Antidepressant-like effect of a Kampo (Japanese herbal) medicine, kososan, against the interferon-α-induced depressive-like model mice

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Kososan (Xiang-Su-San in Chinese), a Kampo (Japanese herbal) medicine, has been used clinically in East Asia for the treatment of depressive symptom associated with the common cold, allergy, insomnia and autonomic imbalance. The therapeutic use of interferon (IFN)-α is known to cause various neuropsychiatric adverse effects, and especially, there is a problem in which IFN-α therapy in patients with hepatitis C often induces the depressive symptom. However, the antidepressive effect of kososan on the IFN-α-induced depression remains unclear. In the present study, we investigated whether kososan improves depressive-like behaviors in IFN-α-induced depressive-like model mice. The degree of the depressive-like state was measured by the animal’s duration of immobility using a forced swimming test (FST). Oral administration of kososan (1.0 g/kg/day, 14 days) significantly reduced the duration of immobility time of IFN-α (1.2 x 10^6 IU/kg/day, 7 days, i.p.)-induced depressive-like model in the FST; however, locomotor activity was not affected. Hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis plays a crucial role in the pathology of depression. IFN-α-induced increases in corticotropin-releasing hormone mRNA expression in the hypothalamus and corticosterone levels of sera were decreased by the administration of kososan. These results suggest that kososan shows an antidepressive-like effect via normalizing the hyperactivity of HPA axis in the IFN-α-induced depressive-like model mice.

Key words kososan, Kampo medicine, interferon-α, depression, HPA axis.

Introduction

Interferons (IFN-α, - β and -γ) are known as multifunctional cytokines that are associated with the host defense against viral and parasitic infections, and tumorigenesis. These IFNs produced by infected cells prevent the multiplication of viruses and also induce adaptive immune response. Thus, IFNs have widely been used for the treatment of a variety of chronic viral infections. In addition to these critical roles of host defense, IFNs have several adverse effects such as increased neuronal excitabilities, fever, imbalance of circadian rhythm, insomnia and emotional disorders such as anxiety and depression. Chronic and high-dose IFN-α therapy is frequently found to lead to severe neurotoxicity and neuropsychiatric complications such as agitation, depression, anxiety and memory loss. Especially, depressive mood is one of the critical risk factors during IFN-α therapy in patients with hepatitis C. In addition to the clinical studies of IFN-α, several experimental investigations have reported that intravenous injection of IFN-α induces depressive-like behaviors in animal model of depression, forced swimming test, in mice and rats. The severity of depression often leads to the discontinuation of the IFN-α therapy and an increase in suicide attempts. Thus, appropriate management of IFN-α-induced depression plays a crucial role in allowing patients to continue receiving treatment for cancers and viral diseases such as hepatitis C and prevention of suicide attempts. 

Kososan, 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 clinically used 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. It has been clinically suggested that kososan can alleviate depression induced by IFN-α therapy for hepatitis C. Our previous study has demonstrated that kososan shows an antidepressive-like effect through normalizing the hyperactivity of hypothalamic-pituitary-adrenal (HPA) axis, which is strongly associated with pathology of depression, in stress-induced depression-like model mice. However, it has not been elucidated whether kososan, also, has the antidepressive-like effect on IFN-α-induced depression-like behaviors. Therefore, clarification of the antidepressive-like effect of kososan on IFN-α-induced depression may contribute to reduce the IFN-α-induced depression.

In the present study, we evaluated the effect of kososan...
on IFN-α-induced depression-like behaviors using modified forced swimming test. Furthermore, its possible mechanism of action on HPA axis was assessed.

