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H. 知的所有権の出願・登録状況（予定を含む）

1. 特許取得
   なし
2. 実用新案登録
   なし
3. その他
   なし

厚生労働科学研究費補助金（こころの健康科学研究事業）
分担研究報告書
気分障害患者末梢白血球における遺伝子発現変動の解析
分担研究者　内田周作　山口大学大学院医学系研究科高次脳機能病態学分野

要旨
うつ病は、心身に著しい苦悩をもたらし、社会生活に甚大な支障をきたすばかりか、しばしば自殺企図に結びつく。生涯罹患率が１０％にも上るこの疾患の的確な診断と適切な治療体制の確立は、国民生活の向上に必須であり、社会全体の急務である。主任研究者らは、ストレス反応と関連する遺伝子の mRNA の発現量を一括解析する DNA チップを開発した。本研究の目的は、気分障害患者を対象として DNA チップとリアルタイム PCR 法を用いた調査を行い、気分障害の早期診断、治療評価および病態研究に応用することである。

A. 研究目的

うつ病は、心身に著しい苦悩をもたらし、社会生活に甚大な支障をきたすばかりか、しばしば自殺企図に結びつく。生涯罹患率が１０％にも上るこの疾患の的確な診断と適切な治療体制の確立は、国民生活の向上に必須であり、社会全体の急務である。病態の評価、早期診断、及び治療評価に応用できる簡便かつ客観的な指標の確立の意義は絶大であり、その必要性は高い。

主任研究者らは、神経伝達物質、サイトカイン、ホルモン、熱タンパク質などと関連する遺伝子 1500 種の mRNA の発現量を、白血球を試料として一括解析する DNA チップを開発した。学位審査発表などの心理的ストレスにさらされると、特定の遺伝子発現が増減し、翌日には回復することを見出し、ストレスに鋭敏に反応する測定系となることを確認している。

本研究の目的は、気分障害患者を対象として DNA チップとリアルタイム PCR 法を用いた調査を行い、気分障害の早期診断、治療評価および病態研究に応用することである。その端緒として、我々のグループは気分障害患者（大うつ病性障害および双極性障害）末梢白血球由来 RNA から cDNA を調整し、遺伝子発現変動を定量的リアルタイムＰＣＲ法により検討する。

B. 研究方法

山口大学医学部附属病院精神科神経科に入院もしくは通院中の気分障害患者 78 名、気分障害患者第一度血縁者 32 名、大うつ病性障害患者第一度血縁者 17 名、および健常者 28 名をリクルートした。また採血はうつ状態と寛解状態の二時点で施行した。診断には精神疾患の分類と診断(DSM-IV)の診断基準を用い、うつ状態の重症度評価にはハミルトンうつ病評価尺度を用いた。難治例については、イミ

プラミン換算で 150mg/日以上の抗うつ薬を 8 週間以上投与したが改善せず ECT 施行により軽快した症例を難治例と定義した。精神症状が重度であるため早急に ECT を導入した症例、及び副作用のため薬剤による治療が困難であったため ECT を導入した 症例は除いた。薬物治療反応群２５名、薬物治療非反応群９名をリクルートした。採血後，末梢白血球から抽出した全 RNA より cDNA を調整した。

（倫理面への配慮）

本研究は遺伝子多型解析ではなく、すべての人に発現しているｍＲＮＡの発現量を測定するものであり、いわゆる遺伝子解析研究ではない。しかし、倫理面への配慮は十分に行い、連結可能匿名化を行ってプライバシーを保護している。本研究は山口大学倫理委員会の承認を得て行い、対象者には研究の趣旨について文書を用いて説明し同意を得ている。

C. 研究結果および考察

本年度はサンプルの採取を中心に行った。今後、更に患者数を増やして研究を進めていく予定である。本研究は、mRNA の発現パターンを指標として、うつ病を健康成人から識別できること、および治療経過にそった変化が捉えられることが得られている。事実、我々はこれまでに気分障害患者末梢白血球におけるグルココルチコイド受容体遺伝子 mRNA の有意な発現低下を報告しており、この発現低下はうつ状態のみならず寛解状態、さらには第一度血縁者においても認められたことから、うつ病の trait-marker としての可能性が示唆された。また、神経栄養因子群や細胞接着因子群の遺伝子発現量を検討したところ、グリア細胞由来栄養因子(GDNF)、NCAM、L1 などの発現量は気分障害患者において有意な変動が認められ、この変化は state-dependent であった。さらに、薬物治療抵抗性患者における検討では、カルシトニン関連ペプチド(CGRP)や細胞接着因子 L1 の発現量に有意な変化が認められ、薬物治療抵抗性を判断できる生物学的指標となり得る可能性が示唆された。このように、末梢白血球由来の試料を用いることは、患者の状態やうつ病のサブタイプ毎の検討が可能となり、気分障害の早期診断、治療評価および病態研究への応用が期待できる。

D. 結論

神経伝達物質、サイトカイン、ホルモン、熱タンパク質などと関連する遺伝子1500種のmRNAを解析する革新的なDNAチップを用いて、気分障害とmRNA発現パターンの変化についての調査研究を開始した。今後症例数を増やして、研究を継続する予定である。

F. 健康危険情報

特になし

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H. 知的所有権の出願・登録状況

1. 特許取得
   なし
2. 実用新案登録
   なし
3. その他
   なし

研究成果の刊行に関する一覧表

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Changes in circulating cytokine levels in midlife women with psychological symptoms with selective serotonin reuptake inhibitor and Japanese traditional medicine

