Manji HK, Drevets WC, and Charney DS (2001) The cellular neurobiology of depression. *Nat Med* **7**:541–547.

Messer CJ, Eisch AJ, Carlezon WA Jr, Whisler K, Shen L, Wolf DH, Westphal H, Collins F, Russell DS, and Nestler EJ (2000) Role for GDNF in biochemical and behavioral adaptations to drugs of abuse. *Neuron* **26**:247–257.

Miyake K, Fukuchi H, Kitaura T, Kimura M, Sarai K, and Nakahara T (1990) Pharmacokinetics of amitriptyline and its demethylated metabolite in serum and specific brain regions of rats after acute and chronic administration of amitriptyline. *J Pharm Sci* **79**:288–291.

Muraoka S, Kamei K, Muneoka K, and Takigawa M (1998) Chronic imipramine administration amplifies the serotonin$_{2A}$ receptor-induced intracellular Ca^{2+} mobilization in C6 glioma cells through a calmodulin-dependent pathway. *J Neurochem* **71**:1709–1718.

Newton SS and Duman RS (2004) Regulation of neurogenesis and angiogenesis in depression. *Curr Neurovasc Res* **1**:261–267.

Öngür D, Drevets WC, and Price JL. (1998) Glial reduction in the subgenual prefrontal cortex in mood disorders. *Proc Natl Acad Sci USA* **95**:13290–13295.

Prouty RW and Anderson WH (1990) The forensic science implications of site and temporal influences on postmortem blood-drug concentrations. *J Forensic Sci* **35**:243–270.

Rajkowska G (2002) Cell pathology in mood disorders. *Semin Clin Neuropsychiatry* **7**:281–292.

Santarelli L, Saxe M, Gross C, Surget A, Battaglia F, Dulawa S, Weisstaub N, Lee J, Duman R, Arancio O, et al. (2003) Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. *Science (Wash DC)* **301**:805–809.

Scharfman H, Goodman J, Macleod A, Phani S, Antonelli C, and Croll S (2005) Increased neurogenesis and the ectopic granule cells after intra hippocampal BDNF infusion in adult rats. *Exp Neurol* **192**:348–356.

Shimizu E, Hashimoto K, Okamura N, Koike K, Komatsu N, Kumakiri C, Nakazato M, Watanabe H, Shinoda N, Okada S, et al. (2003) Alterations of serum levels of brain-derived neurotrophic factor (BDNF) in depressed patients with or without antidepressants. *Biol Psychiatry* **54**:70–75.

Shinagawa S (1994) Serotonin protects C6 glioma cells from glutamate toxicity. *Neuroscience* **59**:1043–1050.

Takebayashi M, Hisaoka K, Nishida A, Tsuchioka M, Miyoshi I, Kozuru T, Hikasa S, Okamoto Y, Shinno H, Morinobu S, et al. (2006) Decreased levels of whole blood glial cell line-derived neurotrophic factor (GDNF) in remitted patients with mood disorders. *Int J Neuropsychopharmacol* **9**:607–612.

Tsuchioka M, Takebayashi M, Hisaoka K, Shinno H, and Nakata Y (2005) Serotonin (5-HT) induced glial cell line-derived neurotrophic factor (GDNF) expression is mediated by transactivation of fibroblast growth factor receptor (FGFR) in rat C6 glioma cells, in *Proceedings of the Society for Neuroscience 35th Annual Meeting*; 2005 Nov 12–16; Washington, DC. Program no. 841.7. Society for Neuroscience, Washington, DC.

Tsuda T, Kusui T, and Jensen RT (1997) Neuromedin B receptor activation causes tyrosine phosphorylation of p125FAK by a phospholipase C independent mechanism which requires p21rho and integrity of the actin cytoskeleton. *Biochemistry* **36**:16328–16337.

Zumwalt JW, Thunstrom BJ, and Spangelo BL (1999) Interleukin-1β and catecholamines synergistically stimulate interleukin-6 release from rat C6 glioma cells in vitro: a potential role for lysophosphatidylcholine. *Endocrinology* **140**:888–896.

Address correspondence to: Dr. Minoru Takebayashi, Department of Psychiatry and Institute of Clinical Research, National Kure Medical Center, 3-1 Aoyama, Kure 737-0023, Japan. E-mail: mtakebayashi@kure-nh.go.jp

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Risperidone in the treatment of psychotic depression

Makiko Goto, Reiji Yoshimura *, Shingo Kakihara, Koji Shinkai, Yasuhisa Yamada, Kyoko Kaji, Nobuhisa Ueda, Jun Nakamura

Department of Psychiatry, University of Occupational and Environmental Health, 1-1 Iseigaoka, Yahatanishi-ku, Kitakyushu, Fukuoka 807-8555, Japan

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Abstract

In the preset study, the authors investigated that effects of the antipsychotic drug risperidone on psychotic depression and examined the mechanism of risperidone to ameliorate psychotic depression. Fifteen patients met the DSM-IV criteria for major depressive disorder with psychotic features and the remaining five patients met those for bipolar I disorder (most recent episode depressed) with psychotic features (M/F: 8/12, age: 54±18). All patients were evaluated regarding their clinical improvement using the Hamilton Rating Scale for Depression (Ham-D), and Positive and Negative Syndrome Scale (PANSS). In addition, plasma concentrations of HVA and MHPG were analyzed by HPLC. Patients with a 50% or more improvement in Ham-D score were defined as responders. Three were prescribed risperidone alone, and the other 17 were administered risperidone as an addition to preexisting antidepressants or mood stabilizers. The preexisting antidepressants or mood stabilizers were as follows: paroxetine (6), lithium (3), valproic acid (3), clomipramine (2), fluvoxamine (1), amitriptyline (1), amoxapine (1). The average dose of risperidone was 1.8±0.5 mg/day. Eleven of twenty patients (55%) turned out to be responders 4 weeks after initiation of risperidone administration. No differences were observed between responders and nonresponders with respect to age, sex, Ham-D score before risperidone treatment, dose and plasma level of risperidone or its active metabolite, 9-hydroxyrisperidone. Plasma HVA levels before risperidone administration in responders were significantly higher than those in nonresponders. In addition, a significant correlation was observed between changes in plasma HVA level and the percentage improvement on Ham-D score. These results indicate that treatment with risperidone is effective to ameliorate psychotic depression, and the influence of risperidone on dopaminergic activity is associated with its efficacy.

Keywords: HVA; MHPG; Paroxetine; Psychotic depression; Risperidone

1. Introduction

Psychotic depression, a disorder associated with considerable morbidity and mortality, is more common than is generally realized and is encountered frequently in clinical practice. Psychotic depression is a particularly severe form of mood disorder, and its prevalence is estimated to be about 20% in patients with depressive episodes and around 0.6% in the general population (Roose and Glassman, 1988; Johnson et al., 1991). Patients with psychotic depression exhibit more frequent relapse and recurrences, and show increased use of mental health services, greater disability, and a poorer clinical course when compared with nonpsychotic depressed patients (Aronson et al., 1988; Rothschild et al., 1993; Coryell et al., 1996). In addition, patients with psychotic depression demonstrate distinct biological abnormalities such as hypothalamic–pituitary–adrenal (HPA) activity, dopaminergic activity, enzymatic activity, and abnormalities on brain imaging study, and electroencephalogram sleep profile (Schatzberg and Rothschild, 1992; Rothschild, 2003). Patients with psychotic depression respond to traditional antidepressant drugs at a lower rate than do non-psychotic patients (Schatzberg and Rothschild, 1992; Rothschild, 2003). Psychotic depression is known to be particularly resistant to treatment with tricyclic antidepressant drugs or selective serotonin reuptake inhibitors (SSRIs) alone (Chan et al., 1987; Matthews et al., 2002); however, it has been reported that patients with psychotic depression respond better to a combination of antipsychotic drugs and antidepressant

Abbreviations: HPA, hypothalamic–pituitary–adrenal; HVA, homovanillic acid; MAOI, monoamine oxidase inhibitor; MHPG, 3-methoxy-4-hydroxyphenylglycol; PANSS, positive and negative syndrome scale.

* Corresponding author. Tel.: +81 936917253; fax: +81 936924894.
E-mail address: yoshi621@med.uoeh-u.ac.jp (R. Yoshimura).

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drugs than to antidepressants alone (Nelson and Bowers, 1978; Charney and Nelson, 1981). Electroconvulsive therapy is considered by many to be the best treatment for acute episodes of psychotic depression, but the initial beneficial effects of ECT often require maintenance ECT treatment, which is costly and difficult to provide (Parker et al., 1992). Thus, some researchers have argued that psychotic depression should be classified as a distinct clinical entity due to a number of biological and behavioral symptoms that are specific to the disorder (Schatzberg and Rothschild, 1992; Rothschild, 2003). However, the pathogenesis of psychotic depression remains unclear, and the treatment strategy for pharmacotherapy is controversial.

Risperidone, a benzisoxazol derivative, belongs to the group of atypical antipsychotics. In vitro and in vivo studies have demonstrated that risperidone is a mixed serotonin ($5\text{-}HT_2$) and dopamine (D_2) receptor antagonist (Janssen et al., 1988; Leysen et al., 1988). We have reported that risperidone is effective for treating the acute phase of schizophrenia with little extrapyramidal side effects (Yoshimura et al., 2003). Furthermore, we reported that risperidone is also effective on negative symptoms of chronic schizophrenic patients (Yoshimura et al., 2000a). In negative schizophrenic symptomatology and in mood disorders, a disturbance of the serotonergic system has been shown to be of relevance. Antiserotonergic drugs are used in the treatment of depression. In addition, various interactions between the dopaminergic and the serotonergic system, indicating the complex nature of the serotonergic system, have been described (Marsden, 1991). Furthermore, our previous findings indicate that the effect of risperidone on noradrenergic system as well as on the serotonergic system might be related to its efficacy for negative symptoms of schizophrenia (Yoshimura et al., 2000a, b). Taken together, the effects of risperidone on three monoaminergic systems (dopamine, serotonin, noradrenaline) are associated with its clinical efficacy for schizophrenia.

It has been reported that risperidone is increasingly being used to control acute manic episodes, and data are emerging to support its mood stabilizing and antidepressant properties (Kasper et al., 2002; Yatham, 2002). Furthermore, recent anecdotal reports suggest that risperidone is useful for treating psychotic depression (Hillert et al., 1992; Keck et al., 1995). In the present study, we investigated the effects of risperidone on psychotic depression in order to clarify the pathogenesis of psychotic depression and to examine the mechanism of risperidone to ameliorate psychotic depression.

2. Methods

2.1. Subjects

Twenty patients were enrolled in the study. Fifteen patients met the DSM-IV criteria (American Psychiatric Association, 1994) for major depressive disorder with psychotic features and three patients met those for bipolar I disorder (most recent episode depressed). Eight were male and 12 were female, and their ages ranged from 28 to 74 (mean±S.D.=54±18) years. All patients were physically healthy and free of current alcohol and/or drug abuse. The protocol of this study was approved by the Ethics Committee of the University of Occupational and Environmental Health. All patients gave their consent to participate after having been informed of the study's purpose.

2.2. Procedures

The patients were treated with risperidone at a dose ranging from 1 to 4 (mean±S.D.=1.8±0.5) mg/day. Seventeen patients were treated with antidepressants for at least 4 weeks and then risperidone was coadministered. The remaining of three patients were treated with risperidone alone. Only benzodiazepines were permitted as hypnotics, and the dosage was kept constant throughtout the study period. The dosage of risperidone varied among patients and, based on ethical considerations, was not fixed. The patients were evaluated regarding their clinical improvement using the Hamilton Rating Scale for Depression (Ham-D) (Hamilton, 1960) and Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987) before and every week after risperidone administration by two experienced psychiatrists (M.G. and R.Y.), and the psychiatrists assessing the Ham-D and PANSS were blind to the results of plasma levels of catecholamine metabolites.

