

Kami-shoyo-san, a herbal medicine, reduces plasma interleukin-6 (IL-6) and soluble IL-6 receptor concentrations in depressive climacteric women

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Abstract

We evaluated the effects of Kami-shoyo-san, a herbal medicine, on improvement of climacteric symptoms and on plasma interleukin-6 (IL-6) and soluble interleukin-6 receptor (sIL-6R) concentrations in comparison with those of anti-depressants. One hundred patients complaining of menopausal symptoms were recruited and separated into two groups (Kami-shoyo-san group was selected on the basis of SHO; 48 cases, Anti-depressants for 52 cases), and plasma IL-6 and sIL-6R concentrations were analyzed before treatment and after 3 months of treatment. The change of plasma concentrations of IL-6 and sIL-6R differed significantly between the Kami-shoyo-san therapy group and anti-depressant therapy group after 3 months (IL-6: -29.1 ± 7.3 and $8.6 \pm 6.5\%$, sIL-6R: -17.6 ± 4.9 and $3.4 \pm 6.1\%$, respectively) of treatment. The relationship between the rate of decrease in climacteric scale score and plasma IL-6 and sIL-6R concentrations were also explored. There were positive correlations between the rate of decrease in climacteric scale score and plasma IL-6 ($R=0.523$, $P=0.0043$) and sIL-6R ($R=0.536$, $P=0.0033$) concentrations in the Kami-shoyo-san group.

This study demonstrated that Kami-shoyo-san reduced plasma IL-6 and sIL-6R concentrations during the treatment. Findings of this study indicated that Kami-shoyo-san appears to have the potential to decrease morbidity by alleviation of stress reactions.

Key words interleukin-6, soluble interleukin-6 receptor, climacteric, Kami-shoyo-san (Jia-Wei-Xiao-Yao-San, 加味逍遥散), Kampo medicine.

Introduction

The causes and pathophysiology of undefined symptoms in the climacterium have not been fully understood. In addition to the dynamic endocrine changes in the hypothalamo-hypophyseo-ovarian axis and environmental changes unique to menopausal women, the factors responsible for these symptoms also include the delicate characterological variations of individual patients. However, that there are significant differences among patients from the onset of complaints, clinical symptoms and response to drugs indicates the involvement of physiological substance (s) other than these factors in the pathogenesis of climacteric symptoms.

Recently, the importance of the role of cytokines in emotional changes has been reported. A number of in-

vestigations have indicated the important relationship between depressive mood and cytokines.^{1,2)} Interleukin-6 (IL-6) has been considered a good overall indicator of immune functioning in older adults because of its contribution to the pathogenesis of several age-related conditions such as osteoporosis. Cytokines have also been reported to play important roles as mediators of stress³⁾; intra-cranial injection of anti-IL-1 antibody reversed the markedly decreased lymphocyte blastogenesis and K-cell activity induced by foot shock stress in mice and rats, suggesting the involvement of brain cytokines in stress.⁴⁾ Regulation of IL-6 is impaired in elderly adults, and levels of IL-6 increase with stress and depression.⁵⁾ We revealed that depressive middle-aged women showed significant higher plasma concentrations in IL-6 and soluble IL-6 receptor than non-depressive women.⁶⁾ Furthermore, estrogen dependent changes in the produc-

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tion of IL-1 β and IL-6 potentially modify cytokine bioactivity. Estrogen inhibits IL-1 β and IL-6 production by human osteoblast-like cells.⁷⁾

On the other hand, there are several reports concerning correlations between the changes in plasma levels of various kinds of cytokines and cyclic respiratory infection or general malaise,⁸⁾ between IL-6 concentration and degree of mental stress,⁹⁾ soluble IL-2 receptor level and emotional suppression,²⁾ and between IL-1 β , TNF- α ¹⁰⁾ or interferon- γ levels¹¹⁾ and anxiety. Elevated level of IL-6 in postmenopausal women¹²⁾ and *in vitro* suppressive effect of FSH and LH on IL-1 β production were also reported.¹³⁾ Furthermore, IL-1 β attenuated FSH secretion in response to addition of activin A in the cultured rat anterior pituitary cells.¹⁴⁾ Dondi *et al.*¹⁵⁾ reported that IL-1 β injected in a lateral ventricle of 3-week-castrated female rats resulted in the expected decrease in serum levels of FSH and LH. These findings indicate a close relationship between endocrinological change or stress and cytokine dynamics, and suggest the possible involvement of immunological factors including cytokines in the pathogenesis of undefined symptoms in the climacterium.