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ABSTRACT

Objective: The aim of the present study was to compare the effects on serum cytokine concentrations of paroxetine, a selective serotonin re-uptake inhibitor, and kamishoyosan, a Japanese traditional medicine, in midlife women with psychological symptoms.
Methods: Seventy-six women with psychological symptoms such as anxiety and mild depression as menopausal symptoms were enrolled in this study. Thirty-eight women received oral administration of 10 mg paroxetine every day, and 38 women received oral administration of kamshoyosan every day for 6 months. Overall climacteric symptoms were assessed using Greene's climacteric scale. Serum levels of cytokines were measured using a multiplexed human cytokine assay.
Results: Greene's total scores in both women treated with paroxetine and in women treated with kamishoyosan decreased significantly. Percentage decreases in Greene's total, psychological and vasomotor scores during the 6-month period in the paroxetine group were significantly greater than those in the kamishoyosan group. Serum IL-6 concentration in women treated with paroxetine decreased significantly. Serum concentrations of IL-8, IL-10, macrophage inflammatory protein (MIP)-1β and monocyte chemoattractant protein-1 in women treated with paroxetine decreased significantly. On the other hand, serum IL-6 concentration in women treated with kamishoyosan decreased significantly, but other serum concentrations did not change significantly.
Conclusion: Decrease in IL-6 concentration may be involved in the mechanism of the actions of both paroxetine and kamishoyosan in women with psychological symptoms, and IL-6 may therefore be useful as a marker of treatment. The action of paroxetine may also be associated with decreases in IL-8, IL-10, MIP-1β.

1. Introduction

In midlife women during the menopausal transition, psychological symptoms such as anxiety and mild depression as well as vasomotor symptoms have been observed as menopausal symptoms. A selective serotonin reuptake inhibitor (SSRI) has been used to treat depression in women, but adverse reactions such as nausea and headache have been observed in women treated with SSRI [1].

In Japan, various Japanese traditional medicines have been used for treating women who complain of menopausal symptoms. Kamishoyosan (Jia-wei-xiao-yao-san) is one of the formulae used for treatment of psychological symptoms such as anxiety, depression and irritability in menopausal women [2,3]. Recently, it has been reported that women with premenstrual dysphoric disorder were successfully treated with kamishoyosan [4]. Kamishoyosan consists of the following 10 medical herbs: Bupleurum root, Peony root, Atractylodis lanceae rhizome, Angelica root, Hoelen, Gardenia fruit, Moutan bark, Glycyrrhiza root, Ginger rhizome and Mentha herb. It is thought that kamishoyosan acts on the central nervous system, but the mechanism of the action of kamishoyosan has not been fully elucidated.

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Cytokines are involved in various functions of the central nervous system. It has been reported that circulating cytokines are dysregulated in major depression [5]. Plasma interleukin (IL)-6 concentration has been reported to be increased in major depressive disorders [6,7]. Levels of mitogen-induced cytokines such as IL-1β, IL-2, IL-10 and interferon (IFN)-γ have also been reported to be high in patients with major depression [8]. In midlife women with depression as a menopausal symptom, plasma IL-6 concentration was found to be increased [9]. We also reported that serum concentrations of IL-6, IL-8 and IL-10 were high in midlife women with psychological symptoms [10]. On the other hand, it has been reported that decreases in serum concentrations of IL-6 and tumor necrosis factor (TNF)-α were observed in depressed patients treated with SSRI [11,12]. Ushiroyama et al. reported that plasma TNF-α concentration was increased in depressed menopausal women treated with kamishoyosan [3]. However, the changes in cytokines in women treated with paroxetine and kamishoyosan have not been fully elucidated.

To date, it has been difficult to detect low levels of circulating cytokines in serum of healthy women. Recently, a multiplexed cytokine assay for measurement of serum concentrations of cytokines has been developed, and the use of this assay has enabled simultaneous measurements of low levels of various cytokines in serum of healthy subjects [13,14].

In the present study, we compared the effects of paroxetine and kamishoyosan on serum cytokine concentrations in midlife women with psychological symptoms using a highly sensitive multiplexed cytokine assay.

2. Subjects and methods

2.1. Subjects

The subjects of this study were recruited from patients visiting the outpatient clinic of the Department of Obstetrics and Gynecology, Tokushima University Hospital. Seventy-six women who had complained of psychological symptoms such as anxiety and mild depression as menopausal symptoms were enrolled in this study between November 2005 and October 2007. Informed consent for participation in this study was obtained from each woman. The Ethics Committee of Tokushima University Hospital approved the study. Women with major depression were excluded. Reviews of medical histories and the results of physical examinations and blood chemistry tests showed that all of the women were in good health. None of the subjects had taken any medication known to influence the immune system for at least 1 year. Subjects suspected of having infectious diseases, inflammatory disorders, malignancy or autoimmune diseases, of being undernourished, or of abusing alcohol or drugs were excluded according to the SENIEUR protocol [15]. Seven premenopausal women had regular menstruation and 32 perimenopausal women had experienced alterations in menstrual frequency and/or flow in the 12 months preceding entry into the study, and natural menopause had occurred in 37 women at least 12 months before entry into the study. Eligible women were randomly assigned in open, parallel-group fashion to a paroxetine group or kamishoyosan group. Thirty-eight women received oral administration of 10 mg paroxetine (Glaxo) every day and 38 women received oral administration of 7.5 g kamishoyosan (Tsumura Co., Tokyo, Japan) every day for 6 months. Climacteric symptoms were assessed using Greene's climacteric scale [16]. Compliance was assessed by pill count or sheet count, and side effects were ascertained by questionnaires at 4-week intervals. Venous blood samples were drawn into tubes between 8 a.m. and 10 a.m. after a 12-h fasting before and at 6 months of treatment. Samples obtained were frozen at −70 °C until use for analysis.

2.2. Preparation of herbal drugs

Kamishoyosan is composed of 10 medical herbs: 3 g of Bupleurum root, Peony root, Atractylodis lanceae rhizome, Japanese Angelica root, and Hoelen; 2 g of Gardenia fruit and Moutan bark; 1.5 g of Glycyrrhiza root and 1 g of Ginger rhizome and Mentha herb. Kamishoyosan used in the present study was prepared as a spray-dried powder from hot water extract and obtained from Tsumura Co. Ltd. (Tokyo, Japan).