**Materials and Methods**

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

**Drugs.** Component herbs of kosoan are as follows: Cyperi rhizoma (rhizome of Cyperus rotundus L.), 4.0 g (Tsumura & Co., Tokyo, Japan); Perillae herba (leaf of Perilla frutescens Britton var. acuta Kudo), 2.0 g (Tsumura & Co.); Aurantii nobilis pericarpium (pericarp of Citrus unshiu Markovich), 3.0 g (Tsumura & Co.); Glycyrrhizae radix (root of Glycyrrhiza uralensis Fisher), 2.0 g (Uchida Wakan-yaku Co. Ltd., Tokyo, Japan) and Zingiberis rhizoma (rhizome of Zingiber officinale Roscoe), 0.5 g (Tsumura & Co.). The Kampo formula was decocted with 600 mL of distilled water until the volume was reduced to half, and the extract was filtrated through filter paper immediately in vacuo. The filtrate was lyophilized and the yield of kosoan extract was approximately 28% from the herbal mixture based on the dry weight. A three dimensional (3D)-HPLC profile of the kosoan extract is shown in Fig. 1 and the extract was used for the present study. Introne® A (recombinant interferon-α-2b, rIFN-α), Toledomin® (milnacipran hydrochloride) and caffeine were purchased from Schering-Plough K.K. (Osaka, Japan), Asahi Kasei Pharma Corp. (Tokyo, Japan) and Wako Pure Chemical Industries (Osaka, Japan), respectively.

**Drug administration.** Administration of rIFN-α to the mice was performed with some modifications as described. Briefly, rIFN-α (1.2 x 10^6 IU/kg) was administered intraperitoneally (i.p.) once daily for 7 consecutive days (Fig. 2A, B). Control mice were administered saline. Our previous study has demonstrated that kosoan shows the significant antidepressant-like effect in stress-induced depression-like model mice at the dose of 1.0 g/kg. Therefore kosoan (1.0 g/kg) or milnacipran (30 mg/kg) as a positive control for forced swimming test was dissolved or suspended in distilled water, and administered orally once daily for 14 consecutive days (Fig. 2B). I.p. administration of caffeine, which is a psychostimulant, has been shown to increase spontaneous locomotor activity dose-dependently from 3.3 to 30 mg/kg. Therefore caffeine (25 mg/kg), as a positive control for spontaneous locomotor activity test, was dissolved in saline, and administered i.p. once daily for 14 consecutive days. All administrations were carried out between 13:00 to 16:00.

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**Fig. 1** 3D-HPLC profile of kosoan
A, glycyrrhizin (Glycyrrhizae Radix); B, liquiritigenin (Glycyrrhizae Radix); C, rosmarinic acid (Perillae Herba); D, hesperidin (Aurantii Nobilis Pericarpium); E, narirutin (Aurantii Nobilis Pericarpium); F, liquiritin (Glycyrrhizae Radix); G, caffeic acid (Perillae Herba).
Reverse transcription-polymerase chain reaction (RT-PCR). Brains were collected immediately after FST on the 14th day. Hypothalamus was then dissected on dry ice. Total RNA was isolated from hypothalamus of depression-like model mice immediately after FST by the acid guanidinium-phenol-chloroform method using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Total RNA was used as a template for cDNA synthesis by the first-strand cDNA synthesis kit (GE Healthcare, Tokyo, Japan). PCR amplification of this cDNA was performed by using Taq DNA polymerase (Takara Bio, Otsu, Japan). Primers (Invitrogen) 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’ GGT CAA CCC CAC CGT GTT CTT CGA 3’ (sense) and 5’ TTG CCA TCC AGC CAT TCA GTG TTG 3’ (antisense) for cyclophilin. CRH and cyclophilin mRNA produced PCR products of 585 and 396 bp, respectively. CRH or cyclophilin cDNA product was amplified for 27 or 18 cycles, each cycle consisting of denaturation at 96°C for 30 s, annealing at 58 and 57°C for 30 s, respectively, and extension at 72°C for 3 min. PCR products were analyzed on a 2% agarose gel containing 0.0025% ethidium bromide (Sigma, St. Louis, MO, USA).

Measurement of corticosterone. Blood samples were collected by decapitation of mice at 60 min after the final drug administration, centrifuged at 4°C, and then sera were stored at -80°C until assayed for corticosterone. Serum corticosterone was measured using a corticosterone Enzyme Immunoassay Kit (Assaypro, St. Charles, MO, USA) according to the manufacturer’s instructions. The sensitivity of the measurement was 40 pg/mL. The intra- and inter-assay coefficients of variations were 5% and 7%, respectively.