2.3. Assay for the plasma levels of HVA, MHPG, and paroxetine

Blood samples drawn into heparinized tubes were obtained at 08.00–10.00 before breakfast (approximately 13–15 h after the last dose of the drugs) before and at 4 weeks after risperidone treatment. The plasma samples were quickly separated in a centrifuge and stored at −80 °C until assayed. The plasma homovanillic acid (HVA) levels were analyed by high-performance liquid chromatography with electrochemical detection (HPLC-ECD) according to the method of Yeung et al. (1996). In short, each cyano-bonded solid-phase extraction cartridge was preconditioned with methanol followed by glass-distilled water. To each cartridge were added 0.3 ml of plasma sample or standard and 0.1 ml of working internal standard solution (5 ng of 5-hydroxyindolecarboxylic acid in 0.01 M KH_2PO_4, pH 7.2). The samples were allowed to pass through the cartridge slowly under mild vacuum (15 mm Hg), and the filtrate was collected. The cartridge was washed with 0.2 ml of distilled water. The filtrate portions were combined and deproteinized with 1 ml of acetonitrile. After mixing by vortex and centrifugation (1760×*g*, 4 °C for 10 min), an aliquot (5 μl) of supernatant was injected into the HPLC. The plasma 3-methoxy-4-hydroxyphenylglycol (MHPG) levels were analyzed according to the method of Minegishi and Ishizaki (1984). In brief, plasma was separated by centrifugation at 600×*g* at 4 °C. Extraction was performed under a vacuum using Bond-Elut columns prepacked with 100 mg of C18-bonded silica (40 μm) in a 1 ml-capacity disposable syringe. The columns, which were inserted into a vacuum chamber connected to an aspirator, were prepared by washing with 1 ml methanol followed by 1 ml of water. After the addition of 50 μl of a solution of vanilly alcohol (internal standard equivalent to 5 ng/ml) to 1 ml of plasma, samples were applied

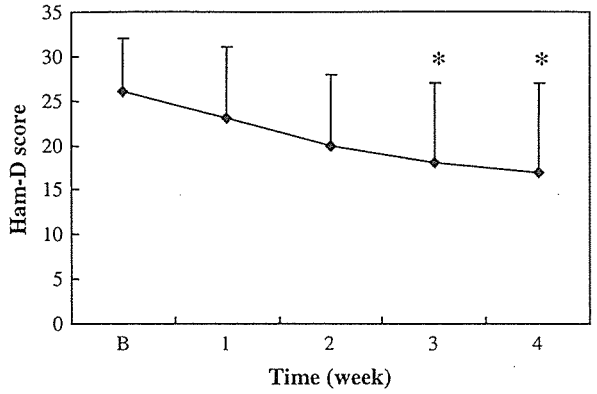

Fig. 1. Changes in scores on Ham-D before (B) and after risperidone administration. *$p<0.05$, compared with Ham-D score before risperidone administration.

to and passed through the columns, followed by 0.75 ml of water to rinse off both residual samples and easily eluted hydrophilic compounds. The adsorbed materials were eluted with 200 μl of methanol to a 0.1 M phosphate buffer (pH 4.8) mixture (40:60, v/v). A 20 μl portion of this solution was injected into the HPLC. Plasma risperidone and 9-hydroxyrisperidone were analyzed by HPLC according to the method of Olesen and Linnet (1997). In brief, 1 ml of plasma was mixed with 0.5 ml, 0.6 M sodium carbonate/bicarbonate buffer, pH 10, and 50 μl haloperidol solution, 3.76 mg/ml (10 mM) as an internal standard. An 8 ml portion of heptane–isoamylalcohol (98:2, v/v) was added, and the mixture was shaken for 5 min in the horizontal position at 250 shakes/min. After centrifugation at 1500 ×*g* for 10 min, the aqueous layer was frozen by immersing the tubes in a cooling bath consisting of dry ice and ethanol. The heptane layer was decanted into centrifuge tubes and evaporated to dryness at 60 °C in a gentle stream of nitrogen. The residue was dissolved in 75 μl of the mobile phase (40 mM ammonium acetate buffer pH 7.0–methanol; 100: 900, v/v), of which 65 μl was injected into the HPLC. Plasma paroxetine was also analyzed by HPLC according to the method of Gupta (1994). In short, a 0.5 ml aliquot of the

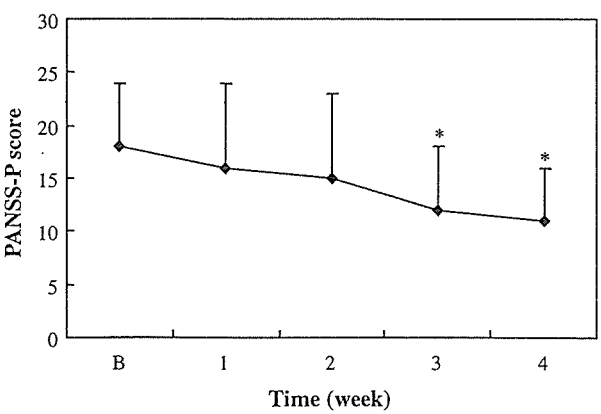

Fig. 2. Changes in scores on PANSS-P before (B) and after risperidone administration. *$p<0.05$, compared with PANSS-P score before risperidone administration.

sample was mixed with 100 μl of the working standard (dibucaine) and 0.5 ml of acetonitrile in a glass tube. After centrifugation at 1500×*g* for 3 min, the supernatant was applied to a 1 ml Bond-Elut C18 extraction column which had been previously activated by washing serially, once with 1 M HCl, twice with methanol, and once with water. The sample was passed slowly through the column by mild suction. The column was then washed serially, twice with water and once with acetonitrile, making sure that each column was grained completely after every wash. An aliquot of 0.25 ml of methanol containing 2.5 ml/100 ml of 35% perchloric acid was applied to each column. The liquid was allowed to pass through the column. A 7 μl aliquot of the elut was injected into the HPLC.

2.4. Data analysis

The Student *t*-test was used to compare plasma HVA and MHPG levels before and at 4 weeks after risperidone administration. The Pearson's coefficient test was used to examine the relationship between plasma HVA or MHPG level and clinical improvement on Ham-D score. ANOVA was used to compare the scores of Ham-D and PANSS-P at each point. Probabilities of less than 0.05 were considered significant.

3. Results

Seventeen of twenty patients were receiving preexisting treatment with antidepressants or mood stabilizers at least 4 weeks, as follows: 6 patients with paroxetine, 3 patients with lithium, 3 patients with valproic acid, 2 patients with clomipramine, 1 patient with fluvoxamine, 1 patient with amitriptyline, and 1 patient with amoxapine risperidone was added to these antidepressants or mood stabilizers. The mean Ham-D score of 26±6 at baseline (before risperidone administration) significantly decreased to 17±10 at 4 weeks after risperidone administration (Fig. 1). The mean PANSS-P score of 18±6 at baseline also significantly decreased to 11±5 at 4 weeks after risperidone administration (Fig. 2). Eleven of twenty (55%) patients turned to be responders to risperidone treatment within 4 weeks. There were no differences between responders and nonresponders with regard to age, sex, daily dosage of risperidone, plasma levels of risperidone and 9-hydroxyrisperidone, or baseline Ham-D and PANSS-P scores (Table 1). Plasma HVA levels were significantly higher in responders than nonresponders before risperidone adminis-

Table 1
Demographics of responders and nonresponders

	Responders (11)	Nonresponders (9)
Sex (M/F)	4/7	3/6
Age	50±14	57±17
Ham-D	28±10	25±4
PANSS-P	17.6±6.6	18.5±7.1
Risp (mg/day)	1.5±0.5	1.8±0.8
Risp (ng/ml)	13±8	15±7
9-OH-risp (ng/ml)	29±14	33±11

tration (responders: 9.7±2.4 ng/ml, nonresponders: 7.4±1.3 ng/ml, $p=0.02$) (Fig. 3). In addition, a significant negative correlation was observed between change in plasma HVA levels and percentage improvement on Ham-D score ($r=-0.56$, $p=0.04$) (Fig. 4). On the other hand, no significant difference was found between responders and nonresponders with respect to plasma MHPG level (responders: 8.0±1.7 ng/ml, nonresponders: 7.1 ±2.5 ng/ml) before risperidone administration (Fig. 5), and no significant correlation was observed between change in plasma MHPG levels and percentage improvement on Ham-D (Fig. 6). Six patients were coadministered risperidone (2 mg/day) after 4 weeks treatment with paroxetine (40 mg/day). We compared the concentrations of paroxetine before and after 2 weeks coadministration with risperidone. There was no difference in plasma paroxetine levels before and at 2 weeks after risperidone coadministration (before: 161±66 ng/ml, 2 weeks after: 163 ±72 ng/ml) (Fig. 7).

4. Discussion

4.1. Clinical efficacy and plasma levels of catecholamine metabolites

The main findings of the present study are that higher pretreatment plasma HVA levels are associated with a favorable response to risperidone treatment, and that there is a negative correlation between change in plasma HVA levels and percentage improvement on Ham-D score in patients with psychotic depression. On the other hand, pretreatment plasma MHPG levels were not associated with response to risperidone treatment. Several studies have demonstrated that there is an activation of the dopaminergic system in psychotic depression. Sweeney et al. (1978) reported that the probenecid-induced accumulation of HVA in cerebrospinal fluid (CSF) was higher in patients with psychotic major depression than in those with nonpsychotic major depression. In addition, Aberg-Wistedt et al. (1985) reported that patients with psychotic major depression had significantly higher levels of HVA in CSF than did patients with nonpsychotic major depression. With regard to plasma HVA levels, Devanand et al. (1985) reported that plasma HVA concentrations were significantly higher in women with psychotic depression than in women with nonpsychotic depression. Mazure et al. (1987) also reported

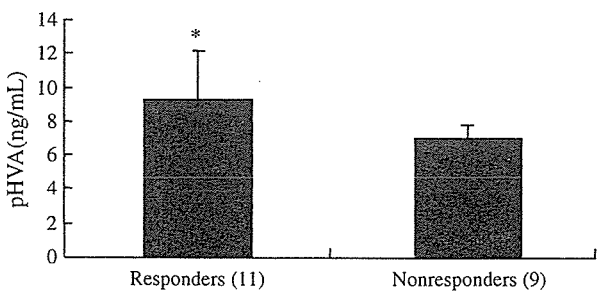

Fig. 3. Plasma HVA levels before risperidone administration compared between responders and nonresponders to risperidone treatment. *$p<0.05$, compared with nonresponders.

