Antidepressant-like activity of a Kampo (Japanese herbal) medicine, Koso-san (Xiang-Su-San), and its mode of action via the hypothalamic–pituitary–adrenal axis

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Abstract

Koso-san (Xiang-Su-San in Chinese), a Kampo (Japanese herbal) medicine, is used clinically in East Asia for the treatment of depression-like symptoms associated with the initial stage of the common cold, allergic urticaria due to food ingestion, irritable bowel syndrome, chronic fatigue syndrome, insomnia, and autonomic imbalance. However, the antidepressant-like activity of Koso-san has never been evaluated scientifically. In this study, ddY mice subjected to a combination of forced swimming and chronic mild stresses were termed depression-like model mice. The degree of the depression-like state was measured by the animal’s duration of immobility using the forced swimming test (FST). Oral administration of Koso-san (1.0 g/kg/body wt./day, 9 days) significantly shortened the duration of immobility of the depression-like model mice in the FST; however, locomotor activity was not affected. Hyperactivity of the hypothalamic–pituitary–adrenal (HPA) axis plays an important role in the pathophysiology of depression. Levels of corticotropin-releasing hormone mRNA expression in the hypothalamus and proopiomelanocortin mRNA expression in the pituitary were significantly increased, and glucocorticoid receptor protein expression in the hypothalamus paraventricular nucleus was downregulated in the depression-like model mice. However, Koso-san ameliorated these alterations to the normal conditions.

The results of this study suggest that Koso-san shows the antidepressant-like effect through suppressing the hyperactivity of the HPA axis in depression-like model mice.

Keywords: Koso-san; Xiang-Su-San; Antidepressant; HPA axis; Forced swimming test; Chronic mild stress; Depression-like model mice

Introduction

Koso-san, a Kampo (Japanese herbal) medicine (Xiang-Su-San in Chinese), is composed of five herbs (Cyperi rhizoma, Perillae herba, Aurantii nobilis pericarpium, Glycyrrhizae radix and Zingiberis rhizoma) and is used clinically to treat depression-like symptoms associated with the initial stage of the common cold, allergic urticaria due to ingestion of food, irritable bowel syndrome, chronic fatigue syndrome, insomnia and autonomic imbalance. In addition, it has been clinically suggested that Koso-san can alleviate depression, a common side effect of interferon-α therapy for hepatitis
C infection (Hanawa, 1995). Pharmacological studies have reported that Koso-san is effective for globus pharyngeus (Motoo et al., 1999), skin injury induced by X-rays (Wang et al., 1990) and secretion of bile (Sasaki et al., 1989). However, no investigation of the possible mechanisms of action of Koso-san’s antidepressant-like effects has yet been reported.

Clinical depression is an endemic disorder in modern societies. The incidence of human depression is worldwide, with a prevalence of 4–16% in some developed countries (Wong and Licinio, 2001). Stressful lifestyles and events often precede the onset of clinical depression and stress-related neuroendocrine mechanisms are proposed to be involved in the pathogenesis of affective disorders (Checkley, 1992; Sapolsky, 1996).

Impaired function of the hypothalamic–pituitary–adrenal (HPA) axis has consistently been observed in patients with clinical depression. Hyperactivation of the HPA axis results in stimulating levels of corticotropin-releasing hormone (CRH), adrenocorticotropic hormone (ACTH) and glucocorticoid in the hypothalamus, pituitary and adrenal cortex, respectively. Abnormalities of the HPA axis have been reported with levels returning to normal values after treatment with antidepressant drugs or electroconvulsive therapy (Gold et al., 1988; Pariante and Miller, 2001; Stout et al., 2002). Glucocorticoid receptors (GR) are associated with the negative-feedback regulation of the HPA axis and believed to play an important role in regulating the response to stress when the endogenous level of glucocorticoid is high (Pariante and Miller, 2001). Treatment of transgenic mice with impaired GR function using tricyclic antidepressant drugs shows increases in hypothalamic GR mRNA expression and dexamethasone-binding activities, while decreasing levels of plasma ACTH and corticosterone, resulting in the disappearance of deficient behaviors (Montkowski et al., 1995; Pepin et al., 1992). These findings suggest that normalization of GR dysfunction may be closely associated with the recovery of impaired neuroendocrine and depressive moods.