2.3. Measurement of serum cytokine concentrations

Serum concentrations of IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, TNF-α, IFN-γ, macrophage inflammatory protein (MIP)-1β and monocyte chemotactic protein (MCP)-1 were measured by using a Bio-Plex human multi-plex cytokine assay kit (Bio-Rad Laboratories, Hercules, CA, USA) as previously reported [14]. The intra- and inter-assay coefficients of variation were 2.0–7.0% and 5.2–16.1%, respectively. The sensitivity levels were 1.1 pg/ml for IL-2, IL-6 and MIP-1β, 0.5 pg/ml for IL-4, IL-7 and IL-8, 0.8 pg/ml for IL-1β and IL-5, 0.9 pg/ml for IL-10, 19.3 pg/ml for IFN-γ, 3.0 pg/ml for TNF-α and 6.7 pg/ml for MCP-1.

2.4. Measurements of concentrations of estradiol and FSH

Serum estradiol concentration was measured by a two-site immunoenzymometric assay using a commercially available kit (TOSOH Co., Tokyo, Japan). The intra- and inter-assay coefficients of variation were 4–9% and 6–9%, respectively, and the detection limit was 20 pg/ml. Serum FSH concentration was measured by an immunoradiometric assay using a commercially available kit (TFB Co., Tokyo, Japan). The intra- and inter-assay coefficients of variation were 3–4% and 3–4%, respectively, and the detection limit was 1.0 IU/l.

2.5. Analysis of kamishoyosan by HPLC

Kamishoyosan was extracted with 20 ml of methanol under ultrasonication for 30 min. The solution was filtered and subjected to treatment with an alumina cartridge (Bond Elute Co. Ltd.). Elution provided the alkaloid fraction. The methanol solution and the alkaloid fraction were tested. HPLC with an LC-10AD pump (Shimazu, Tokyo, Japan) and SPD-M10A absorbance detector was performed using a TSK-GEL ODS-80TM column (150 mm × 4.6 mm). The effluent from the column was monitored at 254 nm with a UV detector.

2.6. Statistical analysis

Based on results of the previous study [17], sample size was estimated to detect at least 20% change in levels of cytokines and chemokines after administration with 80% power at the 0.05 level of significance. We defined the values below the detection limit as half of the detection limit in further analyses. Differences between the paroxetine group and the kamishoyosan group in subject's characteristics, baseline serum hormonal concentrations and Greene's scores and percentage changes in Greene's scores were analyzed by an unpaired t-test, and values are presented as means ± standard deviations. Baseline serum cytokine levels, which were not normally distributed, are presented as medians with 10th and 90th percentile ranges, and significance of those values was evaluated by the non-parametric Wilcoxon rank sum test. Changes by treatments in Greene's scores were analyzed by Student's paired t-test, and changes by treatments in serum cytokine levels were analyzed by the non-parametric Wilcoxon signed-rank test. The relationship among continuous variables was determined by using Spearman's rank order analysis. p values less than 0.05 were considered to be

Table 1
Baseline characteristics in women treated with paroxetine and kamishoyosan.

	Paroxetine	Kamishoyosan	p values
Number	38	38	
Age (years)	50.5 (5.4)	51.4 (5.1)	0.42
Menopausal status			
Premenopause	4	3	
Perimenopause	16	16	
Postmenopause	18	19	
BMI	21.9 (3.4)	21.8 (6.7)	0.92
FSH (mIU/ml)	58.2 (40.5)	70.4 (43.1)	0.21
Estradiol (pg/ml)	55.9 (49.9)	40.2 (37.9)	0.18

Values in age, BMI, FSH and estradiol are means (standard deviations). Values in menopausal status are numbers. BMI: body mass index, FSH: follicle-stimulating hormone.

statistically significant. Box plots show median, 25th and 75th percentiles as boxes and 10th and 90th percentiles as error bars.

3. Results

3.1. General characteristics

As shown in Table 1, there were no significant differences between baseline characteristics such as age, BMI, serum concentrations of FSH and estradiol in the two groups. The proportions of pre-, peri- and postmenopausal women treated with paroxetine were 10.5%, 42.1% and 47.3%, respectively, and the proportions of pre-, peri- and postmenopausal women treated with kamishoyosan were 7.9%, 42.1% and 50.0%, respectively. Sixty-seven of the 76 women completed the 6-month study. Six of the 38 women treated with paroxetine dropped out of the study because of the following adverse effects: headache, nausea and abnormal feeling of gastrointestinal tract. One woman treated with kamishoyosan dropped out of the study because of oral bitterness and diarrhea, and two women dropped out because of no response to kamishoyosan. Data from 67 of the 76 women were therefore used for analysis.

3.2. Changes in Greene's scores in women treated with paroxetine and kamishoyosan

There were no significant differences in total Greene's scores in women before treatments with paroxetine and kamishoyosan. The mean psychological, somatic and vasomotor scores in women before treatments were also not significantly different in the two groups. As shown in Table 2, Greene's total score (mean ± standard deviation) in women treated with paroxetine was significantly

Table 2
Greene's scores before and at 6 months after treatments with paroxetine and kamishoyosan in women with psychological symptoms.

	Paroxetine ($n=32$)		Kamishoyosan ($n=35$)	
	Before	6 months	Before	6 months
Total	18.3 (4.9)	12.6 (4.3)*	17.2 (3.8)	13.2 (3.5)*
Psychological	10.4 (3.3)	7.3 (3.1)*	9.6 (3.1)	7.2 (2.5)*
Anxiety	5.1 (2.2)	4.3 (2.0)*	5.2 (1.9)	4.4 (1.7)*
Depression	5.3 (2.2)	3.3 (1.8)*	4.4 (2.3)	2.8 (1.5)*
Somatic	4.4 (2.1)	3.4 (1.5)*	4.4 (1.9)	3.7 (1.8)*
Vasomotor	2.3 (1.9)	1.0 (0.9)*	2.5 (1.5)	1.5 (1.2)*

Values are means (standard deviations).
* $p<0.0001$ vs. before treatment.