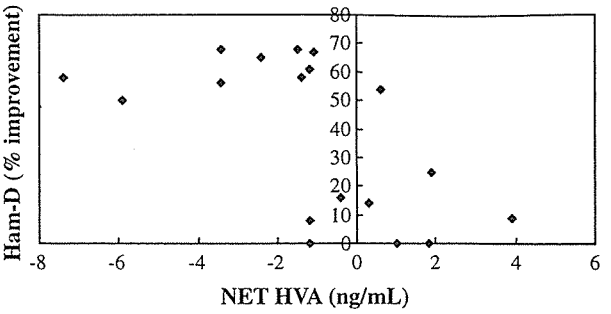

Fig. 4. Correlation between percentage improvement on Ham-D score and change in HVA level. $r=-0.56$, $p<0.05$.

that plasma HVA levels were significantly higher in patients with psychotic major depression than in melancholic patients with nonpsychotic major depression. Although we did not examine the drug-naïve levels of HVA, the average HVA concentrations before risperidone administration of our 20 patients with psychotic depression was 8.2±2.6 ng/ml, which was higher than those in the nonpsychotic patients 4.8±2.8 from our previous study (Shinkai et al., 2004). Eleven of our twenty (55%) patients responded to risperidone treatment within 4 weeks. Ten of eleven responders were being coadministered antidepressant drugs or mood stabilizers as follows; paroxetine (5), lithium (2), clomipramine (1), amitriptyline (1), and amoxapine (1). Several case reports and retrospective chart reviews have suggested that risperidone as monotherapy or in combination with antidepressants may be effective in patients with psychotic depression. In one double-blind, multicenter, parallel group trial (Müller-Siecheneder et al., 1998), the efficacy of risperidone monotherapy was compared with that of a combination of haloperidol and amitriptyline over 6 weeks in patients with psychotic depression. Both treatments were effective in producing clinically relevant score reductions on the PANSS, BPRS (Overall and Gorham, 1962), and Bech–Rafaelson Melancholia Scale (Bech et al., 1980). The reductions were, however, significantly largest in the amitriptyline plus haloperidol group. Furthermore, it has been reported that risperidone combined with SSRIs or MAOI is effective for treatment-refractory depression (O'Conner and Silver, 1998; Stoll and Hanra, 2000). Recently, Tani et al. (2004) reported that 5 treatment-refractory patients responded to an augmentation therapy of

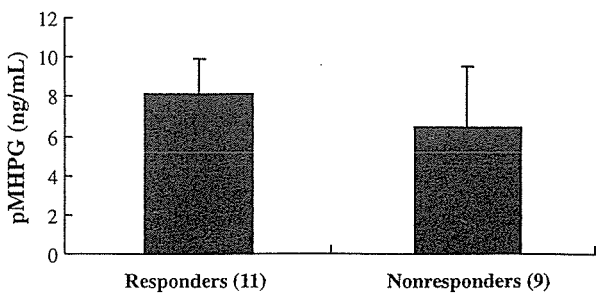

Fig. 5. Plasma MHPG levels before and after risperidone administration compared between responders and nonresponders to risperidone treatment.

milnacipran, a serotonin noradrenaline reuptake inhibitor, plus risperidone. In the present study, the possibility that risperidone alone, not combined with antidepressants or mood stabilizers, was effective for treating psychotic depression remains obscure. Other atypical antipsychotic drugs such as clozapine (Banov et al., 1994; Ranjan and Meltzer, 1995), olanzapine (Rothschild et al., 1999; Nelson et al., 2001), and quetiapine Zarate et al., 2000) also have been reported to be effective for treating psychotic depression. In the present study, pretreatment plasma HVA levels in the responders to risperidone treatment were higher than those of nonresponders, and an association was found between change in plasma HVA level and clinical improvement on Ham-D. These results suggest that higher levels of plasma HVA before risperidone administration might be a predictor of good response to risperidone treatment, and the influence of risperidone on dopaminergic activity might be associated with its efficacy in treating psychotic depression. Scores on PANSS-P and on Ham-D also decreased after risperidone administration. Furthermore, a correlation was observed between change in plasma HVA level and change in score on PANSS-P (data not shown), suggesting that change in the plasma HVA level is also associated with the relief of psychotic symptoms as well as depressive symptoms. Previously, we demonstrated that an elevated plasma HVA level was related to clinical effect of risperidone treatment on positive symptoms of schizophrenia in the acute phase, and a trend toward a negative correlation between decrease in plasma HVA and improvement in total PANSS-P score has been reported (Yoshimura et al., 2003). The results in the present study of psychotic depression are almost in accord with those in our previous study of schizophrenia. Taken together, findings suggest that the dopaminergic system might be important in both psychotic depression and schizophrenia, and, in addition, that psychotic depression might be a subtype of depression, which involves a disturbance predominant in the dopaminergic system. We also reported finding a significant correlation between the changes in plasma MHPG level and the percentage change in the negative symptom score on BPRS (Yoshimura et al., 2000a), suggesting risperidone partly improves negative symptoms by influencing the noradrenergic system in chronic schizophrenic patients. However, no difference in pretreatment plasma MHPG level was observed between responders and non-

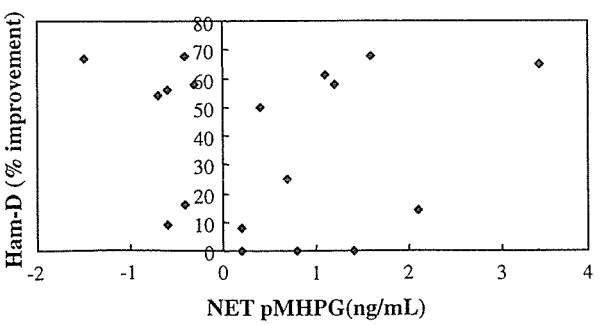

Fig. 6. No correlation was observed between percentage improvement on Ham-D and change in MHPG level.

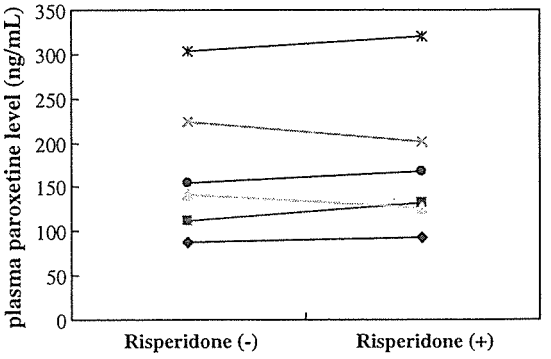

Fig. 7. Change in plasma paroxetine level before and at 2 weeks after risperidone administration.

responders to risperidone treatment, nor was any correlation found between change in the plasma MHPG level and clinical improvement in patients with psychotic depression. Thus, the noradrenergic system might not play an important role in the pathogenesis of psychotic depression, or preexisting antidepressant drugs or mood stabolizers might complicate the results of plasma MHPG level.

4.2. Effect of risperidone on plasma levels of paroxetine in steady states

Six of twenty patients were administer risperidone at 2 mg/day in addition to preexisting paroxetine administration at 40 mg/day. We compared plasma paroxetine concentration before and at 2 weeks after risperidone coadministration. There was no significant difference in plasma paroxetine concentrations before and 2 weeks after risperidone administration. This result indicates that risperidone has little influence on paroxetine metabolism. Both paroxetine and risperidone are mainly metabolized by cytochrome P450 (cyp) 2D6 (Spina et al., 2001; Yoshimura et al., 2001). Therefore, risperidone is not likely to potently inhibit cyp2D6. On the other hand, it has been reported that paroxetine increases the sum of plasma levels of risperidone and 9-hydroxyrisperidone (Spina et al., 2001), and, consequently might give rise to the risk of occurrence of extrapyramidal side effects by risperidone treatment. We also reported that severe extrapyramidal symptoms did not emerge under a dose of 4 mg/day of risperidone, but that at a daily dose greater than 4 mg, extrapyramidal symptoms induced by risperidone increased in conjunction with both the dosage of risperidone and the total plasma concentrations of risperidone and 9-hydroxyrisperidone in Japanese schizophrenic patients (Yoshimura et al., 2001). In the present study, we used a relatively low dose of risperidone, and extrapyramidal side effects were not worsened after risperidone administration.

5. Conclusion

Risperidone is effective for treating psychotic depression, and a relatively high plasma HVA level before risperidone administration might be a predictor of good responsiveness to

risperidone treatment. In addition, the influence of risperidone on the dopaminergic system is associated with its effectiveness on psychotic depression. There are limitations to this study, in that plasma HVA and MHPG levels appear to derive only in part from central sources (Yoshimura et al., 2000a, 2003, 2004). Further limitations include the fact that our sample size was very small, and we used an open flexible dose regime. Further studies are therefore warranted to confirm the findings of this preliminary study.

Acknowledgements

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References

Aberg-Wistedt A, Wistedt B, Bertilsson L. Higher CSF levels of HVA and 5-HIAA in delusional compared to nondelusional depression. Arch Gen Psychiatry 1985;42:925–6.

American Psychiatric Association. Diagnostic and statistical manual of mental disorders. 4th edition. Washington, DC: American Psychiatric Association; 1994.

Aronson TA, Shukla S, Gujavarty K, Hoff A, DiBuono M, Khan E. Relapse in delusional depression: a retrospective study of the course of treatment. Comp Psychiatry 1988;29:12–21.

Banov MD, Zarate Jr CA, Tohen M, Scialabba D, Wines Jr JD, Kolbrener M. Clozapine therapy in refractory affective disorders: polarity predicts response in long-term follow up. J Clin Psychiatry 1994;55:295–300.

Bech P, Gram LF, Reisby N, Rafaelson OJ. The WHO depression scale, relationship to the Newcastle scales. Acta Psychiatr Scand 1980;62:140–53.

Chan CH, Janicak PG, Davis JM. Response of psychotic and non-psychotic depressed patients to tricyclic antidepressants. J Clin Psychiatry 1987;48:197–200.

Charney DS, Nelson JC. Delusional and nondelusional unipolar depression. Am J Psychiatry 1981;138:328–33.

Coryell W, Leon A, Winokur G, Endicott J, Keller MB, Akiskal H, et al. The importance of psychotic features to long term course in depressive disorders. Am J Psychiatry 1996;153:483–9.

Devanand DP, Bowers MB, Hoffman FJ, Nelson JC. Elevated plasma homovanillic acid in depressed females with melancholia and psychosis. Psychiatry Res 1985;15:1–4.

Gupta RN. Colomn liquid chromatographic determination of paroxetine in human serum using solid-phase extraction. J Chromatgr 1994;661:362–5.

Hamilton M. A rating scale for depression. Neurol Neurosurg Psychiatry 1960;23:56–62.

Hillert A, Maier W, Wetzel H, Benkert O. Risperidone in the treatment of disorders with a combined psychotic and depressive syndrome: a functional approach. Pharmacopsychiatry 1992;25:213–7.

Janssen PAJ, Niemegeers CJF, Awouters F, Schellenkens KHL, Menges AAHP, Meert TF. Pharmacology of risperidone (R64766), a new antipsychotic with serotonin-S2 and dopamine-D2 antagonistic properties. J Pharmacol Exp Ther 1988;244:1014–24.

Johnson J, Horwath E, Weissman MM. The validity of major depression with psychotic features based on a community study. Arch Gen Psychiatry 1991;48:1075–81.

Kasper S, Stamenkovic M, Letmaier M, Schreinzer D. Atypical antipsychotics in mood disorders. Int Clin Psychopharmacol 2002;17(Suppl. 3):S1–10.

Kay SR, Fiszbein A, Opler LA. The positive and negative syndrome scale (PANSS) for schizophrenia. Schizophr Bull 1987;13:261–76.

Keck Jr PE, Wilson DR, Strakowski SM, McElroy SL, Kizer DL, Balisteri TM. Clinical predictors of acute risperidone response in schizophrenia, schizoaffective disorder, and psychotic mood disorders. J Clin Psychiatry 1995;56:466–70.

Leysen JE, Gommeren W, Eens A, DeChaffog, De Coucelles D, Stoof JC, et al. Biochemical profile of risperidone, a new antipsychotic. J Pharmacol Exp Ther 1988;247:661–70.

Marsden CA. The neuropharmacology as serotonin in the central neurons system. In: Feighner JP, Boyer WF, editors. Selective serotonin re-uptake inhibitors. Perspectives in PsychiatryChichester: John Willey and Sons; 1991. p. 11–36.

Matthews JD, Bottonari KA, Polania LM, Mischoulon D, Dording CM, Irvin R, et al. An open study of olanzapine and fluoxetine for psychotic major depressive disorder: interim analysis. J Clin Psychiatry 2002;63:1164–70.

Mazure CM, Bowers MB, Hoffman F, Miller KB, Nelson JC. Plasma catecholamine metabolites in subtypes of major depression. Biol Psychiatry 1987;22:1469–72.