Statistical analysis. Results were expressed as mean ± S.E. Differences between 2 groups were analyzed with t-test. Statistical analysis of differences during more than 3 groups was performed by one-way analysis of variance. Post hoc comparison, if applicable, was carried out with Tukey’s test or Dunnett’s test using the StatView software (Abacus Concepts, CA, USA). The p-values lower than 0.05 (p < 0.05) were considered significant.

Results

Effects of rIFN-α on the duration of immobility in the FST and spontaneous locomotor activity in the open field test. The duration of immobility of mice in the FST was measured at 15 min and 7 days after the administration of rIFN-α (1.2 x 10^6 IU/kg/day, i.p.) for 7 days (Fig. 2). The duration of immobility at 7 days after the final rIFN-α injection was significantly increased compared with saline-injected control (p < 0.05); but the duration of immobility at 15 min after the final rIFN-α injection was not increased (Fig. 3A). When rIFN-α (1.2 x 10^6, 1.2 x 10^5 or 1.2 x 10^4 IU/kg, i.p.) was administered for 7 days, the duration of immobility was dose-dependently increased at 7 days after the final rIFN-α injection (data not shown). In the open field test, the total number of line crossing was not affected at 7
Fig. 3 Effects of rIFN-α on the duration of immobility in the FST (A) and spontaneous locomotor activity in the open field test (B). rIFN-α (1.2 x 10^6 IU/kg) or saline was administered i.p. once daily for 7 consecutive days after FS. (A) The total duration of immobility for 5 min of FST was measured at 15 min and 7 days after the final injection of rIFN-α. (B) The total number of line crossing was measured at 7 days after the final injection of rIFN-α. Each column represents the mean ± S.E. (A, n = 8; B, n = 10). *p = 0.0109 with t-test.

Fig. 4 Effects of kososan on the duration of immobility of rIFN-α-induced depressive-like model mice in the FST (A) and spontaneous locomotor activity in the open field test (B). (A) Kososan (1.0 g/kg) or milnacipran (30 mg/kg) were administered p.o. to rIFN-α-induced depressive-like model mice once daily for 14 consecutive days after FS. The total duration of immobility for 5 min of FST was measured at 60 min after the final drug administration. (B) Kososan (1.0 g/kg, p.o.), milnacipran (30 mg/kg, p.o.) or caffeine (25 mg/kg, i.p.) were administered to naïve mice for 14 consecutive days. The total number of line crossing was measured at 60 min after the final drug administration. Each column represents the mean ± S.E. (A, n = 14-16; B, n = 8). *p < 0.05 and **p < 0.01 with Tukey’s test (A) or Dunnett’s test (B). KS, kososan; MIL, milnacipran; CAF, caffeine.

Fig. 5 Effects of kososan on CRH mRNA expression in the hypothalamus of rIFN-α-induced depressive-like model mice. rIFN-α (1.2 x 10^6 IU/kg) or saline was administered i.p. once daily for 7 consecutive days after FS. Brain was collected and dissected immediately after FST on 14 days. CRH mRNA expression in the hypothalamus was measured using RT-PCR. Results represent as ratio of CRH/cyclophilin mRNA. Each column represents the mean ± S.E. (n = 12-21). *p < 0.01 with Tukey’s test.

days after the final rIFN-α injection (Fig. 3B).

Effects of kososan on the duration of immobility of rIFN-α-induced depressive-like model mice in the FST and on the spontaneous locomotor activity of mice. Oral administration of kososan for 14 days (Fig. 2) significantly reduced the duration of immobility compared with water-administered control in the rIFN-α-induced depressive-like model mice, and oral administration of milnacipran, also did the same (p < 0.01, Fig. 4A). While administration of caffeine to naïve mice for 14 days significantly increased the total number of line crossing compared with water-administered control in the open field test (p < 0.05), administration of kososan or milnacipran did not affect the total number of line crossing (Fig. 4B).