Table 3
Percentage changes in Greene's scores at 6 months after treatments with paroxetine and kamishoyosan.

	Paroxetine ($n=32$)	Kamishoyosan ($n=35$)	p values
Δ Total (%)	−33.0 (13.0)	−22.2 (8.9)	0.0002
Δ Psychological (%)	−34.3 (17.0)	−22.1 (10.1)	0.0007
Δ Somatic (%)	−23.8 (21.2)	−16.4 (20.5)	0.167
Δ Vasomotor (%)	−52.3 (33.6)	−33.7 (32.4)	0.05

Values are means (standard deviations). (Δ) percentage change.

($p<0.0001$) decreased (from 18.3 ± 4.9 to 12.6 ± 4.3). Greene's psychological, somatic and vasomotor scores in women treated with paroxetine were also decreased significantly ($p<0.0001$). On the other hand, Greene's total score in women treated with kamishoyosan was decreased (from 17.2 ± 3.8 to 13.2 ± 3.5) significantly ($p<0.0001$). Greene's psychological, somatic and vasomotor scores in women treated with kamishoyosan were also decreased significantly ($p<0.0001$). As shown in Table 3, percentage decreases in total, psychological and vasomotor scores during the 6-month period in the paroxetine group were significantly ($p=0.0002$, 0.0007 and 0.05, respectively) greater than those in the kamishoyosan group.

3.3. Changes in serum cytokine concentrations in women treated with paroxetine and kamishoyosan

There were no significant differences in serum cytokine concentrations in women before treatments with paroxetine and kamishoyosan. As can be seen in Fig. 1, median IL-6 concentration in women treated with paroxetine was decreased (baseline, 1.41 pg/ml; 6 months, 0.55 pg/ml) significantly ($p=0.0003$). Serum concentrations of IL-8, MIP-1β and MCP-1 in women treated with paroxetine was decreased significantly ($p=0.018$, 0.033 and

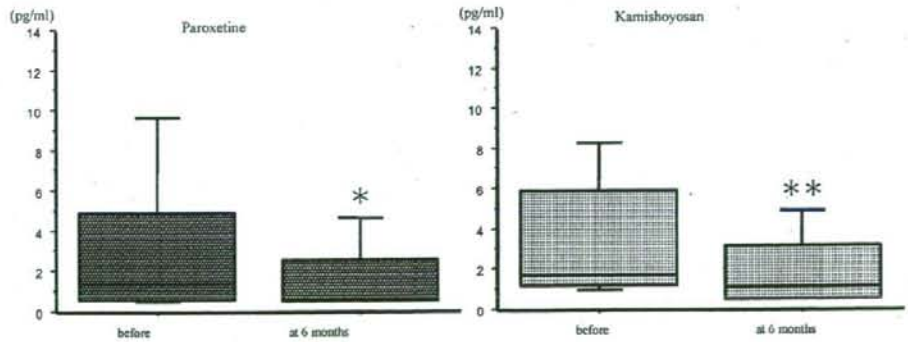

Fig. 1. Changes in serum IL-6 concentrations in women treated with paroxetine and kamishoyosan. Horizontal small bars represented the 10th–90th percentile range, and boxes indicate the 25th–75th percentile range. The horizontal line in each box corresponds to the median; *$p<0.01$, **$p<0.05$.

Table 4
Serum cytokine concentrations before and at 6 months after treatments with paroxetine and kamishoyosan in women with psychological symptoms.

	Paroxetine (n = 32)		Kamishoyosan (n = 35)	
	Before	6 months	Before	6 months
IL-1β (pg/ml)	0.40 (0.40–1.92)	0.40 (0.40–1.42)	0.80 (0.80–1.62)	0.40 (0.40–6.81)
IL-2 (pg/ml)	0.55 (0.55–5.53)	0.55 (0.55–5.46)	0.55 (0.55–3.89)	0.55 (0.55–0.96)
IL-4 (pg/ml)	0.25 (0.25–0.25)	0.25 (0.25–0.25)	0.25 (0.25–0.25)	0.25 (0.25–0.25)
IL-5 (pg/ml)	0.40 (0.40–0.45)	0.40 (0.40–0.49)	0.40 (0.40–0.76)	0.40 (0.40–0.56)
IL-6 (pg/ml)	1.41 (0.55–9.67)	0.55 (0.55–4.68)*	1.73 (1.01–8.35)	1.16 (0.55–4.97)**
IL-7 (pg/ml)	3.59 (0.89–7.14)	3.05 (1.25–4.96)	3.35 (1.29–9.05)	3.09 (0.55–6.36)
IL-8 (pg/ml)	44.3 (5.67–212.8)	18.5 (4.89–102.9)**	46.4 (5.96–216.5)	26.4 (6.75–198.5)
IL-10 (pg/ml)	0.73 (0.45–1.92)	0.45 (0.45–1.32)	0.45 (0.45–1.23)	0.45 (0.45–0.94)
TNF-α (pg/ml)	1.50 (1.50–7.32)	1.50 (1.50–7.57)	1.50 (1.50–8.30)	1.50 (1.50–8.72)
IFN-γ (pg/ml)	9.65 (9.65–9.65)	9.65 (9.65–9.65)	9.65 (9.65–9.65)	9.65 (9.65–9.65)
MCP-1 (pg/ml)	50.1 (20.9–104.5)	38.2 (15.8–66.4)**	45.9 (23.0–90.4)	45.1 (24.0–75.3)
MIP-1β (pg/ml)	195.0 (77.4–449.6)	158.3 (67.4–245.0)**	232.9 (59.2–486.7)	221.7 (76.7–353.9)

Values are medians (10–90 percentiles). IL: interleukin; TNF-α: tumor necrosis factor-α; IFN-γ: interferon-γ; MCP-1: monocyte chemoattractant protein-1; MIP-1β: macrophage inflammatory protein-1β.
* $p < 0.01$ vs. before treatment.
** $p < 0.05$ vs. before treatment.