This study investigated the changes of plasma concentrations of IL-6 and soluble IL-6 receptor in the Kampo treatment with Kami-shoyo-san.

Subjects and Methods

Patients: Between November 1, 1999 and October 22, 2001, there were 332 perimenopausal women with ill-defined complaints who were presented for climacteric consultation in our department. Of these, one hundred patients complaining of menopausal symptoms and who were diagnosed with mood disorder based on DSM-IV¹⁶⁾ were recruited as the usual ordinary treatment in the out-patient clinic. No patient had any disease associated with tumor or inflammation or history of treatment with hormone preparations prior to this study. Plasma interleukin-6 (IL-6) and soluble interleukin-6 receptor (sIL-6R) concentrations were analyzed before treatment and after 3 months of treatment. In Kampo medicine, the selection of the most appropriate drug is based on SHO, a method of diagnosis based on the pathophysiological concepts of Kampo medicine. In this study, we accepted relative asthenic (weak) constitution and the

existence of mild resistance to or to pressure of the right subcostal regions as the SHO for Kami-shoyo-san.

Preparation of herbal drugs: Kami-shoyo-san is a combination of 10 herbal drugs: 3 g of Bupleurum root (Saiko), Peony root (Shakuyaku), Atractylodes Rhizome (Byakujutsu), Japanese Angelica root (Touki), and Hoelen (Bukuryou); 2 g of Gardenia fruit (Sanshishi) and Moutan bark (Botanpi); 1.5 g of Glycyrrhiza root (Kanzo); 1 g of Ginger Rhizome (Shokyo) and Mentha herb (Hakka). A mixture consisting of these (chopped) ingredients was extracted with hot water, filtered, lyophilized and stored at 4°C as 5 g of Kami-shoyo-san extract. Five grams of extract was transformed to 7.5 g of granular-type agent as a commercial drug (Tsumura Co. Ltd., Tokyo, Japan).

Experimental procedures: Forty-eight patients were administered Kami-shoyo-san according to the definition of above SHO. On the other hand, 52 patients who were different in SHO of Kami-shoyo-san were administered anti-depressants (tetracycle antidepressant: 31 cases, SSRI: 21 cases, combined administration with minor tranquilizer: 18 cases). We assessed the improvement of overall symptoms by Greene's Climacteric Scale.¹⁷⁾ This scale is intended specifically to be a brief and standard measure of core climacteric symptoms or complaints (three separate sub-scales measuring vasomotor, somatic, and psychological complaints) to be used for comparative and replicative purposes across different types of studies whether they are medical, psychological, sociological, or epidemiological in nature.

Determination of IL-6 and IL-6 receptor: Blood samples were drawn at 1:00 p.m. in all subjects, and plasma interleukin-6 (IL-6), soluble interleukin-6 receptor (sIL-6R) concentrations were measured by enzyme-linked immunosorbent assay by using sets of paired monoclonal antibodies for capture and detection, as suggested by the manufacturer's protocol (Sumitomo Bioscience Laboratories, Kanagawa, Japan). Samples were assayed in duplicate, and cytokine concentrations were derived from a standard curve comprised of serial dilutions (4.1–400 pg/ml) of purified recombinant human IL-6 and sIL-6R. Assay sensitivity limits are below 0.5 pg/ml for IL-6 and below 20 ng/ml for sIL-6R. The intraassay and interassay CV are 3.1 and 3.6% for IL-6, 7.9 and 7.1% for sIL-6R. Plasma values of IL-6 and sIL-6R are not modified in the presence of the recombinant

sIL-6R and IL-6, respectively, suggesting that the immunoenzymatic assay can measure total (free and bound) cytokine levels present in serum.