Minegishi A, Ishizaki T. Determination of free 3-methoxy-4-hydroxyphenylglycol with several other monoamine metabolites in plasma by high-performance liquid chromatography with amperometric detection. J Chromatgr 1984;311:51–7.

Müller-Siecheneder F, Muller MJ, Hillert A, Szegedi A, Wetzel H, Benkert O. Risperidone versus haloperidol and amitriptyline in the treatment of patients with a combined psychotic and depressive syndrome. J Clin Psychopharmacol 1998;18:111–20.

Nelson JC, Bowers MBJ. Delusional unipolar depression: description and drug response. Arch Gen Psychiatry 1978;35:1321–8.

Nelson EB, Rielage E, Welge JA, Keck Jr PE. An open trial of olanzapine in the treatment of patients with psychotic depression. Ann Clin Psychiatry 2001;13:147–51.

O'Conner M, Silver H. Adding risperidone to selective serotonin reuptake inhibitor improves chronic depression. J Clin Psychopharmacol 1998;18:89–91.

Olesen OV, Linnet K. Simplified high-performance liquid chromatographic method for determination of risperidone and 9-hydroxyrisperidone in serum from patients comedicated with other psychotic drugs. J Chromtgr B 1997;698:209–16.

Overall JE, Gorham DR. The brief psychotic rating scale. Psychol Res 1962;10:799–812.

Parker G, Roy K, Hadzi-Pavlovic D, Pedic F. Psychotic (delusional) depression: a meta-analysis of physical treatments. J Affect Disord 1992;24:17–24.

Ranjan R, Meltzer HY. Acute and long-term effectiveness of clozapine in treatment-resistant psychotic depression. Biol Psychiatry 1995;40:253–8.

Roose S, Glassman AH. Delusional depression. In: Georgotas A, Cancro R, editors. Depression and mania. New York: Elsevier Science; 1988. p. 76–85.

Rothschild AJ. Challenges in the treatment of depression with psychotic features. Biol Psychiatry 2003;53:680–90.

Rothschild AJ, Samson JA, Bond TC, Luciana MM, Schildkraut JJ, Schatzberg AF. Hypothalamic–pituitary–adrenal axis activity and one-year outcome in depression. Biol Psychiatry 1993;34:392–400.

Rothschild AJ, Bates KS, Boehringer KL, Syed A. Olanzapine response in psychotic depression. J Clin Psychiatry 1999;60:116–8.

Schatzberg AF, Rothschild AJ. Psychotic (delusional) major depression: should it be included as a distinct syndrome in DSM-IV? Am J Psychiatry 1992;149:733–45.

Shinkai K, Yoshimura R, Ueda N, Okamoto K, Nakamura J. Associations between baseline plasma MHPG (3-methoxy-4-hydroxyphenylglycol) levels and clinical responses with respect to milnacipran versus paroxetine treatment. J Clin Psychopharmacol 2004;24:11–7.

Spina E, Avenso A, Facciola G, Scordo MG, Ancione M, Madia A. Plasma concentrations of risperidone and 9-hydroxyrisperidone during combined treatment with paroxetine. Ther Drug Monit 2001;23:223–7.

Stoll AL, Hanra G. Tranylcypromine plus risperidone for treatment-refractory major depression. J Clin Psychopharmacol 2000;20:495–6.

Sweeney D, Nelson C, Bowers M, Mass J, Heninger G. Delusional versus nondelusional depression: neurochemical differences. Lancet 1978;2:100–1.

Tani K, Takei N, Kawai M, Suzuki K, Sekine Y, Toyoda T, et al. Augmentation of milnacipran by risperidone in treatment for major depression. Int J Neuropsychopharmacol 2004;7:55–8.

Yatham LN. Mood stabilization and the role of antipsychotics. Int Clin Psychopharmacol 2002;17(Suppl. 3):S21–7.

Yeung PKF, Buckley SJ, Pedder SCJ, Dingemanse J. Determination of 3,4-dihydroxyphenylacetic acid and 5-hydroxyindoleacetic acid in human plasma by a simple and rapid high-performance liquid chromatography assay. J Pharm Sci 1996;85:451–3.

Yoshimura R, Nakamura J, Ueda N, Terao T. Effect of risperidone on plasma free 3-methoxy-4-hydroxyphenylglycol (pMHPG) levels in schizophrenic patients: relationship among plasma concentrations of risperidone and 9-hydroxyrisperidone, pMHPG, and clinical improvement. Int Clin Psychopharmacol 2000a;15:175–80.

Yoshimura R, Yanagihara N, Hara K, Terao T, Nakamura J, Ueno S, et al. Inhibitory effects of clozapine and other antipsychotic drugs on noradrenaline transporter in cultured bovine adrenal medullary cells. Psychopharmacology 2000b;149:17–23.

Yoshimura R, Ueda N, Nakamura J. Possible relationship between combined plasma concentrations of risperidone plus 9-hydroxyrisperidone and extrapyramidal symptoms. Neuropsychobiology 2001;44:129–31.

Yoshimura R, Ueda N, Shinkai K, Nakamura J. Plasma levels of homovanillic acid and the response to risperidone in first episode untreated acute schizophrenia. Int Clin Psychopharmacol 2003;18:107–11.

Yoshimura R, Nakamura J, Shinkai K, Ueda N. Clinical response to antidepressant and 3-methoxy-4-hydroxyphenylglycol (MHPG) levels: mini review. Prog Neuropsychopharmacol Biol Psychiatry 2004;28:611–6.

Zarate Jr CA, Rothschild SJ, Fletcher KE, Madrid A, Zapatel J. Clinical predictors of acute response with quetiapine in psychotic depression. J Clin Psychiatry 2000;61:185–9.

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Effect of risperidone on plasma catecholamine metabolites and brain-derived neurotrophic factor in patients with bipolar disorders

Reiji Yoshimura*, Yuichiro Nakano, Hikaru Hori, Atsuko Ikenouchi, Nobuhisa Ueda and Jun Nakamura

Department of Psychiatry, University of Occupational and Environmental Health, Iseigaoka, Yahatanishi-ku, Kitakyushu, Fukuoka, Japan

A combination treatment with a mood stabilizer and an antipsychotic drug is often used in as many as 90% of subjects with acute mania. Recently, augmentation therapy with atypical antipsychotics has been investigated in both the acute and long-term treatment of bipolar disorder with or without psychosis. In the present study, the authors investigated the efficacy of risperidone treatment for both acute manic and depressive episodes in bipolar disorder. Eighteen patients (M/F: 8/10, age: 34 ± 15 yr) who met the DSM-IV criteria for bipolar I disorder (12 cases of manic episodes, 6 cases of depressive episodes) with risperidone treatment were evaluated regarding their clinical improvement using the Young Mania rating Scale (YMRS) and the Hamilton rating Scale for Depression (Ham-D). Plasma concentrations of HVA and MHPG were analyzed by HPLC-ECD and plasma brain-derived neurotrophic factor (BDNF) levels were detected by sandwich ELISA. The mean scores of the YMRS were 22, 18, 12, 8, and 5 at time points before and 1, 2, 3, and 4 weeks after the risperidone administration, respectively. The mean scores of the Ham-D were 24, 25, 21, 21, and 19 at time points before and 1, 2, 3, and 4 weeks after the risperidone administration, respectively. The plasma levels of HVA and 3-methoxy-4-hydroxyphenylglycol (MHPG) were observed to have decreased 4 weeks after risperidone administration in manic patients. The levels did not change in depressive patients. The plasma levels of BDNF were decreased in depressive patients compared with manic patients or healthy controls. However, the administration of risperidone did not alter plasma BDNF levels.

KEY WORDS—risperidone; bipolar disorder; homovanillic acid (HVA); 3-methoxy-4-hydroxyphenylglycol (MHPG); brain-derived neurotrophic factor (BDNF)

INTRODUCTION

Many patients with bipolar disorder experience recurrent breakthrough episodes of mania or depression despite adequate ongoing treatment with one or more mood stabilizers (Ghaemi and Sachs, 1997). Lithium and valproic acid are the recommended first-line treatments for bipolar disorder (Sachs *et al.*, 2000; American Psychiatric Association, 2002). However, various shortcomings prevent them from being a monotherapy panacea for this disease. For example, lithium is ineffective or poorly tolerated in about one-third of patients (Bowden *et al.*, 2004) and has a relatively slow onset of action, often requiring weeks to produce maximum therapeutic effects (American Psychiatric Association, 2002). Furthermore, monotherapy with a mood stabilizer is not recommended as the initial treatment for severe mania (American Psychiatric Association, 2002). Augmentation treatment with a mood stabilizer and an antipsychotic drug is becoming a common therapy for patients with mania (Yatham *et al.*, 2003a), with as many as 90% of subjects with acute mania receiving mood stabilizer and antipsychotic combinations (Miller *et al.*, 2001).

*Correspondence to: R. Yoshimura, Department of Psychiatry, University of Occupational and Environmental Health, Iseigaoka, Yahatanishi-ku, Kitakyushu, Fukuoka 8078555, Japan. Tel: +81936917523. Fax: +81936934894.
E-mail: yoshi621@med.uoeh-u.ac.jp

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Unfortunately, typical antipsychotics are associated with neurological side effects such as extrapyramidal symptoms and tardive dyskinesia, and the latter can be irreversible in some patients (Brambilla *et al.*, 2003). Furthermore, elderly patients are known to be especially sensitive to these side effects due to pharmacodynamic and pharmacokinetic changes with age (Muscettola *et al.*, 1999). Because of their lower propensity to cause neurological side effects, the atypical antipsychotics are increasingly being used in the treatment of bipolar disorder.

Risperidone, an atypical antipsychotic, has proven to be effective as a monotherapy, or in combination with mood stabilizers, in the short- and long-term treatment of patients with acute mania as demonstrated by the results of a large number of uncontrolled (Jacobsen, 1995; Sajatovic *et al.*, 1996, Licht *et al.*, 2001; Vieta *et al.*, 2002, 2003; Yatham *et al.*, 2003a) and controlled clinical trials (Segal *et al.*, 1998; Sachs *et al.*, 2002; Yatham *et al.*, 2003b). In their review, Brambilla *et al.* (2003) concluded that risperidone showed a 75% antimanic response with a mean dose of 3.4 mg/day in uncontrolled trials and an 83% antimanic response with a mean dose of 5.4 mg/day in controlled trials. These findings suggest that risperidone has good efficacy in controlling manic symptoms in acute manic patients. In addition, Shelton and Stahl (2004) reported that risperidone, paroxetine, and the combination of risperidone and paroxetine are equally but modestly effective when added to a mood stabilizer in bipolar depression.

We found that the effects of risperidone on plasma levels of homovanillic acid (HVA) and 3-methoxy-4-hydroxyphenylglycol (MHPG) are related to its clinical efficacy in ameliorating the positive and negative symptoms of schizophrenia, respectively (Yoshimura *et al.*, 2000a, 2003; Yoshimura *et al.*, 2005). We also demonstrated that risperidone has an inhibitory effect on noradrenaline transporteds (NAT) (Yoshimura *et al.*, 2000b). These results indicate that the effects of risperidone on dopaminergic and noradrenergic neurons are important to its clinical efficacy in schizophrenia. Recently, we investigated the effects of risperidone treatment on plasma concentrations of risperidone and 9-hydroxyrisperidone, MHPG, HVA, and polymorphism of cytochrome P450 (cyp) 2D6. In addition, interactions between risperidone and smoking or caffeine were examined. In that study, it was found that higher pretreatment HVA levels might predict a favourable response to risperidone treatment and that higher plasma levels of the active moiety (risperidone and 9-hydroxyrisperidone) might be associated with an increased risk for extrapyramidal symptoms induced by risperidone, while smoking, caffeine intake and cyp2D6 genotypes had no effect on plasma risperidone levels (Kakihara *et al.*, 2005).