0.014, respectively) and serum IL-10 concentrations tended to be decreased ($p = 0.093$) (Table 4). Serum concentrations of TNF-α and IL-1β did not change significantly. On the other hand, median IL-6 concentration in women treated with kamishoyosan was decreased (baseline, 1.73 pg/ml; 6 months, 1.16 pg/ml) significantly ($p = 0.021$), but other serum cytokines and chemokines concentrations did not change significantly.

3.4. Correlations of Greene's scores and serum cytokine concentrations

As can be seen in Fig. 2, IL-6 levels showed significant positive correlations with Greene's total scores in women treated with paroxetine and kamishoyosan, respectively ($r = 0.380$, $p = 0.0013$; $r = 0.273$, $p = 0.018$). In addition, IL-8 and MIP-1β showed significant positive correlations with Greene's total scores, respectively ($r = 0.455$, $p < 0.0001$; $r = 0.329$, $p = 0.0058$), and IL-10 showed a weak correlation with Greene's total score ($r = 0.267$, $p = 0.027$) in women treated with paroxetine (Fig. 3).

3.5. Changes in serum cytokine concentrations in women in whom hot flashes were improved by kamishoyosan and in women in whom kamishoyosan had no effect on hot flashes

We assessed severity of hot flashes using the Food and Drug Administration published draft guidance for clinical evaluation of vasomotor symptoms [18]. Severity is defined as mild (sensation of heat without sweating), moderate (sensation of heat with sweating, able to continue activity), and severe (sensation of heat with sweating, causing cessation of activity). We divided the subjects into two groups: those in whom hot flashes were improved by kamishoyosan as responders (i.e. from severe to mild or moderate) and those in whom kamishoyosan had no effect on hot flashes as non-responders (i.e. from moderate to moderate or severe). As can be seen in Table 5, total, psychological and somatic scores were significantly decreased in both responders and non-responders. In the responder group, serum concentrations of IL-6, IL-8 and MIP-1β were decreased significantly ($p = 0.049$, 0.018 and 0.044, respectively). However, serum IL-8 level in the non-responder group was increased significantly ($p = 0.026$).

3.6. Three-dimensional HPLC profile of kamishoyosan

Three-dimensional HPLC profiles of the methanol solution and the alkaloid fraction of kamishoyosan are shown in Fig. 4.

4. Discussion

In the present study, we showed that serum IL-6 concentrations were decreased by both treatments with paroxetine and kamishoyosan in women with psychological symptoms. Circulating IL-6 concentration has been reported to be significantly high in subjects with major depression and in midlife women with depressive mood [6,7,9,10]. It has been reported that elevated IL-6 concentration was decreased following successful treatment of major depression with fluoxetine [11]. Lanquillon et al. reported

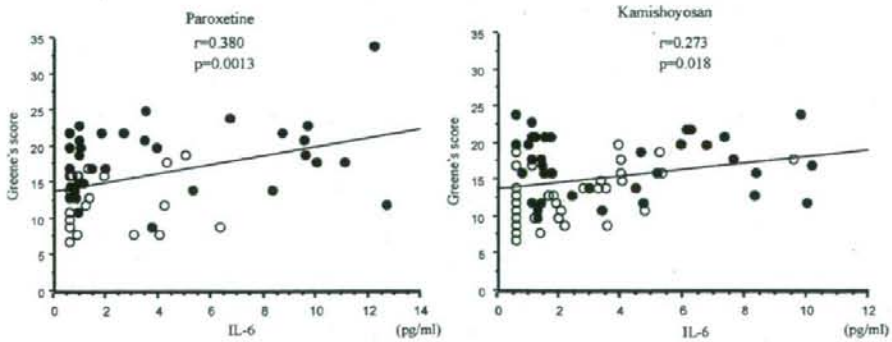

Fig. 2. Correlations of Greene's total scores and serum IL-6 levels in women treated with paroxetine and kamishoyosan: (●) pre-treatment; (○) post-treatment.

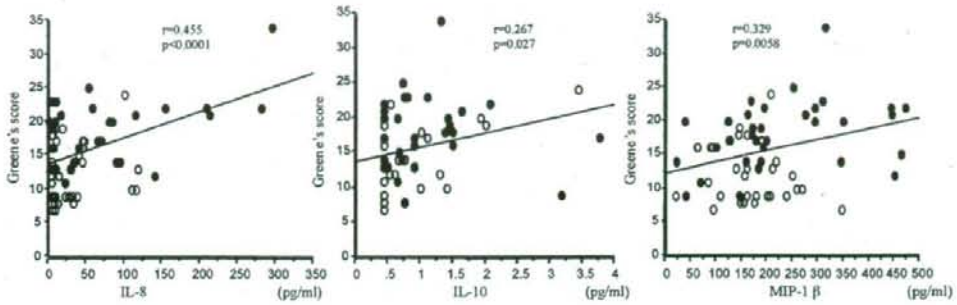

Fig. 3. Correlations of Greene's total scores and serum levels of IL-8, IL-10 and MIP-1β in women treated with paroxetine: (●) pre-treatment; (○) post-treatment.

that IL-6 level decreased in responders to antidepressant treatment but remained high in non-responders [19]. It has also been reported that IL-6 level was decreased significantly after treatment with SSRI [17] and that suppression of IL-6 level was not observed in depressed patients who failed to respond to SSRIs [20]. On the other hand, Ushiroyama et al. reported that there was a decrease in plasma IL-6 level as well as improvement of menopausal symptoms after treatment with kamishoyosan [2]. Therefore, IL-6, which is involved in the pathogenesis of depression, is decreased in response to treatment with paroxetine, and kamishoyosan may also have an effect that is related to the decrease in IL-6 on psychological symptoms.