To create a depressive-like state using an animal model, ddY mice were subjected to chronic and unpredictable stressors such as the forced swimming (FS) (Porsolt et al., 1977; Detke et al., 1997) and chronic mild stress (CMS) (Solberg et al., 1999; Willner et al., 1987) in the present study. The present study describes the antidepressant-like effects of Koso-san using depression-like model mice, and its possible mechanism of action on the HPA axis.

Materials and methods

Animals

Seven-week-old male ddY mice (Japan SLC, Hamamatsu, Japan), weighing 35–40 g each at the beginning of the experiment, were used. The mice were housed under conditions of constant temperature (23 ± 2°C) and humidity (55 ± 10%) with food and water available ad libitum (unless specified otherwise), and a 12/12 h light–dark cycle (8:00–20:00) during the stress procedure. All animal experiments were performed according to the Guidelines for Care and Use of Laboratory Animals at Kitasato Institute and Kitasato University.

Drugs

The following drugs were used: desipramine hydrochloride (Sigma, St. Louis, MO, USA), Toledomin® (milnacipran hydrochloride) (Asahi Kasei Pharma Corp., Tokyo, Japan), Luvox® (fluvoxamine maleate) (Solvay Seiyaku Co., Tokyo, Japan), Horizon® (diazepam) (Yamanouchi Pharmaceutical Co. Ltd., Tokyo, Japan).

Component herbs of Koso-san (Table 1) were purchased from Tsumura & Co., Tokyo, Japan (C. rhizoma, P. herba, A. nobilis pericarpium and Z. rhizoma) and Uchida Wakan-yaku Co. Ltd., Tokyo, Japan (G. radix). The Kampo formula was decocted with 600 ml of distilled water until the volume was reduced by half, and the extract was filtered immediately through paper in vacuo. The filtrate was lyophilized and the yield of Koso-san extract was ca. 28% of the herbal mixture relative to dry weight.

Three-dimensional (3-D) HPLC analysis of Koso-san

The components of Koso-san extract were identified using 3-D HPLC with an Agilent 1100 3-D HPLC
system (Agilent Technologies, Tokyo, Japan) equipped with a photodiode-array detector. A TSK gel ODS-80Ts column (4.6 × 250 mm) (Tosoh Corp., Tokyo, Japan) was used and kept at 40 °C. Elution of the mobile phase was carried out by 10 mM phosphoric acid-acetonitrile linear gradient (95:5–5:95, 60 min), and its flow rate was 0.8 ml/min. The following standard samples were used for identification: rosmarinic acid (purchased from Extrasynthese, Genay, France), caffeic acid (Extra- synthèse), luteolin (purchased from Roth, Karlsruhe, Germany), luteolin-7-glucoside (Roth), l-menthol (purchased from Wako Pure Chemical Industries, Osaka, Japan), apigenin (Roth), l-perillaldehyde (Extrasy nthèse), hesperidin (Extrasyntèse), naringin (Extrasy nthèse), naringenin (Roth), narirutin (Extrasynthèse), glycyrrhizin (Wako), glycyrrhetic acid (Wako), liquiritin (Wako), liquiritigenin (Extrasyntèse), formononetin (Roth) and 6-gingerol (Wako). A 3D-HPLC profile of the Koso-san extract dissolved in distilled water (5 mg/ml) is shown in Fig. 1. In the present study, seven components (glycyrrhizin, liquiritigenin, rosmarinic acid, hesperidin, narirutin, liquiritin and caffeic acid) were identified.