In the present study, the clinical efficacy of risperidone for acute mania was investigated, as well as the effects of risperidone on plasma levels of catecholamine metabolites and brain-derived neurotrophic factor (BDNF). We found that risperidone is effective and well tolerated in the treatment of acute mania, and we also found that plasma levels of HVA and MHPG were reduced after risperidone treatment. In contrast, risperidone did not alter the plasma BDNF levels.

SUBJECTS AND METHODS

The subjects included 18 Japanese bipolar I disorder in/out-patients (12 in manic episode, 6 in depressive episode) of the University Hospital of the University of Occupational and Environmental Health, all of whom fulfilled the DSM-IV criteria for bipolar I disorder. Eight were male and ten were female. Their ages ranged from 23 to 51 (mean $\pm$ *SD* = 34 $\pm$ 15) years. The age- and sex-matched 20 healthy control were prepared as a control group (nine were male and 11 were female, their mean age was 30 $\pm$ 11 years). The patients were treated with risperidone in a dose range from two to six (mean $\pm$ *SD* = 3.1 $\pm$ 1.5) mg/day. The mean daily dose of risperidone for bipolar manic and bipolar depressed were 3.8 $\pm$ 1.1 and 1.4 $\pm$ 0.8 mg, respectively. Ten of twelve manic patients or five of six depressive patients were co-administered valproic acid or lithium. The clinical improvement of patients was evaluated using the Young Mania Rating Scale (YMRS) or the Hamilton rating Scale for Depression (Ham-D) before and 1,2,3, and 4 weeks after risperidone administration by two experienced psychiatrists (Reiji Yoshimura and Yuichiro Nakano). The patients were also evaluated with regard to their extrapyramidal side effects using the Simpson and Angus (SAS) before and 4 weeks after risperidone administration by the same two psychiatrists. The interrater reliability levels determined for the YMRS, Ham-D and the SAS scores were 0.82 and 0.78, and 0.88, respectively. Twenty age- and sex-matched subjects were prepared as a health control group. Plasma concentrations of HVA and MHPG were analyzed by high-performance liquid chromatography with electrochemical detection (HPLC-ECD) before and 4 and 8 weeks after risperiodne administration. Plasma levels of BDNF were assayed with sandwich ELISA methods. The

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plasma was quickly separated in a centrifuge and stored at −80°C until assayed. The plasma HVA and MHPG levels were analyzed by HPLC-ECD according to the method described previously (Yoshimura *et al.*, 2005)

The plasma BDNF levels were measured using a BDNF Emax Immunoassay Kit (Promega, Madison, WI, USA) according to the method described previously (Yukimasa *et al.*, 2006). The protocol of this study was approved by the Ethics Committee of the University of Occupational and Environmental Health. Written informed consent was obtained from all subjects.

STATISTICAL ANALYSIS

The Pearson's correlation coefficient was calculated to investigate the relationship between the changes in the plasma levels of HVA and MHPG and the changes in the YMRS scores. Repeated measures of ANOVA were used to compare the YMRS scores, SAS scores, and plasma levels of HVA and MHPG at each point. Bonferroni's test was carried out in the *post hoc* analysis. Mann-Whitney *u*-test was used to compare the serum BDNF levels. The level of significance for all analysis was set at $p < 0.05$.

RESULTS

The mean scores of the YMRS were 22, 18, 12, 8, and 5 at time points before and 1, 2, 3, and 4 weeks after the risperidone administration, respectively (Table 1). The mean scores of the Ham-D were 24, 25, 21, 21, and 19 at time points before and 1, 2, 3, and 4 weeks after the risperidone administration, respectively (Table 1). The mean SAS scores were 1.5 and 2.0 before and 4 weeks after risperidone administration, respectively. The mean plasma HVA levels before risperidone administration in control, bipolar manic, and bipolar depressed subjects were 8.4, 11.9, and 7.9 ng/mL, respectively, and mean plasma MHPG levels before risperidone administration in those subjects were 6.9, 12.6, and 6.0 ng/mL, respectively

Table 1. Changes in scores of YMRS and Ham-D

	B	1w	2w	3w	4w
YMRS	22±5	18±3	12±4*	8±5*	5±4*
Ham-D	24±6	25±4	21±5	21±6	19±5

*$p < 0.01$, compared with B.
B: before; 1w, 2w, 3w, 4w: 1, 2, 3, and 4 weeks after risperidone administartion.

Table 2. Plasma levels of catecholamine metabolites before risperidone administration

	Control	BP(M)	BP(D)
HVA (ng/mL)	8.4.±3.1	11.9±3.9*	7.9±2.8
MHPG (ng/mL)	6.9±2.4	12.6±4.3**	6.0±3.6

*$p < 0.05$, compared with control or BP(D).
**$p < 0.01$, compared with control or BP(D).
BP(M), bipolar manic patients, BP(D), bipolar depressed patients.

(Table 2). The plasma levels of HVA were 11.9, 8.3, and 8.1 at time points before and 4 weeks and 8 weeks after risperidone administration, respectively (Table 3). On the other hand, plasma levels of MHPG were 12.6, 10.6, and 9.8 ng/mL before 4 weeks and 8 weeks after risperidone administration, respectively (Table 3). No significant correlation was found between the changes the changes in YMRS scores (before and 4 weeks after risperidone administration) and the changes in plasma HVA or MHPG (before and 4 weeks after risperidone administration). The plasma levels of HVA or MHPG were not changed in depressive patients (data not shown). The plasma levels of BDNF were significantly decreased in depressive patients compared with manic patients or healthy controls (depressive patients; 16.1 pg/mL, manic patients; 24.3 pg/mL, control; 25.4 pg/mL). However, the administration of risperidone did not alter serum BDNF levels in either depressive or manic patients (Figure 1).

DISCUSSION

In the present study, we found that risperidone used in combination with mood stabilizers such as lithium or valproic acid is effective in the treatment of acute mania without worsening the extrapyramidal side effects. Plasma levels of HVA and MHPG were significantly higher in bipolar manic group that those in bipolar depressed or control group. We also found that risperidone combined with mood stabilizers

Table 3. Changes in plasma catechomamine metabolite in bipoar manic patients

	B	4w	8w
HVA (ng/mL)	11.9±3.9	8.3±3.6*	8.1±3.8*
MHPG (ng/mL)	12.6±3.9	10.6±1.6	9.8±2.5**

*$p < 0.05$, compared with B.
**$p < 0.05$, compared with B.
B: before; 4w, 8w: 4 weeks, and 8 weeks after risperidone administartion.

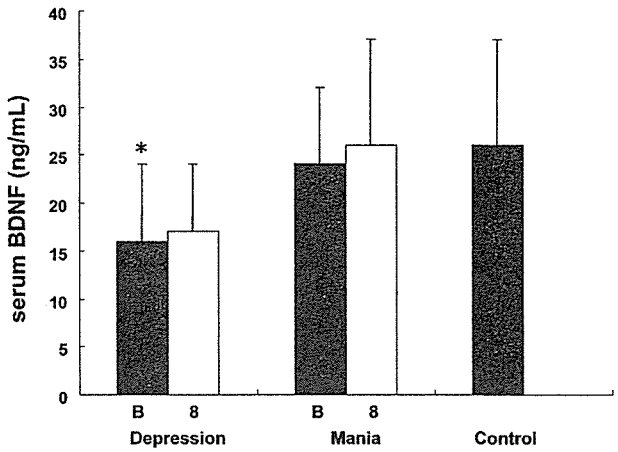

Figure 1. Comparison among depression, mania, and control group with regard to the serum BDNF levels. * < 0.05, compared with bipolar mania and control.

significantly decreased the plasma levels of HVA and MHPG. A trend was found between the changes in YMRS scores and the changes in plasma levels of HVA or MHPG. These results suggest that risperidone combined with mood stabilizers improves manic symptoms by means of its effect on catecholaminergic neurons. In other words, risperidone with mood stabilizers reduced the activity in catecholaminergic neurons in manic patients. Previously, we demonstrated that risperidone decreased the plasma levels of HVA in schizophrenic patients in the acute stage, thereby improving the positive symptoms of schizophrenia, and then increased the plasma levels of MHPG, thereby ameliorating the negative symptoms of schizophrenia, suggesting that the reduction of the dopaminergic neurons by risperidone is related to the improving the positive symptoms and the activation of the noradrenergic neurons by risperidone is related to the improving negative symptoms in schizophrenic patients. Risperidone is associated with decreases in HVA and increases in MHPG in schizophrenic patients; this dose not indicate necessarily that risperidone causes these changes, which could be the result in the change in the state of schizophrenia, but not necessarily related to risperidone *per se*. Moreover, the statement in the reduction of the dopaminergic neurons by risperidone related to the improving the positive symptoms and the activation of the noradrenergic neurons by risperidone is related to the improving negative symptoms in schizophrenic patients is not warranted. The present findings are in accordance with previous studies (Vieta *et al.*, 2002, 2003; Yatham *et al.*, 2003a) in terms of responder rates defined as a YMRS score reduction $\geq$50% at endpoint and euthymia criteria (YMRS ≤ 7; eight were responders and seven fulfilled the euthymia criteria; Baldessarini, 2003). Furthermore, the YMRS score was significantly decreased by 46% from baseline to week 2 and this trend was observed throughout our study. This rapid reduction of YMRS was in agreement with previous controlled and uncontrolled studies (Sachs *et al.*, 2002; Vieta *et al.*, 2002; Yatham *et al.*, 2003a,b). The mean daily dose of risperidone in manic patients was 3.8 ± 1.1 mg/day in the present study, which is similar to a previous open study by Yatham *et al.* (2003b). With regard to the catecholamine metabolites in manic patients, several reports have demonstrated that pretreatment cerebrospinal fluid (CSF) HVA is elevated in some groups of manic patients (Banki, 1977; Bowers and Heninger, 1977; Vestergaard *et al.*, 1978; Swann *et al.*, 1983). Mazure *et al.* (1998) reported a significant relationship between elevated pretreatment plasma HVA and the response to antipsychotic drugs in manic patients. The finding of the present study that the plasma HVA levels in responders to risperidone treatment are significantly higher than those in nonresponders to risperidone treatment (responders; 13.3 ± 3.1 ng/mL, nonresponders; 10.4 ± 2.8 ng/mL) are in accordance with those in Mazure and Bowers (1998). As for plasma MHPG levels, responders to risperidone treatment were also higher than non-responders (responders; 14.1 ± 4.0 ng/mL, nonresponders; 11.6 ± 3.2 ng/mL). Risperidone monotherapy or risperidone with mood stabilizers did not improve depressive symptoms in bipolar disorder. In addition, risperidone with or without mood stabilizers did not alter plasma levels of catecholamine metabolites in bipolar depressed

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patients. On the other hand, McIntyre *et al.* (2004) reported that total mean Ham-D scores significantly decreased from 17 to 5 by 6 months in the risperidone-treated group and from 10 to 7 in the olanzapine-treated group adjunctive to either lithium or divalproex. The authors suggested that adjunctive administration of either agent might reduce the severity of depressive symptoms. We also demonstrated that serum BDNF levels in depressive patients were lower than those in manic patients or normal controls. Karege *et al.* (2002a) reported that the serum BDNF levels were significantly decreased in antidepressant-free depressed patients, and that the serum BDNF levels were negatively correlated with the Montgomery-Asberg Depression Rating Scale. Shimizu *et al.* (2003) also demonstrated that serum BDNF was significantly lower in an antidepressant-naïve group than in either a treated or in a control group, and that there was a significant negative correlation between serum BDNF and Ham-D scores in all patients. Furthermore, they reported preliminary findings that decreased serum BDNF levels in antidepressant-naïve patients recovered to normal levels in association with lower Ham-D scores after treatment with antidepressant medication. Although BDNF is highly concentrated in the brain, it is also present in the plasma and serum. The source of circulating BDNF remains unknown. Platelets, brain neurons, and vascular endothelial cells are considered to be candidate sources. Previously, it was reported that BDNF could cross the blood-brain barrier (Pan *et al.*, 1998), and that BDNF levels in the brain and serum underwent similar changes during the maturation and aging process in rats (Karege *et al.*, 2002b), indicating that plasma BDNF levels might in part reflect the BDNF levels in the brain. However, the serum BDNF levels were not altered 4 weeks after the risperidone treatment. The results are not contradicted by the finding that depressive symptoms were not improved after the risperidone treatment.

