODUCTION

Major depression is a severe disorder including disturbances of emotional, cognitive, autonomic and endocrine functions. In addition, depression is a difficult disease to be cured because it may be chronicity, relapse, and recurrence, resulting in a high suicide rate. The pathophysiology of depression is still unknown and may include functional abnormalities of different brain regions and circuits. The hippocampal formation is one of those brain systems that were suggested to play a critical role in depressive disorder (Dremencov *et al.*, 2003). There are two reasons for interest in the hippocampal formation. At first, hippocampal region controls various brain functions that are disturbed in depressed patients, such as altered regulation of neuroendocrine and autonomic functions, mood and cognition difficulties, adverse responses to stressful stimuli and others (Kalia *et al.*, 2005). Secondly, hippocampal functions are highly regulated by serotonergic (5-HT) systems, the involvement of which in the pathology and treatment of depression has been published (Kim *et al.*, 2005; Hjorth *et al.*, 2000).

In the Oriental society, herbal prescriptions are widely used by consumers and have a long history of use as traditional medicines. Some of them may be effective alternatives in the treatment of depression, as in the case of St John's wort, a western herb (Müller, 2003). Moreover, various findings in recent preclinical studies have supported the therapeutic values of herbal medicines in a clinical setting. For example, Banxia Houpu decoction (Banha-Hubak-Tang in Korean), which is prescribed for the depressive patients by the Oriental herbal practitioners, has shown to have antidepressant-like effect in rats (Luo *et al.*, 2000).

Keum-Ryung-Ja-San (KRJS), a traditional herbal medicine, is made out of *Melia toosendan* SIEB. et ZUCC. (MT) and *Corydalis ternata* Nakai (CT) with equal amounts. It is often used for relieving a various pain, especially, abdominal pain which is appeared below the xyphoid process and costral region resulted from liver-Qi stagnation. From the traditional Chinese medicine, liver-Qi stagnation causes various symptoms, including digestive problems, mental and hormonal dysfunctions which are very similar to the depression (Kim *et al.*, 2005). These findings persuaded us to investigate the effects of KRJS on the depressive behaviors. The purpose of the present

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study was to examine the effect of KRJS on immobility in the forced swimming test which has been a useful in vivo method for screening the antidepressant effects by measuring the duration of immobility time.

MATERIALS AND METHODS

Materials

Imipramine (IMI) was purchased from Sigma Chemical Co. (USA). Dried MT and CT were purchased at the Kyungdong Oriental drug store (Seoul). The materials were authenticated by Emeritus Professor Chang Soo Yook of the Department of Oriental Pharmaceutical Science, College of Pharmacy, Kyung Hee University. All other materials were of the highest grade commercially available.

Animals

Male ICR mice, weighing 23-25 g, were purchased from the Orient Co., Ltd. (a branch of Charles River Laboratories; Seoul). Animals were housed 10 per cage, allowed free access to water and food, and maintained under constant temperature (23±1°C) and humidity (60 ± 10%) under a 12-h light/ dark cycle (light on 07:30-19:30 h). Animal treatment and maintenance were conducted in accordance with the Principles of Laboratory Animal Care (NIH publication No. 85-23, revised 1985) and with the Animal Care and the Use Guidelines of Kyung Hee University, Korea.

Herbal Sample preparation

The voucher specimens (MT: No. KHOPS-06-017, CT: No. KHOPS-06-018) have been deposited at the herbarium located in the College of Pharmacy, Kyung Hee University. The water extracts of KRJA, MT and CT were prepared by boiling dried MT and/or CT in 10 volumes of water for 2 h. The aqueous solutions so obtained were filtered, concentrated on a water bath under vacuum, frozen, lyophilized (Eyela, model FDU-2000, Japan), and stored at -20°C until required. The extract yields were as follows: KRJA extract; 24.6%, MT extract; 29.5%, CT extract; 40.7%.

Locomotor activity

Testing was carried out in clear black Plexiglas boxes (40× 40×40 cm) equipped with the video-based Ethovision System (Noldus, Wageningen, The Netherlands). One hour after KRJS treatment (50, 100, 200, and 400 mg/kg), mice were placed in the center of the apparatus and tested for horizontal locomotor activity by video-recording for 5 min. The horizontal locomotor activity was expressed in terms of the total ambulatory.