The mechanism underlying the decrease in IL-6 in response to paroxetine or kamishoyosan is not clear because the source of IL-6 is not fully understood. In the present study, we showed the significant correlations of Greene's scores and IL-6 levels in women treated with paroxetine and kamishoyosan. IL-6 may be involved in the mechanism by which Greene's scores are reduced by treatments of paroxetine and kamishoyosan. IL-6 has been reported to stimulate the hypothalamic-pituitary-adrenocortical (HPA) axis and the release of corticotropin-releasing factor [21]. It has also been reported that IL-6 was produced and released from the rat adrenal zona glomerulosa by stimulation with corticotropin [22]. In addition, disruption of glucocorticoid-mediated feedback inhibition of IL-6 production has been reported in patients with depression [23]. Therefore, paroxetine and kamishoyosan may have effects on the HPA axis and feedback inhibition in women with psychological symptoms.

We found that serum concentrations of IL-8 and MIP-1β were decreased significantly and that both levels showed significant positive correlations with Greene's scores in women treated with paroxetine. We reported previously that serum concentrations of IL-8 and MIP-1β in midlife women with hot flashes were higher than those in midlife women without hot flashes [24]. In addition, we reported that cytokine-induced neutrophil chemoattractant (CINC), which corresponds to IL-8 in humans, was produced in the hypothalamus and might be involved in the pathoetiology of hot flashes [25]. It has been reported that a substantial reduction in hot flashes was observed following paroxetine treatment in menopausal women [26]. Therefore, paroxetine may improve hot flashes due to suppression of the production of IL-8 in the hypothalamus. In the present study, serum concentrations of IL-8 and MIP-1β decreased significantly in women whose hot flashes were improved by kamishoyosan. Kamishoyosan may also reduce concentrations of IL-8 and MIP-1β by acting on the hypothalamus in women whose hot flashes have been improved.

On the other hand, the change in IL-10 in depression is controversial. IL-10 has been shown to be a negative immunoregulatory

Table 5
Greene's scores and serum cytokine concentrations before and at 6 months after treatments with kamishoyosan in non-responders and responders for hot flashes.

	Non-responders for hot flashes ($n=10$)		Responders for hot flashes ($n=25$)	
	Before	6 months	Before	6 months
Greene's score				
Total	18.7 (3.3)	15.6 (3.6)**	16.6 (3.8)	12.3 (3.1)*
Psychological	10.4 (3.0)	7.7 (2.3)**	9.2 (3.2)	7.0 (2.6)*
Somatic	5.4 (1.1)	4.6 (1.5)#	4.0 (2.0)	3.4 (1.8)**
Vasomotor	2.2 (1.4)	2.3 (1.4)	2.6 (1.7)	1.2 (0.8)*
IL-1β (pg/ml)	0.80 (0.80–2.19)	0.40 (0.40–7.67)	0.80 (0.80–1.62)	0.40 (0.45–6.06)
IL-2 (pg/ml)	0.55 (0.55–1.75)	0.55 (0.55–1.06)	0.55 (0.55–5.90)	0.55 (0.55–3.42)
IL-4 (pg/ml)	0.25 (0.25–0.25)	0.25 (0.25–0.25)	0.25 (0.25–0.25)	0.25 (0.25–0.25)
IL-5 (pg/ml)	0.40 (0.40–1.00)	0.41 (0.40–0.62)	0.40 (0.40–0.76)	0.40 (0.40–0.49)
IL-6 (pg/ml)	3.01 (1.08–8.62)	2.44 (0.55–5.01)	1.85 (1.01–8.35)	0.55 (0.55–4.57)#
IL-7 (pg/ml)	3.53 (2.18–8.39)	3.05 (1.98–7.15)	3.29 (1.08–9.50)	3.09 (0.10–6.36)
IL-8 (pg/ml)	24.7 (3.75–158.5)	89.1 (9.66–283.5)#	51.7 (6.06–242.5)	22.1 (6.73–98.1)#
IL-10 (pg/ml)	0.45 (0.45–0.46)	0.50 (0.45–0.93)	0.45 (0.45–1.50)	0.45 (0.45–2.11)
TNF-α (pg/ml)	1.50 (1.50–4.39)	1.50 (1.50–2.78)	1.50 (1.50–8.58)	1.50 (1.50–9.52)
IFN-γ (pg/ml)	9.65 (9.65–9.65)	9.65 (9.65–9.65)	9.65 (9.65–9.65)	9.65 (9.65–9.65)
MCP-1 (pg/ml)	50.4 (29.2–81.0)	56.6 (23.1–96.4)	45.7 (21.6–93.3)	45.1 (25.1–75.3)
MIP-1β (pg/ml)	227.8 (37.4–432.1)	251.6 (51.1–388.8)	230.0 (82.4–534.2)	174.7 (102.9–327.7)#

Values in Greene's scores are means (standard deviations). Values in cytokines are medians (10–90 percentiles). IL: interleukin; TNF-α: tumor necrosis factor-α; IFN-γ: interferon-γ; MCP-1: monocyte chemoattractant protein-1; MIP-1β: macrophage inflammatory protein-β.

* $p<0.0001$ vs. before treatment.

** $p<0.001$ vs. before treatment.

\# $p<0.05$ vs. before treatment.

