Regular Article

Antidepressant-like effects of the traditional Chinese medicine kami-shoyo-san in rats

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Abstract

Kami-shoyo-san (KSS), a traditional Chinese medicine, has been used to treat patients with neuropsychiatric disorders. The aim of the present paper was to investigate whether KSS has antidepressant-like effects, and to assess its mechanism of action, using male Sprague–Dawley rats given 10-fold (KSS 10X) or 20-fold (KSS 20X) the typical human daily dosage. Immobility time was measured by the forced swimming test, and hippocampal neurogenesis was quantified under immobilization stress. Rats given KSS 20X, but not those given KSS 10X, had a significantly lower immobility time and improved neurogenesis in the hippocampus. These results suggest that KSS possesses an antidepressant-like effect at a behavioral and molecular level.

Key words  forced swimming test, immobilization stress, kami-shoyo-san, neurogenesis.

INTRODUCTION

Major depressive disorder is a common clinical problem with a lifetime risk of 13–20% in the general population.1 Depression is one of the world’s leading disabilities, as measured by the number of years people are afflicted with a disabling condition.2 In spite of treatment using various antidepressants, such as tricyclic antidepressants (TCA), monoamine oxidase inhibitors, selective serotonin re-uptake inhibitors, and specific serotonin–norepinephrine re-uptake inhibitors, one-third of depressive patients are resistant to drug therapy.3 Moreover, most of these drugs have a high propensity to cause serious side-effects such as cardiotoxicity, hypertensive crisis, sexual dysfunction, and sleep disorder. Therefore, an alternative antidepressant drug is urgently required.

According to theories of traditional Chinese medicine (TCM), clinical depression can be classified primarily as liver qi stagnation, the symptoms of which include mental stress, hypochondriac distensive pain, lumps in the breasts, hernial pain, and irregular menstruation. Many Chinese medicinal plants have been used to manage depression by dispersing stagnant liver qì. Kami-shoyo-san (KSS) is a popular TCM in Korea, Japan, and China for treating stress-related neuropsychiatric disorders such as depression or anxiety.10,11 However, the pharmacological mechanisms of its therapeutic effects are not well understood.

We investigated whether KSS has antidepressant-like effects, by assessing its effects on the immobility time of rats in the forced swimming test, and neurogenesis in the hippocampus of adult rats under immobilization stress.

METHODS

Preparation of medicinal extracts

The components of the recommended human daily dose (34 g) of KSS are listed in Table 1.12 The nine
Plants were obtained from Medicinal Materials, Gyeongju Province, Korea, and identified by Y-K. Park, College of Oriental Medicine, Dongguk University (Gyeongju, Korea). The plants were air-dried and extracts were prepared by boiling samples in 200 mL water at 100°C for 2 h. The procedure was repeated twice. The extracts were filtered, concentrated into residues in a vacuum evaporator, and lyophilized into powder. The yield of extracts was 12% (w/w). Rats were given doses at 10-fold and 20-fold the recom- mended daily dose (34 g) for humans, expressed as KSS 10X and KSS 20X, respectively. The actual doses of KSS 10X and KSS 20X were 670 mg/kg and 1340 mg/kg, respectively. Imipramine, a TCA, was sus- pended in normal saline (0.9% NaCl), and the animals were given the drugs orally using an intubation needle (Stoelting, Wood Dale, IL, USA).

### Chemicals

For immunohistochemistry the Vectastain ABC and DAB substrate kits (Vector Laboratories, Burlingame, CA, USA) and monoclonal mouse ant bromodeoxyuridine (Dako, Glostrup, Denmark) were used. All other chemicals were purchased from Sigma-Aldrich (St Louis, MO, USA).