Forced swimming test

The forced swimming test was performed according to the methods described by Porsolt *et al.* (1977a). Each mouse was placed in a 25-cm glass cylinder (14 cm diameter) containing 20 cm of water maintained at 24 ± 2°C, and was forced to swim for 6 min. The duration of immobility was recorded using the video-based Ethovision System (Noldus, Wageningen, Netherlands) on PC computer during the last 4 min of the 6-min test. KRJS, MT and CT (50, 100, 200 and 400 mg/kg) were administered (p.o.) 1 hour before the testing. IMI (15 mg/kg) was administered (i.p.) 30 min before the testing.

Statistics

Values are expressed as means ± S.E.M. Data were analyzed by one-way analysis of variance (ANOVA) followed by the Student-Newman-Keuls test for multiple comparisons. Statistical significance was set at $P < 0.05$.

RESULTS

Effects of KRJS on the locomotor activity

To differentiate between the possible stimulatory effects of tested drugs on the modulation of exploratory behavior, a locomotor activity test was performed. KRJS (200 mg/kg) produced no significant changes in the total ambulatory distances compared with the saline control group (1743.5 ± 65.2 cm vs. 1636.4 ± 76.9 cm).

Antidepressive effect of acute treatment with KRJS

The antidepressive effect of KRJS was evaluated in the forced swimming test in mice. The duration of immobility time in the forced swimming test were reduced approximately by 18.2% at 200 mg/kg of KRJS ($P < 0.05$) compared to the control (Fig. 1). The effects of KRJS were similar to those observed in mice given IMI, a classical antidepressant, .i.e., IMI reduced the immobility time by 21.7% at 15 mg/kg, i.p. At the other dose levels of KRJS, changes in duration of immobility time were not significant.

Antidepressive effect of subchronic treatment with KRJS or its ingredients

The subchronic effects of the KRJS and its ingredients, MT and CT, and IMI treatment for 7 days on the duration of immo-

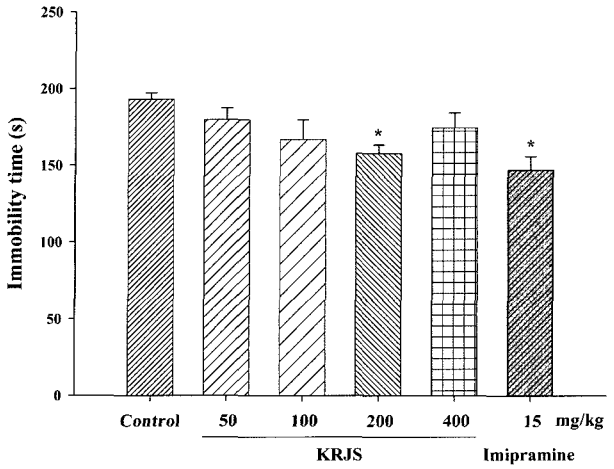

Fig. 1. Antidepressive effect of single treatment with Keum-Ryung-Ja-San (KRJS) on the forced swimming test in mice. KRJS was orally treated 1 h prior to the test at designated dosage regimens. Imipramine was used as a positive control and intraperitoneally treated 30 min prior to the test (15 mg/kg). Data represent the means ± SEM (n=8). *$P < 0.05$ versus saline treated control.