Effects of kososan on CRH mRNA expression in the hypothalamus of rIFN-α-induced depressive-like model mice. CRH mRNA expression in the hypothalamus was assayed at 7 days after the final rIFN-α injection. The CRH mRNA expression was significantly increased in the rIFN-α-injected mice relative to saline-injected control (p < 0.01, Fig. 5). When kososan was administered orally for 14 days, expression of CRH mRNA in the hypothalamus was significantly decreased as compared to the water-administered mice (p < 0.01). Milnacipran (30 mg/kg) showed a tendency to decrease the CRH mRNA expression in the rIFN-α-induced depressive-like model mice, but not statistically significant (Fig. 5). On the other hand, the effect of milnacipran was not also statistically significant as compared with kososan.

Effects of kososan on corticosterone level in the serum of rIFN-α-induced depressive-like model mice. Corticosterone level in the serum was assayed at 7 days after the
final rIFN-α injection. The corticosterone level was significantly increased in the rIFN-α-injected mice relative to saline-injected control (p < 0.05, Fig. 6). When kososan was administered orally for 14 days, increase in corticosterone level was significantly decreased in the kososan-administered mice as compared with water-administered control (p < 0.05). Administration of milnacipran (30 mg/kg) had a tendency to decrease the corticosterone level in the rIFN-α-induced depressive-like model mice, but not statistically significant (Fig. 6). On the other hand, the effect of milnacipran was not also statistically significant as compared with kososan.

**Discussion**

The present study demonstrates that oral administration of kososan for 14 days produces an antidepressive-like effect via the normalization of HPA axis in the IFN-α-induced depressive-like model mice.

In our study, administration of IFN-α significantly increased the duration of immobility in the FST at 7 days after the final injection of IFN-α without affecting spontaneous locomotor activity (Fig. 3A, B). Motor dysfunction such as decrease or increase in locomotor activity results in an increase or decrease in the immobility of FST, respectively. Thus, drugs that cause the motor dysfunction are not capable of successfully evaluating the antidepressive-like effects. These results in combination with these findings suggest that the increasing effect of IFN-α on the immobility was due to its depressive-like behavior, and was not due to the motor dysfunction. Differences between isoforms of IFN-α influence the biological and behavioral reactivities induced by IFN-α in animal studies. Several studies have reported that IFN-α caused a decrease in spontaneous locomotor activity in mice, which is inconsistent with our results. The reason might be due to the differences in the number and routes of IFN-α injection, and in the timing of the locomotor test. Notably, mice were allowed a 7-day non-treated period after the final injection of IFN-α in the present study. The procedure in our study has reflected the evidence that IFN-α induces depression from 2 weeks after the IFN-α therapy. Our results provide evidence in favor of the involvement of the temporal factor in the onset of depression by IFN-α.

Clinical studies have shown that patients with depression have impaired function of HPA axis and, thus, persistent hyperactivity in the HPA axis may play a crucial role in the pathogenesis of depression. In addition, IFN-α has been reported to activate the HPA axis in clinical and animal studies. In the present study, increased expression of hypothalamic CRH mRNA and increased level of serum corticosterone in the IFN-α-injected mice appears to result from the hyperactivity of HPA axis (Fig. 4A, B). Therefore, from the behavioral and neuroendocrinological effects of IFN-α, IFN-α-induced depressive-like model mice may be useful as an animal model of depression triggered by IFN-α.