Statistical analysis: All values are expressed as the mean \pm S.D. One-way analysis of variance (ANOVA) was performed to determine the significance of group differences in initial values. The statistical significance of differences was determined using the Wilcoxon test. All differences were considered significant at $P < 0.05$.

Results

Demographic and clinical characteristics of the study sample are presented in Table I. The study groups did not differ significantly in mean age, menopausal age, postmenopausal years, Hamilton depression scale score and endocrinological values.

Whereas significant decreases were observed in climacteric scale scores after treatment in either group (Kami-shoyo-san: from 26.5 ± 8.3 to 11.1 ± 3.9 , $P = 0.0038$; anti-depressants: from 25.9 ± 10.2 to 10.2 ± 2.8 , $P = 0.0064$), there were no significant differences in mean decreasing rate of climacteric scale score after treatment between the two groups (Kami-shoyo-san vs. antidepressants: 58.1% vs. 60.6%) (Figure 1). Table II presents the plasma concentrations and their percent changes of IL-6 and sIL-6R. There were no significant differences in the basal levels of plasma IL-6 and sIL-6R between the two groups. They differed significantly between the Kami-shoyo-san therapy group and anti-depressant therapy group after 3 months treatment (IL-6 = $-29.1 \pm 7.3\%$ and

$8.6 \pm 6.5\%$, respectively, ($P < 0.0001$); sIL-6R = $-17.6 \pm 4.9\%$ and $3.4 \pm 6.1\%$, respectively, ($P = 0.0012$)), although no significant differences have been observed in the changes of the both mean plasma levels in antidepressants therapy group between basal and after 3 months treatment. The relationship between the rate of decrease in climacteric scale score and plasma IL-6 and sIL-6R concentrations were also explored. There were positive correlations between the rate of decrease in climacteric scale score and plasma IL-6 ($R = 0.523$, $P = 0.0043$) and sIL-6R ($R = 0.536$, $P = 0.0033$) concentrations in the Kami-shoyo-san group (Figure 2 Upper). There was no relationship between the change rate of climacteric scale score and plasma IL-6 and sIL-6R concentrations in the anti-depressant group (Figure 2 Lower).

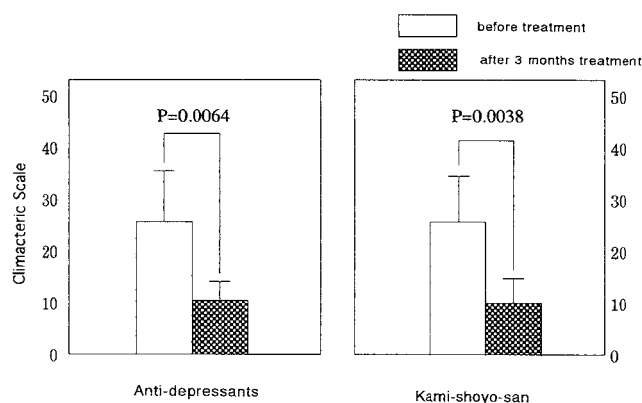


Fig.1 The changes of climacteric scale by treatment with Kami-shoyo-san or anti-depressants.

Table I Demographic and clinical characteristics of the subjects

Group differences	Treatment with Kami-shoyo-san	Treatment with anti-depressants	Significant
No. of patients	48	52	
Age (S.D.) years	54.1(7.4)	53.3(4.5)	n.s.
Number of years (S.D.) from menopause	2.59(4.8)	2.41(3.5)	n.s.
Age within 3 years of menopause (%)	19/48(39.6%)	22/52(42.3%)	n.s.
Mean (S.D.) Hamilton depression scale score	26.7(6.6)	29.2(5.8)	n.s.
Mean (S.D.) plasma hormone levels			
FSH (mIU/ml)	63.8(21.4)	59.7(24.2)	n.s.
LH (mIU/ml)	30.1(15.3)	24.2(12.1)	n.s.
Estradiol (pg/ml)	10.5(5.9)	11.3(7.8)	n.s.