### Animals

Male Sprague–Dawley rats (Hyocang Laboratory, Daegu, Korea) weighing 200–250 g were housed (three per cage) with food and water freely accessible, and maintained at 21°C in a 12:12 light : dark cycle. All animal manipulations were conducted in accordance with the animal care guideline of the National Insti-
The dimensions of the plastic restraint tubes were 20 cm (length) × 7 cm (diameter). This stress paradigm was chosen based on past data that demonstrated that 3 weeks of chronic immobilization stress produced a decrease in hippocampal neurogenesis.18

Bromodeoxyuridine labeling
Bromodeoxyuridine (BrdU) was prepared in saline at a dilution of 20 mg/mL BrdU and 0.007 mol/L NaOH. Solutions were dissolved by sonication and prepared on the same day they were used. To examine cell proliferation, BrdU was injected (i.p., 200 mg/kg) on the last day of the stress session, 1 day before death. Twenty-four hours after BrdU injection, the rats were killed after deep anesthetization with pentobarbital (i.p., 75 mg/kg) and transcardially perfused with ice-cold phosphate-buffered saline (PBS, pH 7.4) followed by ice-cold 4% paraformaldehyde in PBS. After perfusion, all brains were post-fixed in paraformaldehyde until use. Serial sections (50 μm) were cut through the entire hippocampus on a vibratome (Series 1000 Sectioning System; Technical Products International, O’Fallon, MO, USA), and stored in 4% formalin.

Immunohistochemistry
Free-floating sections were used to determine BrdU labeling. Immunohistochemistry was similar to that described by Malberg et al.20 DNA was denatured by incubating samples for 2 h in 50% formamide/2X SSC at 65°C, followed by several PBS rinses. Sections were then incubated for 30 min in 3 N HCl at 37°C and again for 10 min in 0.1 mol/L boric acid. After rinsing with PBS, sections were incubated for 30 min in 3% H2O2 to eliminate endogenous peroxidases. Sections were then washed for 15 min in 0.3% TritonX-100. After blocking with 3% blocking serum (Vector Laboratories) in PBS, cells were incubated overnight with antimouse BrdU (1:200, Dako) at 4°C. Sections were then incubated for 1 h with secondary antibody (biotinylated horse antimouse; Vector Laboratories). Samples were amplified using an avidin–biotin complex (Vector Laboratories), and cells were visualized with DAB (Vector Laboratories).

Photomicroscopic imaging and quantification of BrdU labeling
Neurogenesis can be traced by immunohistochemical detection of BrdU incorporated into the DNA of proliferating cells during the DNA synthesis phase of the cell cycle.21 In the hippocampus these cells generally reside in the subgranular zone (SGZ), at the border between the granule cell layer (GCL) and the hilus. Photomicroscopic images were obtained using image-analysis software (Image-Pro Plus version 3.0; Media Cybernetics, Silver Spring, MD, USA). Mounted sections spaced at least 160 μm apart were subjected to blinded analysis. Nine sections per animal (18 hippocampi) were analyzed for BrdU immunostaining in an area encompassing the entire granule cell layer, and extending approximately two-cell-layer widths deep into the hilus. Small BrdU-labeled nuclei (presumed to be glial precursors) at the hilar border, and linear immunostained forms, were excluded from the analysis.22 Results are presented as the sum of the mean number of BrdU-labeled cell nuclei in either the left or right dentate gyrus of each slice. Mean values are means ± SE.

Statistical analysis
For the forced swimming test, the Kruskal–Wallis test was used to find significant differences in the immobility time among the four groups, and the Mann–Whitney U-test was performed for the post-hoc comparison. For the immobilization stress model, the mean number of BrdU-labeled cell nuclei of the four groups was compared with a one-way ANOVA with Fischer post-hoc least significant difference. Values were considered significant at P < 0.05.

RESULTS
Effects of KSS on immobility time in the forced swimming test
The immobility times in the forced swimming test for the four groups are shown in Fig. 1. The immobility times of the control, imipramine, KSS 10X, and KSS 20X-treated groups were 217.0 ± 15.8 s, 125.0 ± 10.0 s, 179.3 ± 18.8 s, and 144.9 ± 28.6 s, respectively. Groups treated with imipramine (20 mg/kg) and KSS 20X had significantly shorter immobility times compared to the control group (P = 0.009 and P = 0.020, respectively). The immobility time of the KSS 10X-treated group was not significantly different from that of the control group (P = 0.109).