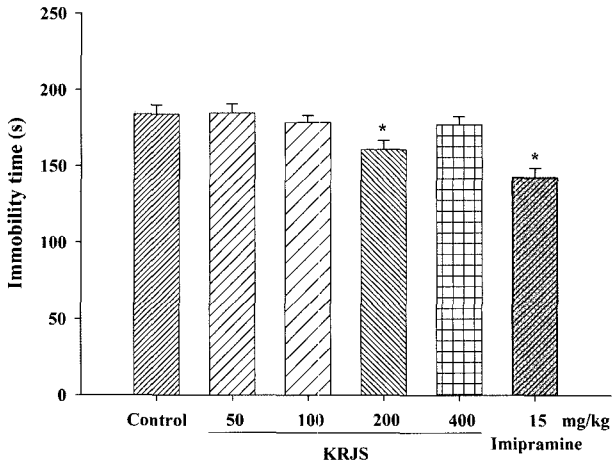

Fig. 2. Antidepressive effect of subchronic treatment with Keum-Ryung-Ja-San (KRJS) on the forced swimming test in mice. KRJS was orally treated once a day for 7 days and the last treatment was finished 1 h prior to the test at designated dosage regimens. Imipramine was used as a positive control and intraperitoneally treated 30 min prior to the test (15 mg/kg). Data represent the means ± SEM (n=8). *$P < 0.05$ versus saline treated control.

bility in the forced swimming test were shown in Figs. 2 and 3. KRJS (200 mg/kg,) and CT (200 mg/kg,) significantly decreased the duration of immobility by 12.6% and 13.9% in a dose-dependent manner ($P < 0.05$). However, mice treated with MT did not show significant decreases in the immobility time compared with those in controls (Fig. 3B). However, the effects of KRJS and CT appeared to be less potent than that of IMI after 7-day treatment in the study.

DISCUSSION

Depression constitutes the second-most common chronic condition in clinical practice, exceeded only by hypertension. Despite recent progress achieved in the development of clinically relevant antidepressant drugs in recent years, the currently available antidepressant is not yet totally effective and it is associated with many undesirable collateral effects (Whooley and Simon, 2000; Nestler *et al.*, 2002). In addition, only 60% of patients are responsive to the treatment with the available antidepressants (Moller and Volz, 1996; Gareri *et al.*, 2000). For this reason, the search for new drugs for the control of the symptoms associated with depressive disorders is still desirable.

The forced swimming test is a well-known behavioral test in rodent that predicted the clinical efficacy of many types of antidepressant treatments (Porsolt *et al.*, 1977b; Butterweck *et al.*, 1996). In addition, tail-suspension test is another rodent model for the antidepressive activity. However, the former is more reliable and specific for the serotonin specific re-uptake inhibitors (Yamamoto and Une, 2002). The immobility behavior means the state of depression in human beings (Porsolt *et el*, 1978).

KRJS, an Oriental herbal medicine, is used in traditional Chinese medicine for relieving a pain in the abdomen, below the xyphoid process and costral region, resulted from liver-Qi stagnation. MT and CT which are ingredients of KRJS are well-known herbal medicine exhibited a wide range of biologic activities (Zhu, 1998). In contrast, very little information was available about the antidepressant activity of these herbs and its prescription, KRJS. In the present study, we observed that both the acute and the subchronic treatment with KRJS resulted in a significant inhibition of the immobility time, with a profile comparable to that observed in imipramine, a classical antidepressant agent. Moreover, the subchronic treatment with CT also resulted in a significant inhibition of the immobility time. However, the acute treatment of MT and CT did not show the changes of the immobility time (data not shown). From these results, it can be concluded that CT might play a role in the antidepressive properties of KRJS. Because there were no changes in the locomotor activities, the antidepressive activity of KRJS was not resulted from its hyperactive properties. However, until now, it is not known which mechanism may be