Preparation of depression-like model mice

The depression-like model mice were prepared using modified FS twice with an 11-day interval (Porsolt et al., 1977; Detke et al., 1997) and CMS (Solberg et al., 1999; Willner et al., 1987). Briefly, the mice were placed individually into separate 51 glass beakers (height 27 cm, diameter 18 cm) filled with 41 of water (23 ± 1 °C) for 15 min. Beakers were separated by non-transparent panels to prevent the mice from seeing each other. After 15 min in the water, mice were removed and allowed to dry with a drier before being returned to their home cages. Mice were then separated into groups by measuring the duration of immobility for the first 5 min of FS, in order to minimize the variation in immobility among the groups. A mouse was judged to be immobile when it ceased struggling and remained floating motionless in the water making only those movements necessary to keep its head above water. After 2 days, mice were exposed to CMS, which consisted of three different stress situations: tilting the cage twice to 30° from the horizontal (CMS 1), pouring 200 ml of water onto the sawdust bedding of a cage (CMS 2), and shaking the cages at 200 rpm with a Green S. Seriker II (Vision Scientific, Kyunggi, Korea) (CMS 3). These stress situations were applied for 48, 24 and 24 h, respectively, with 24-h intervals (Scheme 1). Mice were then placed again into 51 beakers with 41 of water at 60 min after the final drug administration, and the total duration of immobility during 5 min of forced swimming test (FST) was observed.
drug administration

All drugs were suspended in distilled water. Diazepam (0.5 mg/kg/body wt.), desipramine (30 mg/kg/body wt.), fluvoxamine (60 mg/kg/body wt.), milnacipran (60 mg/kg/body wt.) and Koso-san (0.1, 0.5 and 1.0 g/kg body wt.) were administered orally to depression-like model mice by intragastric gavage at a volume of 0.5 ml/mouse once daily on days 1, 2, 4, 5, 6, 7, 8, 9 and 11 (9 times) (Scheme 1).

spontaneous locomotor activity test

Spontaneous locomotor activities of mice were evaluated using the open field test. Briefly, mice were placed individually in the center of an open field (40 x 40 x 20 cm), with the field divided into 25 equal squares (Sanki Kagaku Kogei, Kawasaki, Japan), and allowed to move freely for 15 min. The total number of line crosses for the first 5 min was counted in order to minimize variation in the total number of line crosses among the groups. Eleven days later, mice were again placed individually in the open field and the total number of line crosses was counted during a 5-min interval, 60 min after the final drug administration.

reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was isolated from the hypothalamus or pituitary of depression-like model mice by the acid guanidinium–phenol–chloroform method using ISO-GEN (Nippon gene, Toyama, Japan). Total RNA was used as a template for cDNA synthesis by the first-strand cDNA synthesis kit (Amersham Biosciences, Piscataway, NJ, USA). PCR amplification of this cDNA was performed using Taq DNA polymerase (Takara Bio, Otsu, Japan). Primers (Invitrogen, Carlsbad, CA, USA) used for PCR were as follows: 5′ GCA TCC TGA GAG AAG TCC CTC TG 3′ (sense) and 5′ GCC CTG GCC ATT TCC AAG AC 3′ (antisense) for CRH, 5′ CGG CCC CAG GAA CAG CAG CAG T 3′ (sense) and 5′ GGG CCC GTC GTC CTT CTC C 3′ (antisense) for proopiomelanocortin (POMC), 5′ GGT CAA CCC CAC CGT GTT CTT CGA 3′ (sense) and 5′ TTG CCA TCC AGC CAT TCA GTC TTG 3′ (antisense) for cyclophi-

Measurement of corticosterone

Blood samples were collected by decapitation of mice at 60 min after final drug administration and were centrifuged at 4 °C. Sera were stored at −80 °C until assayed for corticosterone.

Serum corticosterone was measured using a Corre-late-EIA™ Corticosterone Enzyme Immunoassay Kit (Assay Designs, Ann Arbor, MI, USA) according to the manufacturer’s instructions. The sensitivity of the measurement was 26.99 pg/ml. The intra- and inter-assay coefficients of variations were 8% and 13%, respectively.