We are aware of the limitations of this study, in that plasma catecholamine metabolite, BDNF, cytokines appear to derive only in part from central sources (Yoshimura *et al.*, 2003, 2004, 2005); in other words, plasma levels of catecholamine metabolites and BDNF only partially reflect the activities of the neurons in the brain. In addition, our sample size was very small and heterogeneous, we used an open flexible dose regime of risperidone, and most patients had been treated with lithium or valproic acid. The fact that the majority of patients in this study received concomitant treatment with lithium or valproic acid is a potential confound, as mood stabilizer treatment has been associated with decreased levels of catecholamine metabolites in manic patients (Swann *et al.*, 1987). The same would have been true had a difference been detected in BDNF levels with treatment (Hashimoto *et al.*, 2004). Furthermore, we did not examine the effects of risperidone on plasma catecholamine metabolites in healthy control. Then, there are two possibilities redarding changing the plasma levels of catecholamine metabolites after risperidone treatment. One possibility is that risperidone itself decreased the plasma levels of HVA and MHPG, another possibility is that recovering from manic state brought the decrease of plasma levels of catecholamine metabolites. Finally, we performed Mann-Whitney *u*-test comparing serum BDNF levels between in bipolar depressed and bipolar manic or control group. When we used multiple comparisons, no differences were found in serum BDNF levels among the three groups. Further studies are therefore warranted to confirm our preliminary results.

In conclusion, the reduction of the plasma catecholamine metabolites might be related to the antimanic effect of risperidone with valproic acid or lithium. In addition, the antidepressive effect of risperidone with valproic acid or lithium was not elucidated in bipolar patients.

REFERENCES

American Psychiatric Association. 2002. Practice Guideline for the Treatment of patients with Bipolar Disorder (revision). *Am J Psychiatry* **159**(suppl 4): 1–50.

Baldessarini RJ. 2003. Assessment of treatment response in mania: commentary and new findings. *Bipolar Disord* **5**: 79–84.

Banki CM. 1977. Correlation between cerebrospinal amine metabolites and psychomotor activity in affective disorders. *J Neurochem* **28**: 255–257.

Bowden CL, Myers JE, Grossman F, Xie Y. 2004. Risperidone in combination with mood stabilizers: a 10-week continuation phase study in bipolar I disorder. *J Clin Psychiatry* **65**: 707–714.

Bowers MB, Jr. Heninger GR. 1977. Lithium: clinical effects and cerebrospinal fluid acid monoamine metabolites. *Community Psychopharmacol* **1**: 135–145.

Brambilla P, Barale F, Soares JC. 2003. Atypical antipsychotics and mood stabilization in bipolar disorder. *Psychopharmacology* **166**: 315–332.

Ghaemi SN, Sachs GS. 1997. Long-term risperidone treatment in bipolar disorder: 6-month follow up. *Int Clin Psychopharmacol* **12**: 333–338.

Hashimoto K, Shimizu E, Iyo M. 2004. Critical role of brain-derived neurotrophic factor in mood disorder. *Brain Res Brain Res Rev* **45**: 104–114.

Jacobsen FM. 1995. Risperidone in the treatment of affective illness and obsessive-compulsive disorder. *J Clin Psychiatry* **56**: 423–429.

Kakihara S, Yoshimura R, Shinkai K, *et al.* 2005. Prediction of response to risperidone treatment with respect to plasma concentrations of risperidone, catecholamine metabolites, and poly-

morphism of cytochrome P450 2D6. *Int Clin Psychopharmacol* **20**: 71–78.

Karege F, Schwald M, Cisse M. 2002a. Postnatal development profile of brain-derived neurotrophic factor in rat brain and platelets. *Neurosci Lett* **328**: 261–264.

Karege F, Perret G, Bondolfi G, Schwald M, Bertschy G, Aubry JM. 2002b. Decreased serum brain-derived neurotrophic factor levels in major depressed patients. *Psychiatry Res* **109**: 143–148.

Licht RW, Bysted M, Christensen H. 2001. Fixed-dosed risperidone in mania: an open experimental trial. *Int Clin Psychopharmacol* **16**: 103–110.

Mazure CM, Bowers MB. 1998. Pretreatment plasma HVA predicts neuroleptic response in manic psychosis. *J Affect Disord* **48**: 83–86.

Mclntyre RS, Mancini DA, Srinivasan J, McCann S, Konarski JZ, Kennedy SH. 2004. The antidepressant effects of risperidone and olanzapine in bipolar disorder. *Can J Pharmacol* **11**: 218–226.

Miller DS, Yatham LN, Lam RW. 2001. Comparative efficacy of typical and atypical antipsychotics add-on therapy to mood stabilizers in the treatment of acute mania. *J Clin Psychiatry* **62**: 975–980.

Muscettola G, Barbato G, Pampallona S, Casiello M, Bollini P. 1999. Extrapyramidal symptoms in neuroleptic-treated patients: prevalence, risk factors, and association with tardive dyskinesia. *J Clin Psychopharmacol* **19**: 203–208.

Pan W, Banks WA, Fasold MB, Blush J, Kastin AJ. 1998. Transport of brain-derived neurotrophic factor across the blood-brain barrier. *Neuropharmacology* **37**: 1553–1561.

Sachs GS, Printz DJ, Kahn DA, Carpenter D, Docherty JP. 2000. The Expert Consensus Guideline Series: Medication Treatment of Bipolar Disorder. *Postgard Med Apr.* (spec no.): 1–104.

Sachs GS, Grossman F, Gheami SN, Okamoto A, Bowden CL. 2002. Combination of a mood stabilizer with risperidone or haloperidol for treatment of acute mania: a double-blind, placebo-controlled comparison of efficacy and safety. *Am J Psychiatry* **159**: 1146–1154.

Sajatovic M, DiGiovanni SK, Bastani B, Hattab H, Ramirez LF. 1996. Risperidone therapy in treatment refractory acute bipolar and schizoaffective mania. *Psychopharmacol Bull* **32**: 55–61.

Segal J, Berk M, Brook S. 1998. Risperidone compared with both lithium and haloperidol in mania: a double-blind randomized controlled trial. *Clin Neuropharmacol* **21**: 176–180.

Shelton RC, Stahl SM. 2004. Risperidone and paroxetine given singly and in combination for bipolar depression. *J Clin Psychiatry* **65**: 1715–1719.

Shimizu E, Hashimoto K, Okuma N, *et al.* 2003. Alterations of serum levels of brain-derived neurotrophic factor (BDNF) in depressed patients with or without antidepressants. *Biol Psychiatry* **54**: 111–114.

Swann AC, Secunda S, Davis JM, *et al.* 1983. CSF monoamine metabolites in mania. *Am J Psychiatry* **140**: 396–400.

Swann AC, Koslow SH, Katz MM, *et al.* 1987. Lithium carbonate treatment of mania: cerebrospinal fluid and urinary monoamine metabolites and treatment response. *Arch Gen Psychiatry* **44**: 345–354.

Vestergaard P, Sorensen T, Hoppe E, Rafaelsen OJ, Yates CM, Nicolou N. 1978. Biogenic amine metabolites in cerebrospinal fluid of patients with affective disorders. *Acta Psychitr Scand* **58**: 88–96.

Vieta E, Herraiz M, Parramon G, Goikolea JM, Fernandez A, Benabarre A. 2002. Risperidone in the treatment of mania: efficacy and safety eresults from a large, multicenter, open study in Spain. *J Affect Disord* **72**: 15–19.

Vieta E, Goikolea JM, Olivares JM, Gonzales-Pinto A, Rodriguez A, Colom F. 2003. 1-year follow-up patients treated with risperidone and topiramate for a manic episode. *J Clin Psychiatry* **64**: 834–839.

Yatham LN, Grossman F, Augustyns I, Vieta E, Ravindran A. 2003a. Mood stabilizers plus risperidone or placebo in the treatment of acute mania: international, double-blind, randomized controlled trial. *Br J Psychiatry* **182**: 141–147.

Yatham LN, Binder C, Riccardelli R, *et al.* 2003b. Risperidone in acute and continuation treatment of mania. *Int Clin Psychopharmacol* **18**: 227–235.

Yoshimura R, Nakamura J, Ueda N, Terao T. 2000a. Effect of risperidone on plasma free 3-methoxy-4-hydroxyphenylglycol (pMHPG) levels in schizophrenia patients: relationship among plasma concentrations of risperidone and 9-hydroxyrisperidone, pMHPG levels, and clinical improvement. *Int Clin Psychopharmacol* **15**: 175–180.

Yoshimura R, Yanagihara N, Hara K, *et al.* 2000b. Inhibitory effects of clozapine and other antipsychotic drugs on noradrenaline transporters in cultured bovine adrenal medullary cells. *Psychopharmacology* **149**: 17–23.

Yoshimura R, Ueda N, Shinkai K, Nakamura J. 2003. Plasma levels of homovanillic acid and the response to risperidone in first episode untreated acute schizophrenia. *Int Clin Psychopharmacol* **18**: 107–111.

Yoshimura R, Nakamura J, Shinkai K, Ueda N. 2004. Clinical response to antidepressant treatment and 3-methoxy-4-hydroxyphenylglycol levels: mini review. *Prog Neuropsychopharmacol Biol Psychiatry* **28**: 611–616.

Yoshimura R, Nakamura J, Shinkai K, *et al.* 2005. An open study of risperidone liquid in the acute phase of schizophrenia. *Hum Psychopharmacol* **20**: 243–248.

Yukimasa T, Yoshimura R, Tamagawa A, *et al.* 2006. High-frequency repetitive transcranial magnetic stimulation improves refractory depression by influencing catecholamine and brain-derived neurotrophic factors. *Pharmacopsychiatry* **39**: 52–59.

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Druckerei Sommer

T. Yukimasa[1]
R. Yoshimura[1]
A. Tamagawa[2]
T. Uozumi[2]
K. Shinkai[1]
N. Ueda[1]
S. Tsuji[2]
J. Nakamura[1]

High-Frequency Repetitive Transcranial Magnetic Stimulation Improves Refractory Depression by Influencing Catecholamine and Brain-Derived Neurotrophic Factors

Original Paper

Introduction: Repetitive transcranial magnetic stimulation (rTMS) is a non-invasive and easily tolerated method of altering cortical physiology. To date, numerous open and sham controlled clinical trials have explored the antidepressant potential of rTMS. In the present study, we investigated clinical trials of high-frequency rTMS (20 Hz) for treatment of refractory depression, and also examined the effect of rTMS on plasma levels of catecholamine metabolites and brain-derived neurotropic factor (BDNF). **Methods:** Twenty-six depressed inpatients who met the DSM-IV criteria for major depressive disorder and had failed to respond to treatment with at least two antidepressant drugs given at adequate doses (above 150 mg/day in an equivalent dose of imipramine) and durations (at least 4 weeks for each drug) were enrolled in this study. Eleven were males, 15 females. The ages of the subjects ranged from 19 to 78 years old (mean ± SD = 52.9 ± 17.8). All patients were administered left prefrontal 20 Hz rTMS at 80% MT (total 800 pulses a day) over ten daily sessions. The plasma levels of 3-methoxy-4-hydroxyphenylglycol (MHPG) and homovanillic acid (HVA) were analyzed by high-performance liquid chromatography. The plasma levels of BDNF were also measured with the sandwich ELISA method. **Results:** The mean 17-item Hamilton Rating Scale for Depression (Ham-D) score of 20.5 ± 5.2 before rTMS was significantly decreased to 15.6 ± 7.3 after rTMS. Nine of 26 patients (35%) demonstrated some improvement (Ham-D .25%) by rTMS. The levels of plasma MHPG, but not those of HVA, were significantly reduced after rTMS treatment, and a negative correlation was observed between the change in plasma MHPG levels and the change in scores of agitation. In addition, the plasma levels of BDNF were significantly increased by 23% in responders and partial responders, but not in nonresponders, after rTMS treatment, and a trend for association was found between the changes in Ham-D scores and changes in plasma BDNF levels in all patients after rTMS treatment. **Conclusion:** These results suggest that rTMS treatment brings about some improvement in refractory depression, especially for symptoms such as agitation, by influencing MHPG and BDNF, which is in accordance with previous reports showing that BDNF was increased by various antidepressants treatments.