FSH and LH were greater than 30 IU/ml and 15 IU/ml, respectively, in all women.

Figures in parenthesis indicate 1 standard deviation. P value assessed using ANOVA. No significant differences were found in any of the parameters between the groups.

Table II The change of plasma IL-6 and sIL-6R concentration after 3 months treatment with Kami-shoyo-san or anti-depressants

	Kami-shoyo-san	Anti-depressants	Significance
IL-6			
% change	-29.1±7.3%	8.6±6.5%	P<0.0001
plasma level			
before treatment	1.88±1.56pg/ml	2.06±1.99pg/ml	n.s.
3-months treatment	1.20±0.85pg/ml	2.35±3.79pg/ml	P=0.039
sIL-6R			
% change	-17.6±4.9%	3.4±6.1%	P=0.0012
plasma level			
before treatment	104.3±25.5ng/ml	99.8±29.5ng/ml	n.s.
3-months treatment	92.0±33.7ng/ml	102.4±32.7ng/ml	P=0.12

*1: P=0.018, *2: P=0.021, *3: P=0.762, *4: P=0.681

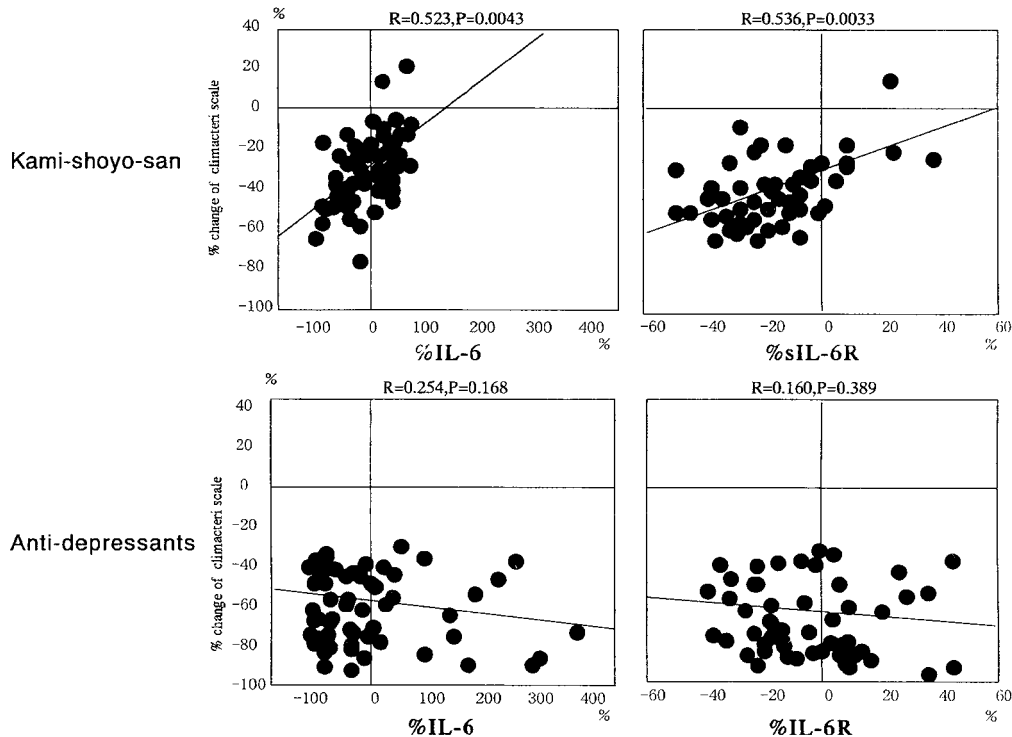


Fig. 2 Relationship between the percent changes of climacteric scale and plasma IL-6 and sIL-6R concentrations in the treatment with Kami-shoyo-san (Upper) or anti-depressants (Lower).