Oral administration of milnacipran, a serotonin-noradrenaline reuptake inhibitor, reduced IFN-α-induced increase in the immobility in the FST (Fig. 4A). This result raises the possibility that IFN-α-induced depressive-like model mice may be available for evaluating antidepressant compounds. Oral administration of kososan, also, reduced the IFN-α-induced increase in the immobility in the FST (Fig. 4A) and did not affect spontaneous locomotor activity in naive mice (Fig. 4B). These results suggest that kososan shows an antidepressive-like effect in the IFN-α-induced depressive-like model mice. With regard to the mechanism of antidepressive action of kososan, oral administration of kososan reduced IFN-α-induced increase in expression of CRH mRNA (Fig. 5) and in level of serum corticosterone (Fig. 6). These results indicate that kososan attenuates IFN-α-induced hyperactivity of the HPA axis. Moreover, in our previous finding, we reported that kososan exhibits an antidepressive-like effect through suppressing the hyperactivity of the HPA axis in stress-induced depressive-like model mice. Studies of two animal models of depression induced by IFN-α in the present study or stress support that antidepressive-like effect of kososan is more closely associated with the regulation of HPA axis.

It has been reported that IFN-α stimulates the synthesis of pro-inflammatory cytokines, such as interleukin (IL)-1, IL-6 and tumour necrosis factor (TNF)-α, and these cytokines play as potent stimulators of the HPA axis. One mechanism by which pro-inflammatory cytokines may induce hyperactivity of HPA axis is by reducing the function of the glucocorticoid receptor (GR) for endogenous glucocorticoids. Our previous study has demonstrated that GR protein expression in the hypothalamic paraventricular nucleus (PVN) is downregulated in the stress-induced depression-like model mice, and kososan ameliorated these alterations to the normal conditions. These results suggest that the antidepressant-like effect of kososan in the IFN-α-induced depressive-like model mice would be associated with the up-regulation of GR protein expression in the PVN.
Further studies of kosisan on the pro-inflammatory cytokine production and GR protein expression in the IFN-α-induced depressive-like model mice could help to further elucidate the mechanism(s) of kosisan's antidepressive-like activity through the modulation of HPA axis.

In the present study, oral administration of milnacipran at the dose of 30 mg/kg showed the tendency of reduction of IFN-α-induced increase in expression of CRH mRNA in the hypothalamus (Fig. 5) and level of serum corticosterone (Fig. 6) but was not statistically significant. These results suggest that the antidepressive-like effect of milnacipran in the IFN-α-induced depressive-like model mice may be caused by combination of HPA axis modulation with other mechanisms, such as serotonin-noradrenaline reuptake inhibition. Further study of the dose-dependent effect of milnacipran on the modulation of HPA axis in the model could help to further elucidate the mechanism of antidepressive-like activity of milnacipran.

IFN-α administered peripherally (i.p. or i.v.) translocates to the brain across the blood-brain-barrier by limited diffusion or saturable transport system and affects brain function such as monoamines biosynthesis and transcriptional response. In a recent study it has been reported that one of mechanisms underlying depression caused by peripheral administration of IFN-α is the decrease in neurogenesis, which is closely concerned with depression, in the dentate gyrus of the hippocampus. These findings imply that a direct action of IFN-α plays an important role in the neuroregulatory function in the brain. Further studies of kosisan on the regulation of neurotransmitters containing monoamines, transcriptional response and hippocampal neurogenesis in the IFN-α-induced depressive-like model mice could help to further elucidate the mechanism(s) of kosisan's antidepressive-like activity other than the modulation of HPA axis.

Pretreatment or treatment of antidepressants is likely to be effective in the IFN-α-induced depression in patients with hepatitis C, but IFN-α-induced depression is sometimes refractory to antidepressive treatments. In addition, treatments of antidepressants for IFN-α-induced depression in patients with hepatitis C are required to be careful for drug metabolisms in patients with other disorders because several antidepressants have inhibitory effects on the drug metabolisms of cytochrome P450. To our knowledge, there are few findings that kosisan affects the drug metabolisms, pharmacokinetics and drug-drug interaction. Therefore, treatment of IFN-α in combination with kosisan in patients with hepatitis C may be capable of controlling IFN-α-induced depression even though the patients take other medications. The speculation appears to be somewhat supported by a case report.

In conclusion, this study represents the first report showing the antidepressant-like effect of kosisan on IFN-α-triggered depression through the regulation of HPA axis, using the IFN-α-induced depressive-like model mice. The findings of our study provide further insight into our understanding of the mechanism of the antidepressant-like effect of kosisan.

References