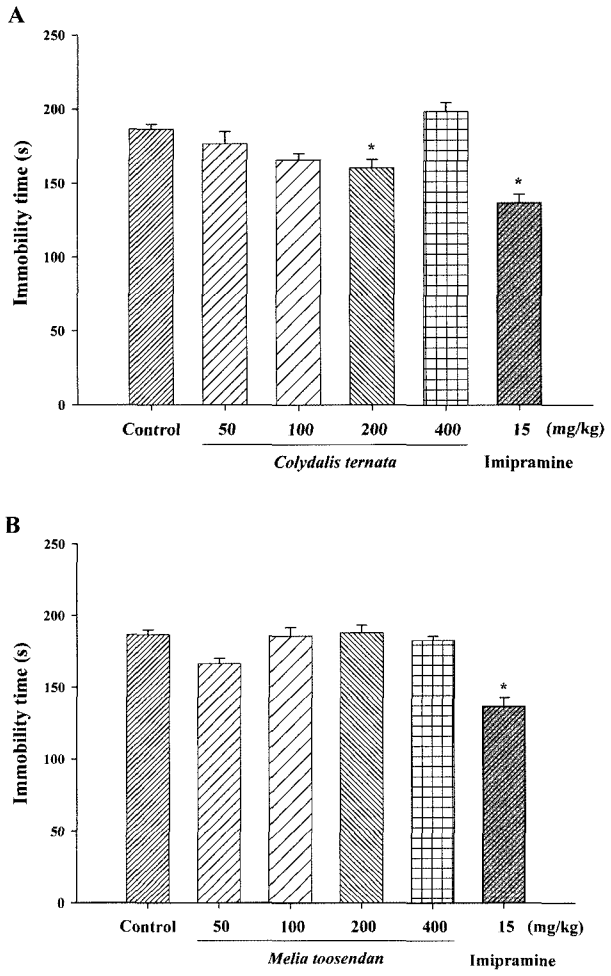

Fig. 3. Antidepressive effect of subchronic treatment with *Corydalis ternata* (A) and *Melia toosendan* (B) on the forced swimming test in mice. The extract of *Corydalis ternata* or *Melia toosendan* was orally treated once a day for 7 days and the last treatment was finished 1 h prior to the test at designated dosage regimens. Imipramine, as a positive control, was treated 30 min prior to the test (15 mg/kg, i.p.). Data represent the means ± SEM (n=8). $*P < 0.05$ versus saline treated control.

involved in the antidepressive properties of KRJS or CT.

Fatigue associated with cholestasis is now recognized as a major problem in health-related quality of life. Although the pathogenesis of this symptom is largely unknown, it has been suggested that central serotonergic neurotransmission may be contributed to the genesis of fatigue with cholestasis (Swain and Maric, 1997). Celik *et al.* (2005) also reported that bile duct-resected rats spent more immobility time in the forced swimming test. From these data, it can be assumed that cholagogue can be applied to the depressive states. Among the ingredients of KRJS, MT was reported to have the cholagogue activities (Kim *et al.*, 1996). In the present study, however, MT did not show antidepressive activity, but CT only exhibited such activity. The assumption which cholagogue plays a role in antidepressive activity might be excluded. It was reported that intravenous administration of DL-tetrahydropalmatine, a major compound in the CT, produced an increase in hypothalamic dopamine release in rats (Lin *et al.*, 1996). Shioda *et al.* (2004) reported that fluoxetine increases dopamine release in the hypothalamus. Therefore, DL-tetrahydropalmatine can work like as antidepressant, probably. However, it is not known that DL-tetrahydropalmatine is responsible for antidepressive effect through dopamine release.

At present study, the exact reasons why higher dose of KRJS (400 mg/kg) were less effective than that of low dose (200 mg/kg) are unclear. However, it could be speculated that these phenomena were observed because of autoreceptor of any neurotransmitter. Several publications have reported that cholinomimetics improve memory function at low doses, but impair function at higher doses (Braida *et al.*, 1996; Bejar *et al.*, 1999). Moreover, Braida et al (1996) speculated that the activation of presynaptic autoreceptors might play a role in the reduced efficacy of ChE inhibitors by excess ACh release, thereby resulting in an inverted U-shaped dose-response curve. Most of the antidepressants affect to the amount of neurotransmitters in the synaptic cleft. Although we did not check the monoamine contents in the synaptic cleft, we assumed that KRJS also influenced on the amount of neurotransmitters in the synaptic cleft. Therefore, we consider that those U-shaped dose-dependent manners for KRJS in the present studies resulted from its influences on neurotransmitters and receptors. Further studies are needed to clarify these issues.

In conclusion, the present results present convincing pharmacological evidence supporting antidepressant-like actions for the KRJS and open up new possibilities for the use of KRJS in the treatment of mood disorders, such as mild and moderate states of depression. Although the findings of herb effects may not in general provide clinically useful outcomes in patients or in normal humans, the findings of this study may be important because they confirm the validity of KRJS or CT as a medicinal plant.

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