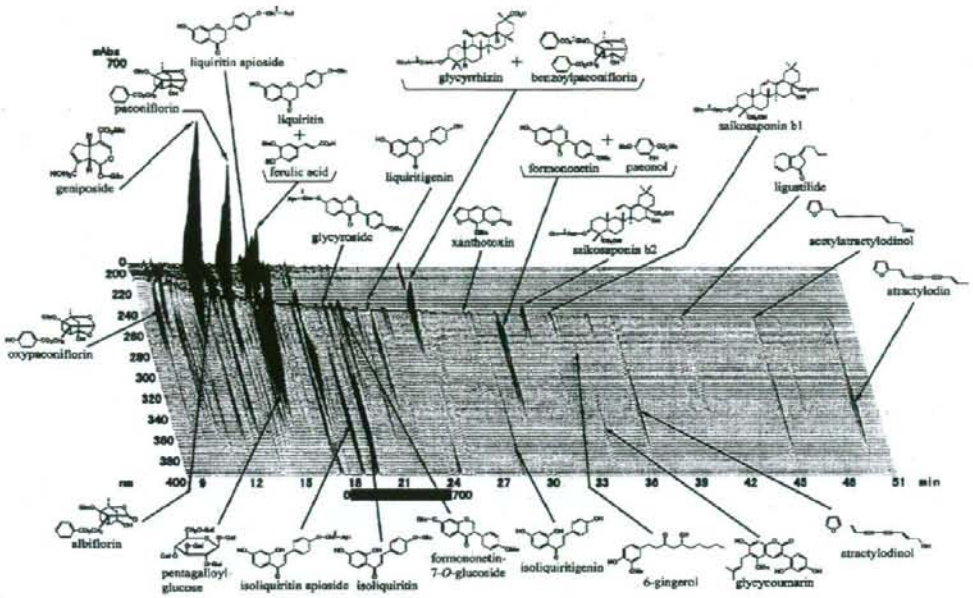

Fig. 4. Three-dimensional HPLC profile of the alkaloid of kamishoyosan.

cytokine [27]. It has been shown that venlafaxine significantly enhances IL-10 production in vitro [28]. However, Seidel et al. demonstrated by using a whole blood cell assay that the production level of IL-10 was elevated in patients with major depressive disorder compared to that in healthy subjects [8]. We also reported that serum IL-10 concentration in women with psychological symptoms was higher than that in women without psychological symptoms [10]. It has recently been reported that IL-10 receptor is expressed in the mouse adrenal gland and that IL-10 plays an important role in the regulation of steroid biosynthesis and in the maintenance of homeostasis and immunity during a period of stress [29]. Further study on the site where paroxetine acts is needed.

In the present study, we found that paroxetine reduced serum MCP-1 concentration. MCP-1 is the primary chemokine responsible for the recruitment of monocytes to sites of active inflammation, including the developing atheroma [30]. The expression of MCP-1 has been reported to be enhanced in endothelial cells [31] and white adipose tissue [32]. Recently, SSRI therapy has been reported to be associated with significant reduction in pro-inflammatory markers [33]. Paroxetine may play a suppressive role in inflammation.

It has been reported that there was a significant decrease in plasma TNF-α after SSRI treatment [12]. Denys et al. demonstrated that SSRI might decrease TNF-α due to activation of the 5-HT_{2A} receptor and increase in 5-HT since 5-HT might inhibit TNF-α [34]. We did not find a significant change in serum TNF-α level in women treated with paroxetine or kamishoyosan. The difference in these results may be due to the difference in subjects.

In the present study, kamishoyosan as well as paroxetine significantly improved psychological symptoms, although the magnitude of the effect of kamishoyosan was weak. On the other hand, compliance in women treated with paroxetine was rather poor due to adverse reactions, such as nausea and headache, but only one woman treated with kamishoyosan dropped out of the study because of adverse reaction. Thus, kamishoyosan may be a candidate for treatment of psychological symptoms because treatment for psychological symptoms must be continued without adverse reactions as long as possible.

It has been reported that kamishoyosan increased social interaction time and that its anxiolytic effect was as strong as that of diazepam [35]. In addition, it has been shown that the anxiolytic effect of kamishoyosan is due to neurosteroid synthesis followed by stimulation of γ-amino-butyric $acid_A$/benzodiazepine receptor [35]. Recently, many components have been shown to be included in Japanese traditional medicines by three-dimensional HPLC fingerprint analysis as shown for kamishoyosan in Fig. 4. Toriizuka et al. reported that Gardeniae fruit's major component, geniposide, increased social interaction time in a dose-dependent manner [36]. It has been reported that ligustilide and butylidenephthalide, components of Angelica root, reversed the decrease in pentobarbital sleep in mice [37]. Further study is needed to clarify which components of kamishoyosan have effects on cytokines and whether these components have effects similar to those of paroxetine.

In conclusion, decrease in IL-6 concentration may be involved in the mechanism of the actions of both paroxetine and kamishoyosan in women with psychological symptoms, and IL-6 may therefore be useful as a marker of treatment. In addition, the action of paroxetine may be associated with decrease in other cytokines and chemokines, including IL-8, IL-10, MIP-1β.

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Metabolite changes and gender differences in schizophrenia using 3-Tesla proton magnetic resonance spectroscopy (^{1}H-MRS)

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ABSTRACT

A change in the glutamatergic system is thought to play an important role in the pathophysiology of schizophrenia. The aim of this study was to investigate the changes in metabolites, including glutamate (Glu), in the anterior cingulate cortex (ACC) and the left basal ganglia (ltBG) of patients with chronic schizophrenia using proton magnetic resonance spectroscopy (^{1}H-MRS). In addition, since gender differences in this illness were known, we examined the effects of gender on these metabolites.

The ^{1}H-MRS was performed on the ACC and ltBG of 30 patients with schizophrenia and 25 healthy individuals who acted as the control group. The levels of Glu, glutamine (Gln), creatine plus phosphocreatine (Cre), myo-inositol (mI), N-acetylaspartate (NAA), and choline-containing compounds (Cho) were measured.

Two-way analysis of variance revealed that the illness significantly affected the levels of Glu and mI in the ACC; both metabolites were lower in the patients with schizophrenia as compared to the control subjects. The results also revealed that gender significantly affected the level of Gln in the ACC and the levels of Cre and NAA in the ltBG; the level of Gln in the ACC were higher in male subjects versus female subjects, whereas Cre and NAA levels in the ltBG were lower in male subjects as compared to female subjects.

These results confirmed a change in the glutamatergic system and suggested an involvement of mI in the pathophysiology of schizophrenia.