Introduction

Depression has a lifetime prevalence estimated to be between 1.5–19% [48], and depression, like anxiety disorder, is one of the most common of all psychiatric disorders. Despite the administration of many kinds of antidepressants and various kinds of psychotherapy, treatment failures occur because of the delayed onset of efficacy or because of intolerable side effects. Unlike psychotherapy or drug treatment, electroconvulsive therapy (ECT) has a short onset latency and has been used for patients with serious or treatment-resistant depression [8]. However, it requires anesthetic agents and seizure induction, and can some-

Affiliation
[1] Department of Psychiatry, University of Occupational and Environmental Health, 1-1 Iseigaoka, Yahatanishi-ku, Kitakyushu, Fukuoka 807-8555, Japan
[2] Department of Neurology, University of Occupational and Environmental Health, 1-1 Iseigaoka, Yahatanishi-ku, Kitakyushu, Fukuoka, 807-8555, Japan

Correspondence
Reiji Yoshimura · Department of Psychiatry · University of Occupational and Environmental Health · 1-1 Iseigaoka, Yahatanishi-kuKitakyushu · Fukuoka 807-8555 · Phone: +81 93 691 7253 · Fax: +81 93 692 4894 · E-Mail: yoshi621@med.uoeh-u.ac.jp

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Bibliography
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times cause memory impairment [32]. Repetitive transcranial magnetic stimulation (rTMS) has the advantages of not requiring anesthesia and of not inducing seizures and the associated memory loss. In addition, it allows better control of stimulus frequency and location [4]. rTMS is a neurologic and psychiatric research tool that has gained attention in recent years for its potential application as a treatment not only for neurological disorders, but also for psychiatric disorders such as depression, schizophrenia, and obsessive-compulsive disorder [18]. The initial application of high-frequency (20 Hz) rTMS over the left prefrontal cortex in depressed patients resulted in promising findings [12,20]. Subsequent sham-controlled studies of daily left prefrontal high-frequency rTMS have demonstrated significant clinical improvement in depressed patients [7,16]. In general, high-frequency (3–20 Hz) rTMS is considered to increase cortical excitability and metabolism, whereas low-frequency (≤ 1 Hz) stimulation does the opposite [18]. Current data support the antidepressant effects of excitatory stimulation to the left prefrontal cortex. According to the review of Gershon et al. [21], 41% of 139 patients treated with high-frequency rTMS to the left prefrontal cortex achieved either a 50% decrease in their Hamilton Rating Scale for Depression (Ham-D) scores or a final score under 8. More recently, rTMS has been studied mainly to assess its putative therapeutic effects in the treatment of refractory depression [31]. Although the mechanism through which rTMS may exert therapeutic effects in depression has also been studied in a number of animal models by investigating plasticity and changes in cortical activity [13], it remains to be fully elucidated.

The original catecholamine hypothesis of depression postulated that depression is characterized by a deficiency of functional noradrenaline. Recently, this hypothesis has been tempered and modified to place more emphasis on possible disturbances in the regulation of the catecholaminergic systems as a phase affective/arousal system. A dysregulated noradrenergic system may contribute significantly to the vegetative and anxiety-related symptoms of depression [3,44]. Previously, we proposed that depression might be dichotomized into two groups using plasma levels of 3-methoxy-4-hydroxyphenylglycol (MHPG), a major metabolite of noradrenaline [52]. Recently, many animal studies and several human studies have demonstrated that brain-derived neurotrophic factor (BDNF) plays important roles in the pathophysiology of depression. Several lines of evidence indicate that the expression of BDNF might be a downstream target of a variety of antidepressant treatments or ECT [1,22,41].

In the present study, we performed a clinical trial of high-frequency rTMS (20 Hz) for treatment of refractory depression. Moreover, we clarified the effects of rTMS on catecholaminergic systems by analyzing the plasma levels of 3-methoxy-4-hydroxyphenylglycol (MHPG) and homovanillic acid (HVA). In addition, we also investigated the effects of rTMS on plasma BDNF levels. We found that rTMS brings some improvement to persons with antidepressant-resistant depression. A surprising result was a reduction in the plasma MHPG levels and an increase in the plasma BDNF levels after rTMS, and these effects were found to be related to the improvement of the depressive symptoms.

Subjects and methods

Twenty-six depressed inpatients at a psychiatric ward in our university hospital who met the DSM-IV criteria for major depressive disorder, and who were significantly symptomatic despite being on medications, or who were unable or unwilling to try additional medications, were enrolled in this study. The MINI International Neuropsychiatric Interview was used to confirm the DSM-IV diagnosis of a major depressive disorder and rule out combined conditions such as anxiety disorder or personality disorder. The subjects had to have failed to respond (below 50% in their Ham-D score) to at least two prior medication trials judged to be of adequate duration (at least 4 weeks for each drug) and with adequate dosages (above 150 mg/day in an equivalent dose of imipramine). These determinations were made in conference between the first author (T.Y.) and the current treating psychiatrist. Eleven were males and 15 females. A score of at least 15 on the 17-item Ham-D was needed for a subject to be admitted to the study. The ages of the subjects ranged from 19 to 78 years (mean ± SD = 52.9 ± 17.8). All patients were physically healthy and none had a history of alcohol and/or drug abuse. Patients with a history of epilepsy, neurosurgery, and cardiac pacemaker implantation were excluded. A Nihon Koden magnetic stimulator AAA-81077 with a figure-eight coil YM-111B was used in this study. All patients were administered left prefrontal 20 Hz rTMS at 80% MT (total 800 pulses a day) over ten daily sessions. The left prefrontal cortex area was considered to be 5 cm in front of the left motor cortex area of the abductor pollicis brevis muscle. Each patient was assessed to determine his or her motor magnetic threshold at rest only before treatment. The motor threshold was defined as the lowest stimulus intensity capable of producing motor evoked potentials (MEP) of 50 V in the relaxed abductor pollicis brevis muscle in at least 5 to 10 consecutive trials. The motor evoked potentials were collected by electromyographic devices (Neuropack, Nihon Koden). Eighty percent of the individual patient motor threshold was then administered on the left dorsolateral prefrontal cortex within a two-week period. Each patient was evaluated every week during the rTMS treatment by one experienced psychiatrist (T.Y.) using the Ham-D. The psychiatrist assessing the Ham-D was blind to the results of the test of plasma levels of catecholamine metabolites and BDNF. All patients continued to receive their preexisting antidepressants or mood stabilizers at the same dosages from 3 weeks before to 3 weeks after the rTMS treatment. We defined the patients with a 50% or more decrease in their Ham-D score as responders, the patients with a 25–49% decrease in their Ham-D score as partial responders, and the remaining patients as nonresponders comparing the score of Ham-D at the two points, just before the first rTMS treatment and a week after the last rTMS treatment.

All blood samples were taken at 7:00 am before breakfast (at least 12 hours after the last medication) before and after the rTMS treatment. Fifteen milliliters of venous blood was drawn from the patient in the supine position, after the patient had been lying at rest overnight. The plasma samples were quickly separated in a centrifuge (2000 g, 10 min, 4°C) and stored at –80°C until assay. The plasma HVA levels were analyzed by high-performance liquid chromatography with electrochemical detection (HPLC-ECD) according to the method of Yeung et al. [50]

with slight modification [53]. In short, each cyano-bonded solid-phase extraction cartridge was preconditioned with methanol and then glass-distilled water. To each cartridge were added 0.3 mL of plasma sample or standard, and 0.1 mL of working internal standard solution (5 ng of 5-hydroxyindoleacetic acid in 0.01 M KH_2PO_4, pH 7.2). The samples were deproteinized with 1 mL of acetonitrile. After mixing by vortex and centrifugation (1760 g, 4 °C for 10 min), an aliquot (5 μL) of supernatant was allowed to pass through the cartridge slowly under a mild vacuum (15 mmHg). The cartridge was washed with 0.2 mL of distilled water, extracted with 1 mL of ethyl acetate, and then an aliquot was evaporated to dryness under nitrogen gas. After dissolving in the mobile phase (200 μL), a 10 μL portion of this solution was injected into the HPLC. The plasma MHPG levels were analyzed according to the method of Minegishi and Ishizaki [34]. In brief, plasma was separated by centrifugation at 2000 g, 10 min at 4 °C. Extraction was performed under a vacuum using Bond-Elut columns prepacked with 100 mg of C18-bonded silica (40 μm) in a 1 mL capacity disposable syringe. The columns, which were inserted into a vacuum chamber connected to an aspirator, were prepared by washing with 1 mL of methanol followed by 1 mL of water. After the addition of 50 μL of a solution of vanillyl alcohol (internal standard equivalent to 5 ng/mL) to 1 mL of plasma, the samples were applied to and passed through the columns, followed by 0.75 mL of water to rinse off both residual samples and easily eluted hydrophilic compounds. The adsorbed materials were eluted with 200 μL of methanol to a 0.1 M phosphate buffer (pH 4.8) mixture (40 : 60, v/v). A 20 μL portion of this solution was injected into the HPLC. The plasma BDNF levels were measured using a BDNF Emax Immunoassay Kit (Promega, Madison, WI, USA) according to the manufacturer's instructions. In short, 96-well microplates were coated with anti-BDNF monoclonal antibody and incubated at 4 °C for 18 hours. The plates were incubated in a blocking buffer for 1 hour at room temperature. The samples diluted with assay buffer by 100-times and BDNF standards were kept at room temperature under conditions of horizontal shaking for 2 hours, followed by washing with the appropriate washing buffer. The plates were incubated with antihuman BDNF polyclonal antibody at room temperature for 2 hours and washed with the washing buffer. The plates were then incubated with anti-IgY antibody conjugated to horseradish peroxidase for 1 hour at room temperature, and incubated in peroxidase substrate and tetramethylbenzidine solution to induce a color reaction. The reaction was stopped with 1 mol/L hydrochloric acid. The absorbance at 450 nm was measured with an Emax automated microplate reader. Measurements were performed in duplicate. The standard curve was linear from 5 to 5000 pg/mL, and the detection limit was 10 pg/mL. Cross-reactivity to related neurotrophins (NT-3, NT-4, NGF) was less than 3%. Intra- and interassay coefficients of variation were 5 and 7%, respectively. The recovery rate of the exogenous added BDNF in the measured plasma samples was more than 95%.

This study was approved by the ethics committee of the University of Occupational and Environmental Health, and written informed consent was obtained from all participants.

Statistical analysis

The plasma levels of MHPG, HVA, and BDNF before and after rTMS were compared using the Wilcoxon signed-rank test. Spearman's rank correlation coefficient was calculated to investigate the relationship between the changes in the plasma levels of MHPG, HVA, and BDNF and the HAM-D improvement rates. The Mann–Whitney's U test was used to compare the response group and the non-response group with respect to the pretreatment plasma levels of MHPG, HVA, and BDNF, and age. The chi-square test was performed to compare the response group and the nonresponse group with respect to gender. All data were expressed as mean ± standard deviation, and a P-value below 0.05 was considered significant.