Discussion

It has been suggested that cytokines play an important role in the biology of mood and the pathophysiology of depression.^{18,19} Interleukins, which are important mediators of biological reactions in stress, might play roles in the pathogenesis of climacteric symptoms for which stress has critical significance. A correlation between

cytokine dynamics and endocrinological profile during climacterium also exists since menopause itself led to elevation of IL-6 concentration¹²⁾ and reduction of estrogen concentration induced the production and release of IL-6, resulting in activation of osteoblasts.²⁰⁾ In particular, gonadotropin secretion in patients with mood or emotional disorder such as depressive mood, anxiety, and who were jittery exhibited an endocrinologically peculiar profile.²¹⁾ The inflammatory cytokines IL-1 β , IL-6,

and TNF- α induced anxiety and behavior disorder by exerting effects on the neuroendocrine system and subsequently affecting the production and secretion of neurotransmitters.¹⁰⁾ Concentration of IL-2 receptor was found to be related to mood suppression.²⁾ Some investigators reported significant positive correlations between IL-6 secretion and anxiety, depressive mood, and decreases in memory performance.^{1,2,5,22)} On the other hand, some reports suggest that plasma sIL-2R is increased,²³⁾ or not changed,^{24,25)} and TNF- α is decreased²⁴⁾ in depressed patients.

The primary mechanism of Kampo medicine is to restore, as a biological response modifier (BRM), the patient's physiological environment by regulating the physiological balance of neurological, endocrinological, and immunological systems, rather than to act directly on the affected cells or organs. *In vitro* experiments revealed that Saiko-group, one of the Kampo medicines, stimulated the production of IL-6 (Sho-saiko-to),²⁶⁾ IFN- γ (Sai-rei-to),²⁷⁾ and IL-10, but suppressed IL-4 and IL-5 production (Sho-saiko-to).²⁸⁾

Practical Kampo medicine is known to be effective for undefined symptoms associated with the several pathological condition of the climacterium. This study demonstrated that Kami-shoyo-san reduced plasma IL-6 and sIL-6R concentrations during the treatment. This is remarkably different from the conventional drugs used in Western medicine, which did not yield significant changes in cytokine levels. There was a positive correlation between the effect of this drug and the magnitude of the rate of reduction of cytokine concentration, suggesting that Kami-shoyo-san acts as a BRM, regulating a cytokine network by normalizing levels of inflammatory interleukins. Although it is reported that recovery from stress-induced immunoresponse modification requires a long period of time,²⁹⁾ Kami-shoyo-san, an herbal medicine appears to have the potential to decrease morbidity by alleviation of stress reactions and coordination of immune function.

和文抄録

更年期の不定愁訴の発症や増悪には内分泌学的要因のほか免疫学的要素の関与が示唆されている。更年期に不定愁訴で来院したうつ病における、加味逍遙散と抗うつ薬によるサイトカイン動態を検討した。

通常の診療の一環として、東洋医学的「証」診断を行った更年期うつ病のうち、腹証として「胸脇苦満」と「瘀血圧痛」があった48例を加味逍遙散群とし、証が加味逍遙散に適合しなかった52例を抗うつ薬投与群として、投与前、投与3か月目に更年期指数の評価ならびに血中インターロイキン6 (IL-6)、可溶性 IL-6 受容体 (sIL-6R) 濃度を測定し、両群を比較した。

結果：薬物療法により両群において有意な更年期指数の低下が認められたが、群間の差はなかった。血中 IL-6 濃度は、加味逍遙散および抗うつ薬でそれぞれ $-29.1 \pm 7.3\%$ および $8.6 \pm 6.5\%$ の変動がみられた ($P < 0.0001$)。血中 sIL-6R 濃度でも同様の結果が得られた (加味逍遙散および抗うつ薬でそれぞれ $-17.6 \pm 4.9\%$ および $3.4 \pm 6.1\%$ の変動: $P = 0.0012$)。また、加味逍遙散による血中 IL-6、sIL-6R 濃度は更年期指数の低下と有意に相関した ($P < 0.01$)。

考察：加味逍遙散は更年期症状を改善したが、同時に血中 IL-6、sIL-6R 濃度を低下させることが判明した。不定愁訴の解消にこれらのサイトカイン動態の正常化が関与している可能性が示唆された。

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