1. Introduction

Pathological changes in the brain may be an underlying cause of schizophrenia. Proton magnetic resonance spectroscopy (^{1}H-MRS) is a promising method that may be used to investigate such changes to clarify the pathophysiology of this illness.

Glutamate (Glu) is thought to play an important role in the pathophysiology of schizophrenia (Lang et al., 2007). According to the glutamatergic hypothesis of schizophrenia, the glutamatergic system becomes hyperactive in the acute stage and causes neuroinflammation and apoptosis of neurons through excitotoxity. This hypothesis suggests that the Glu concentration may become higher in the acute stage and lower in the chronic stage. The use of a low magnetic field device for evaluation makes separating the signal of Glu and glutamine (Gln) difficult; thus, the combined signals of these compounds (Glx, or the combiniation of Gln and Glu) or the ratio of Glx/Cre (i.e., ceratine plus phosphocreatine) were often reported. The

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Table 1a
Epidemiology of schizophrenic subject and healthy controls

	Control		Schizophrenia		p
	Male	Female	Male	Female	
Number	25		30		
	13	12	14	16	n.s.
Age	34.9±10.7		33.8±9.5		n.s.
	36.8±12.1	32.8±9.1	34.9±9.2	33.1±10.1	n.s.

Ages are shown as mean±S.D. (range).
Abbreviation: n.s., no significant difference.

increase (Choe et al., 1994; Chang et al., 2007), lack of change (Block et al., 2000; Yamasue et al., 2003; Ohrmann et al., 2007; Wood et al., 2007) and decrease (Choe et al., 1996) of the level of Glx or Glx/Cre ratios have been reported. Previous studies with high-magnetic magnetic resonance spectroscopy (MRS) reported that in first-episode schizophrenia patients, Glu concentration significantly increased in the left anterior cingulate cortex (ACC) (Theberge et al., 2002), whereas in medicated, chronic schizophrenia patients, Glu significantly decreased in the left ACC (Theberge et al., 2003).

Since changes in the ACC and basal ganglia were reported in patients with schizophrenia (Molina et al., 2003; Siever and Davis, 2004; Baiano et al., 2007; Harrison et al., 2007; Meda et al., 2008), we measured the levels of Gln, Glu, Cre, myo-inositol (mI), N-acetylaspartate (NAA), and choline-containing compounds (Cho) in the ACC and the left basal ganglia (ltBG) of patients with chronic schizophrenia. Additionally, we paid special attention to the gender differences in patients with schizophrenia. Seeman (1997) reviewed the influence of gender differences in the pathology of schizophrenia during onset, severity, effects of drugs, and typical course of the illness. These differences may be caused by biological factors (e.g., the hormones estrogen and progesterone), but this has not been fully confirmed. Although a few previous MRS studies reported differences between males and females in terms of the manifestation of schizophrenia (e.g., Buckley et al., 1994), very few follow-up studies were attempted. In most MRS studies, the percentage of female subjects was small, thereby making a consideration of the gender differences as related to the pathology of schizophrenia difficult. In this study, we ensured that about half of the participants were female subjects, and we reexamined this issue.

Table 1b
Epidemiology of schizophrenic subject

	Male		Female	p
Age at onset		23.3±7.3		
	24.2±9.0		22.8±5.5	n.s.
Duration of illness (years)		10.2±8.2		
	10.3±8.7		10.0±7.9	n.s.
Duration of therapy (years)		8.6±7.3		
	8.7±8.4		8.5±6.5	n.s.
PANSS Positive		12.5±3.5		
	13.1±3.9		11.9±3.1	n.s.
PANSS Negative		15.0±5.4		
	16.0±6.4		14.1±4.1	n.s.
PANSS General		27.7±7.2		
	28.1±8.4		26.4±6.1	n.s.
Dose of antpychotics (mg)		383.3±341.0		
	548.0±398.1		239.1±199.6	.011*
Other medications (number)				
Benzodiazepine	6		5	
Antidepressant	1		2	

Age at onset, duration of illness, duration of therapy, PANSS and dose of antipsychotics are shown as mean ± S.D.(range).
Dose of antipsychotics is shown as chloropromazine equivalent.
*Significant in two group t-test ($p<.05$).
Abbreviation: PANSS, Positive and Negative Syndrome Scale; n.s., no significant difference.

2. Materials and methods

2.1. Subjects

Thirty patients with chronic schizophrenia and twenty-five healthy control subjects participated in this study after

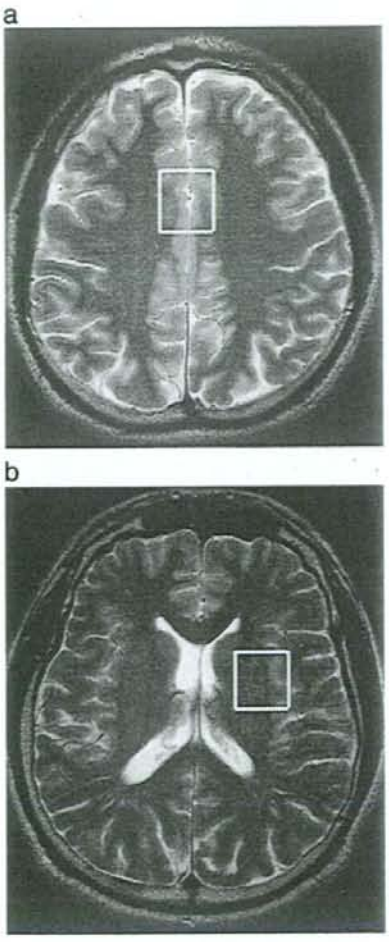

Fig. 1. Region of interest (ROI) positions for spectroscopic measurement by STEAM sequence in the anterior cingulate cortex (ACC) (a) and the left basal ganglia (ltBG) (b). The white box represents the location of the ROI that was used in the STEAM sequence in the horizontal image.