Immunohistochemical study

Mice were quickly anesthetized with sodium amobarbi-tal (Sigma) 5 min after the FST and perfused transcardially with 0.9% saline, followed by 4% paraformaldehyde (Wako) at twice the volume of body weight. Serial coronal brain sections of 50-μm thickness were cut with a vibratome. Free-floating sections were preincubated for 2 h at −20 °C in 80% methanol/3% H2O2 to eliminate endogenous peroxidases. After washing in phosphate buffered saline (PBS), sections were incubated for 2 h at room temperature with 1% bovine serum albumin (BSA) in PBS containing 0.3% Triton X-100 (PBS-T) and incubated overnight at 4 °C with rabbit antiserum against glucocorticoid receptor (GR) 1:1600 (Santa Cruz Biotechnology, Santa Cruz, CA, USA) in PBS-T containing 1% BSA. After washing in PBS-T, sections were incubated for 2 h at room temperature with biotinylated goat anti-rabbit IgG (Vector Laboratories, Burlingame, CA, USA) 1:200 in PBS-T containing 1% BSA, and washing PBS-T, sections were incubated for 2 h at room temperature with avidin–biotin complex using Vectastain Elite ABC kit (Vector Laboratories) in PBS-T. Sections were counterstained with 0.05% toluidine blue, dehydrated, and mounted in permount (Fisher Scientific International Inc., Fair Lawn, NJ, USA). GR-positive cells were detected and processed using an Olympus BX 41 microscope (Olympus Corp., Tokyo, Japan).
Statistical analysis

Results were expressed as means ± SEM. Behavioral data, CRH, POMC mRNA data and serum corticosterone data were analyzed using one-way analysis of variance (ANOVA). Post hoc comparisons, if applicable, were carried out using the Scheffé test. The p-values lower than 0.05 (p < 0.05) were considered significant.

Results

Effects of diazepam, desipramine, fluvoxamine and milnacipran on the duration of immobility of depression-like model mice in the FST

Depression-like model mice were prepared by combination of FS with three different combined chronic mild stresses (CMS) to create depressive-like states. To evaluate this animal model, a known minor tranquilizer and antidepressants were administered to determine the effects on the duration of immobility of mice in the FST. Mice administered minor tranquilizer, diazepam, versus water (control mice) showed no significant difference in the duration of immobility, using the FST as an index. By contrast, administration of the antidepressants, desipramine (tricyclic antidepressant), fluvoxamine (selective serotonin reuptake inhibitor) and milnacipran (serotonin-noradrenaline reuptake inhibitor) reduced the duration of immobility significantly as compared to the water-administered control (p = 0.0028, 0.0211 and p < 0.0001, respectively; data not shown).

Effect of Koso-san on the duration of immobility of depression-like model mice in the FST

Oral administration of Koso-san reduced the duration of immobility in a dose-dependent manner from 0.1 to 1.0 g/kg/body wt./day, and Koso-san administered at 1.0 g/kg/body wt./day significantly reduced the duration of immobility as compared with the water-administered control (p = 0.0074) (Table 2). Milnacipran, a positive control, also significantly reduced the duration of immobility as compared to control (p < 0.0001).

Effect of Koso-san on the spontaneous locomotor activity of mice

The total number of line crosses was not affected by the administration of Koso-san (0.1, 1.0 g/kg body wt.) or milnacipran (60 mg/kg body wt.) (data not shown).

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (g/kg)</th>
<th>Duration of immobility (s/5 min)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td></td>
<td>184.0 ± 15.4</td>
<td>—</td>
</tr>
<tr>
<td>Koso-san</td>
<td>0.1</td>
<td>163.1 ± 10.5</td>
<td>0.9113</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>148.6 ± 20.6</td>
<td>0.5685</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>101.7 ± 14.0</td>
<td>0.0074</td>
</tr>
<tr>
<td>Milnacipran</td>
<td>0.06</td>
<td>47.1 ± 9.2</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

Drugs were administered orally to depression-like model mice once daily according to the schedule shown in Scheme 1. The duration of immobility was measured during a 5 min of FST at 60 min after the final drug administration. Each duration of immobility represents the mean ± SEM (n = 9–10).