Effects of KSS on hippocampal neurogenesis under immobilization stress
Photomicroscopic images and the BrdU-labeled cells of the four groups are shown in Fig. 2. Analysis by one-way ANOVA showed significant effects on the number of BrdU-labeled cell in hippocampal dentate
gyrus \((F = 8.12, \ P = 0.002)\). The total numbers of BrdU-labeled cells from the control, stress, KSS 10X-treated stress, and KSS 20X-treated stress groups were \(440.8 \pm 14.2, 338.8 \pm 22.7, 313.4 \pm 28.4, \) and \(450.6 \pm 29.8\), respectively. Immobilization stress for 21 days (Stress group) resulted in a significant 23% decrease in the number of BrdU-labeled cells compared to the unstressed control group \((P = 0.040)\). The KSS 20X-treated stress group had a significant 25% increase in the number of BrdU-labeled cells compared to the stress group \((P = 0.020)\). However, no significant difference was observed between the stress group and the KSS 10X-treated stress group \((P = 0.880)\).

**DISCUSSION**

Administering kami-shoyo-san to rats at 20-fold the recommended daily dose for humans (KSS 20X) significantly reduced the immobility time in the forced swimming test, and significantly improved neurogenesis in the hippocampus following immobilization stress. KSS demonstrated antidepressant-like effects, at both the behavioral and molecular level, in two different animal models.

In the forced swimming test, KSS 20X induced a statistically significant reduction in immobility time compared to control animals. The level of this antidepressant effect was comparable to that of imipramine 20 mg/kg. Recently, an O-linked glycoside with the sugar chain structure GalNAcα 1-3GalNAc was separated from KSS, and glycoside was shown to increase activity in the forced swimming test in a dose-dependent manner.\(^{23}\) The present results are consistent with this finding, although there were considerable differences between the experimental methods of the two studies, such as the preparation of the extracts, duration of drug treatment, strain of animals, and conduction of the swimming sessions.
KSS has been used to treat neuropsychiatric diseases such as anxiety, insomnia, and irritability or depression during menopause, although its mechanism of action was unclear. Stress and depression are often associated with hyperactivity of the hypothalamic–pituitary–adrenal axis, resulting in elevated levels of adrenal steroid hormones (cortisol in humans and tree shrews, corticosterone in rats), which have been shown to inhibit granule cell proliferation. Increased hippocampal BrdU labeling (neurogenesis) is observed after chronic treatment with antidepressants in unchallenged rats. The present findings suggest that KSS may act therapeutically via a mechanism leading to hippocampal neurogenesis.

Neurogenesis is regulated at several levels, such as cell proliferation, differentiation, migration, and survival. Three weeks after BrdU injection, approximately 80% of the BrdU-labeled cells expressed the neuron-specific marker enolase, and were incorporated into the granule cell layer. In the present study, rats were perfused 24 h after BrdU injection. Thus, histological analysis could evaluate only the rate of cell proliferation. Survival and the cellular phenotype of these newly generated cells were not investigated. Therefore, we can only speculate that the majority of these cells would have differentiated into mature neurons and become integrated into the hippocampal circuitry.

The present data showed that KSS at a dose of 20X had an antidepressant effect in the forced swimming test and hippocampal neurogenesis, but not at a dose of 10X. Previous animal studies also reported antidepressant action above 10-fold the clinical human dose. The present findings suggest that the antidepressant effects of KSS might be dose dependent.

In conclusion, KSS appears to have antidepressant-like effects in animal models of depression. With respect to its mechanism of action, the present results suggest that KSS-induced upregulation of neurogenesis may offset the reduction in hippocampal function and volume typical in patients suffering from depression and other stress-related disorders. Further research is needed to elucidate the precise mechanism of action of KSS, and characterize its active constituents.

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