Effects of Koso-san on expressions of CRH mRNA in the hypothalamus and POMC mRNA in the pituitary of depression-like model mice

The mRNA expression of CRH and POMC was measured in the hypothalamus and pituitary gland of the model mice using RT-PCR. Stress induced by combination of FS and CMS significantly enhanced the CRH and POMC mRNA expressions, as compared with control mice (p = 0.0020, Fig. 2; p = 0.0123, Fig. 3, respectively). The increased expressions of CRH mRNA in the hypothalamus and POMC mRNA in the pituitary indicated hyperactivation of the HPA axis in the depression-like model mice. Administration of Koso-san (0.1, 1.0 g/kg body wt.) significantly and dose-dependently reduced the expression of CRH mRNA significantly (p < 0.0001). Koso-san (1.0 g/kg body wt.) or milnacipran also significantly reduced the expression of POMC mRNA in the pituitary, as compared to the water-administered control (p = 0.0407, 0.0178) (Fig. 3).

Effect of Koso-san on corticosterone levels in the sera of depression-like model mice

Stress caused by the combination of FS and CMS resulted in significant increases of corticosterone levels, as compared with non-stressed mice (p = 0.0029) (data not shown). Milnacipran (60 mg/kg/body wt.) reduced the level significantly, as compared to the water-administered control (p = 0.0003). Administration of Koso-san (0.1, 1.0 g/kg body wt.) did not significantly reduce corticosterone levels.
Effect of Koso-san on GR protein expression in the hypothalamic paraventricular nucleus (PVN) of depression-like model mice

Compared with non-stressed mice (Fig. 4A), stress caused by the combination of FS and CMS resulted in the decrease of GR protein expression in the PVN (Fig. 4B), indicating that GR protein expression in the PVN of depression-like model mice may be downregulated by the addition of stresses. Administration of Koso-san (0.1, 1.0 g/kg body wt.) or milnacipran (60 mg/kg/body wt.) increased the expression of GR protein (Fig. 4C–E, respectively) as compared to the water-administered control (Fig. 4B).

Discussion

The purpose of this study was to determine the efficacy of Koso-san using ddY mice subjected to...
various stresses as the depression-like animal model. FST is a behavioral despair procedure that has been used widely to predict the efficacy of antidepressants (Porsolt et al., 1977; Detke et al., 1997). Chronic stress is thought to play an important role in the etiology of depression. Several studies have demonstrated the usefulness of CMS to measure the effects of antidepressants (Solberg et al., 1999; Willner et al., 1987). In this study, in order to take account of the depressive mood induced by different daily stresses, depression-like model states were prepared in mice subjected to a combination of FS and a series of daily CMS (Scheme 1).

To examine the specificities of drug responses using our animal model, the effects of the minor tranquilizer diazepam and the antidepressants desipramine, fluvoxamine and milnacipran were evaluated by subjecting mice to the FST and measuring the duration of immobility. Significant reductions in the durations of immobility in the FST were observed with the oral administration of desipramine, fluvoxamine and milnacipran but not with diazepam (data not shown), suggesting that the present animal model of combined FS and CMS responds to antidepressants.

Clinical studies have shown that patients with major depression have impaired function of the HPA axis (Gold et al., 1988; Nemeroff, 1996) and, thus, persistent hyperactivity in the HPA axis may play a critical role in the pathogenesis of endogeneous depression (Barden et al., 1995; Heuser, 1998; Holsboer and Barden, 1996). Because the HPA axis was activated in the
depression-like model mice used in the study, it afforded a suitable model for evaluating antidepressant compounds.

A dose-dependent reduction in the duration of immobility was observed with a 1.0 g/kg body wt. dose of Koso-san showing the greatest effect (Table 2). Psychostimulants such as caffeine decrease the duration of immobility in the FST, but in contrast to antidepressants, caffeine causes marked locomotor stimulation indicating that the effects may be nonspecific (Porsolt et al., 1977). Koso-san did not change the locomotor activities of the naïve mice at the doses that produced reduction in the duration of immobility (data not shown). Other Kampo medicines, Boi-ogi-to and Tokaku-joki-to, containing herbs such as Zizyphus fructus or Cinnamomi cortex, in which saponins or essential oil are known for sedative (Hong, 1987; Harada et al., 1976) or antioxidant activity (Shimizu et al., 1990), did not reduce the duration of immobility (Ho et al., unpublished). These results suggest that Koso-san has the antidepressant-like property, but that all Kampo medicines do not necessarily reduce the duration of immobility in depression-like model mice. Since sedatives usually exhibit the suppression of locomotor activities, sedative compounds do not necessarily exhibit antidepressant-like activity. Although some antioxidants exhibit antidepressant-like effects (Kolla et al., 2005), Kampo medicines containing C. cortex might contain low antioxidants.

**Fig. 4.** Effect of Koso-san on GR protein expression in the hypothalamus of depression-like model mice. Drugs were administered as described for Fig. 2. Mice were killed under deep amobarbital anesthesia by transcardiac perfusion with 4% paraformaldehyde after FST and the brains were removed. Immunohistochemical analysis of GR protein was performed on serial 50 μm vibratome sections of the brains of mice without (A) or with stress administered water (B), Koso-san 0.1 g/kg/body wt./day (C), Koso-san 1.0 g/kg/body wt./day (D) or milnacipran (E). GR-positive cells represented as brown spots were detected using Olympus BX 41 microscope. Scale bar equals 100 μm and applies to all sections.
It has been reported that the compounds (rosmarinic acid, caffeic acid and apigenin) in Perillae herba, one of the component herbs of Koso-san, showed antidepressant-like effects in mice subjected to the FST (Takeda et al., 2002a,b; Nakazawa et al., 2003). The 3-D HPLC analysis showed that the water extract of Koso-san contained rosmarinic acid and caffeic acid, but not apigenin (Fig. 1). Therefore, Perillae herba may play a partial role in the antidepressant-like effect of Koso-san. However, it is not clear whether rosmarinic acid is an active component due its low concentration in Koso-san and its very high dose-response antidepressant effect (Takeda et al., 2002b).

Hyperexpressions of CRH and POMC mRNA in model mice were reduced by the oral administration of Koso-san, suggesting that hyperactivation of the HPA axis was, in part, suppressed by the Koso-san in depression-like model mice. Moreover, the antidepressant-like effect of Koso-san would be associated with the recovery of the negative feedback against the hyperactivation of HPA axis, by up-regulation of GR protein expression in the PVN. However, no significant difference in GR protein expression was observed in the hippocampus of depression-like model mice following administration of Koso-san in the present study (data not shown). Therefore, GR protein in the PVN may play a more important role in the negative feedback of the HPA axis than in the hippocampus. Oral administration of Koso-san did not significantly reduce serum corticosterone levels (data not shown), indicating that Koso-san may not be effective in acute stress situations. It has been reported that a Kampo medicine, Saiko-ka-ryukotsu-borei-to, prevented chronic stress-induced disruption of the negative feedback mechanism, evaluated by a dexamethasone suppression test using blood samples 10 days (recovery period) after exposure to the last stress situation in order to avoid the acute influences of stressors in rats (Mizoguchi et al., 2002). Further studies on the influence of Koso-san on corticosterone levels by dexamethasone after the recovery period could help to further explain the modulation of Koso-san against HPA axis. These studies are now underway.

This study represents the first report showing the efficacy of Koso-san as a possible clinically useful antidepressant Kampo medicine, using depression-like mice as an animal model. The results of this study show that Koso-san exhibits antidepressant-like action when administered to depression-like mice by suppressing the hyperactivity of the HPA axis. Further studies are warranted and underway to identify the active components found in Koso-san and to elucidate in more detail the mechanisms and modes of action of each active component with respect to antidepressant-like activities. Studies on the synergistic effects of various combinations of the active components found in Koso-san may lead to more effective clinical treatments of depressive states.

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