Results

All patients were treated with preexisting antidepressants or mood stabilizers for at least 4 weeks or more as follows : 7 patients with milnacipran, 5 patients with paroxetine, 5 patients with sulpiride, 5 patients with lithium carbonate, 5 patients with fluvoxamine, 4 patients with mianserin, 2 patients with amoxapine, 2 patients with imipramine, 2 patients with trazodone, 1 patient with sodium valproate, 1 patient with clomipramine, and 1 patient with maprotiline (Table 1). The patients had been given prior treatments with 2–6 (mean ± SD = 3.1 ± 1.3) antidepressants or mood stabilizers in adequate dosages and for an adequate duration. The number of prior episodes the patients had suffered ranged from 2 to 6 (mean ± SD = 2.3 ± 2.0), and the mean duration of the current episode ranged from 4–36 months (mean ± SD = 16.2 ± 15.9). Next, rTMS was performed (in addition to the antidepressants or mood stabilizers). The mean Ham-D score of 20.5 ± 5.2 before treatment was significantly decreased to 15.6 ± 7.3 at 2 weeks after rTMS treatment. Five of 26 patients showed an improvement of 50% or higher on Ham-D scores (responders), 4 patients showed an improvement of 25–49% on Ham-D scores (partial responders), and the remaining 17 patients demonstrated an improvement of below 25% (nonresponders). Finally, 7 of 26 patients (27%) achieved remission (Ham-D score < 8).

Table 1 Medications used and the number of patients treated

Antidepressants/Mood stabilizers	*Number of Patients*
milnacipran	7
paroxetine	5
sulpiride	5
lithium carbonate	5
fluvoxamine	5
mianserin	4
amoxapine	2
imipramine	2
trazodone	2
sodium valproate	1
clomipramine	1
maprotiline	1

Some patients are treated with more than two drugs.

No statistically significant differences were observed between responders plus partial responders and nonresponders with respect to sex, age, and the number of patients who had only one depressive episode (first depressive episode) or Ham-D score before rTMS treatment and plasma levels of MHPG and BDNF before rTMS treatment (Tables **2**, **3**). There was no significant correlation between the plasma levels of BDNF, MHPG, or HVA and age or body weight. No significant difference was observed between males and females with respect to the plasma levels of BDNF, MHPG, or HVA (data not shown). No associations were found between changes in the total score of Ham-D and changes in the plasma MHPG or HVA levels. However, on the individual items of the Ham-D, a negative correlation was observed between the changes in the plasma MHPG levels and the changes in the scores of agitation on the Ham-D before and 2 weeks after rTMS (Table **4**).

The plasma levels of MHPG were significantly reduced 2 weeks after rTMS treatment (before: 8.27 ± 5.98 ng/mL, after: 5.69 ± 4.70 ng/mL) (Fig. **1A**). However, there was no significant change in the plasma levels of HVA between before and 2 weeks after the rTMS treatment in all patients (before: 6.71 ± 5.49 ng/mL, after: 5.46 ± 5.17 ng/mL) (Fig. **1B**). The plasma levels of HVA in responders were significantly higher than those in nonresponders (Fig. **2**), though no correlations between the changes in plasma HVA and the changes in the scores of each item of Ham-D were observed. There was a trend for increasing plasma BDNF levels in all patients 2 weeks after rTMS treatment (before; 2.53 ± 2.01 ng/mL, after; 3.11 ± 2.00 ng/mL; $P < 0.1$). In particular, the plasma BDNF levels were increased 2 weeks after rTMS treatment in the responders and partial responders (before; 2.35 ± 1.47 ng/mL, after; 3.87 ± 2.13 ng/mL; $P < 0.05$), while, no significant increase was observed in nonresponders (before; 2.46 ± 2.16 ng/mL, after; 2.60 ± 1.84 ng/mL; n.s.), and there were no differences between the two groups in plasma BDNF levels before rTMS treatment (Fig. **3**). Finally, a trend for association was found between changes in Ham-D scores and changes in plasma BDNF levels in all patients before and 2 weeks after rTMS treatment ($\rho = 0.340$, $P < 0.1$) (Fig. **4**). No correlation was found between stimulation intensity and changes in the plasma levels of MHPG, HVA, and BDNF or changes in the Ham-D score (data not shown).

Table **4** Correlation between the changes in plasma MHPG levels and the changes in scores on each item of Ham-D

Items	*ρ Values*	*P Values*
Depressive mood	−0.02	0.76
Feeling of guilt	0.4	0.23
Suicide	0.14	0.84
Insomnia (early/middle/late)	0.17	0.5
Work and Interest	0.04	0.83
Retardation	0.06	0.6
Agitation	−0.08	0.03*
Anxiety (psychic)	−0.1	0.47
Anxiety (somatic)	0.13	0.96
Somatic symptoms (gastrointestinal)	0.03	0.39
Somatic symptoms (gastrointestinal)	0.07	0.28
Genital symptoms	0.21	0.68
Hypochondriasis	0.2	0.53
Loss of weight	0.18	0.73
Insight	0.29	0.67

* $P < 0.05$ significant correlation.

Discussion

The main findings of the present study are that nine out of twenty-six treatment-resistant patients (35%) at least partially responded to rTMS treatment, and plasma levels of MHPG were significantly decreased in all patients after rTMS treatment. A negative correlation was observed between the change in plasma MHPG levels and the change in the Ham-D scores with regard to agitation. In addition, the plasma BDNF levels were increased in the responders and partial responders by 23%, but not in the nonresponders after rTMS treatment, and a trend for association was found between the changes in the Ham-D score and BDNF

Table **2** Characteristics of the patients

Responders (n = 9)	5	4	48.9 ± 20.8	3	21.0 ± 5.5
Nonresponders (n = 17)	6	11	54.1 ± 16.5	9	19.6 ± 4.9

Ham-D: 17-item Hamilton Rating Scale for Depression.

Table **3** Plasma catecholamine metabolites before and after rTMS

	pMHPG (ng/mL)		*pHVA (ng/mL)*		*BDNF (ng/mL)*	
	Before	*After*	*Before*	*After*		
Responders + Partial responders	8.0 ± 3.7	*6.3 ± 5.1	9.2 ± 4.7	9.8 ± 6.5	2.35 ± 1.47	*3.87 ± 2.13
Nonresponders	8.4 ± 7.0	5.3 ± 4.6	5.4 ± 5.6	3.1 ± 2.1	2.61 ± 2.26	2.76 ± 1.90

* $P < 0.05$ before vs. after rTMS.

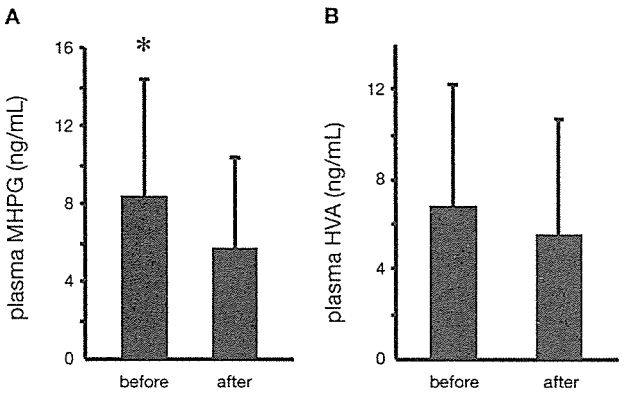

Fig. 1 (A) Plasma levels of MHPG before and after rTMS. Data are presented as the means ± SD. (B) Plasma levels of HVA before and after rTMS. Data are presented as the means ± SD. Plasma levels of MHPG and HVA were analyzed at one experiment in duplicate.

levels before and 2 weeks after rTMS treatment in all patients. No adverse side effects such as memory impairments or seizures were reported during or after rTMS treatment. To date, the clinical efficacy of rTMS treatment for depression has been well demonstrated by performing sham controlled studies [7,10,16,19]. In contrast, Couturier [9] recently reported from six small, but generally well-designed studies, that rapid-rate rTMS was no more efficacious than sham therapy in treating adults with a major depressive episode. Our result, in which only 35% of patients showed some improvement (Ham-D .25%) is in line with the results of the last meta-analysis performed by Couturier [9]. Recently, rTMS has been proposed and subsequently researched as a putative therapeutic approach for refractory major depression. In addition, Rumi et al. [40] demonstrated that rTMS is effective in accelerating the onset and augmenting the therapeutic response to amitriptyline for severe depressed patients by performing a double blind placebo-controlled study. In general, however, the response rates in rTMS for treatment-resistant depression remain low [17,20,38]. Psychotic symptoms have been reported to be a negative predictor for rTMS [21]. However, Fitzgerald et al. [14] performed a double-blind, placebo-controlled study in treatment-refractory depression and found that treatment for at least 4 weeks is necessary for clinically meaningful benefits to be achieved. The response rates in the present study were relatively low compared with those of other studies. In the present study, 4 out of 26 patients had experienced psychotic symptoms during the depressive phase. The response rates in patients with and those without psychotic features were 50 and 32%, respectively, indicating that the response rate in patients with psychotic features was not significantly different from that in those without psychotic features. One of the reasons why the response rate was lower in this study than in previous studies may have been the length of the treatment period; two weeks are not enough time to achieve an adequate response, as mentioned by Fitzgerald et al. [14].

With regard to the actions of rTMS on catecholamine systems, Keck et al. [27] reported that acute rTMS (20 Hz) of frontal brain regions leads to alterations in the mesolimbic and mesostriatal release patterns of dopamine *in vivo*. On the other hand, Ben-Shachar et al. [5] demonstrated that the dopamine content in the frontal cortex of TMS-treated rats was reduced by 26%, while the contents in the striatum and hippocampus were increased by 25 and 18%, respectively. It can be speculated that the rTMS-mediated dopamine release evident in preclinical trials may provide the underlying mechanism for both the poorer response of patients with psychotic depression and newly occurring psychotic symptoms during rTMS treatment. However, Ben-Shachar et al. [6] demonstrated that brain tissue monoamine levels were unchanged after 10 days of treatment with rTMS. In the present study, the plasma HVA levels were not changed between before and after rTMS treatment, and the plasma HVA levels in responders were significantly higher than those in nonresponders, though no correlations were found between the changes in plasma HVA and the changes in the total Ham-D score or individual items in the Ham-D. These results do not resolve the controversy over the effects of rTMS on the dopaminergic system. However, the plasma MHPG levels were significantly decreased after rTMS treatment, and were associated with the improvement of agitation in depressed patients. In a previous study [52], we considered that depressed patients might be dichotomized into two groups, one characterized by anxiety and/or the perception of powerlessness with high plasma MHPG levels, and another by psychomotor retardation with low plasma MHPG levels. We also found correlations between scores of agitation/anxiety in Ham-D and plasma levels of MHPG in 87 patients with major depressive disorder, indicating that depressed patients who revealed predominantly agitation/anxiety were characterized by higher plasma MHPG levels [52]. Eschweiler et al. [11] proposed that anxiety is a positive predictor for a successful clinical outcome after rTMS. Fitzgerald et al. [14] also reported that baseline psychomotor agitation predicted a successful response to rTMS. Taken together, these findings suggest that rTMS might be especially effective for depressed patients characterized by agitation/anxiety with high plasma MHPG levels. In fact, Fleischmann et al. [15] demonstrated an effect of rTMS on noradrenergic neurons, suggesting that chronic rTMS treatment directly downregulates β-adrenoceptors. In addition, Kole et al. [28] reported that chronic rTMS treatment also downregulates 5-HT_{1A} and 5-HT_{2A} receptors in the frontal cortex, which might indirectly influence the noradrenergic neurons, given that there are well-known interactions between serotonergic and noradrenergic neurons in the re-

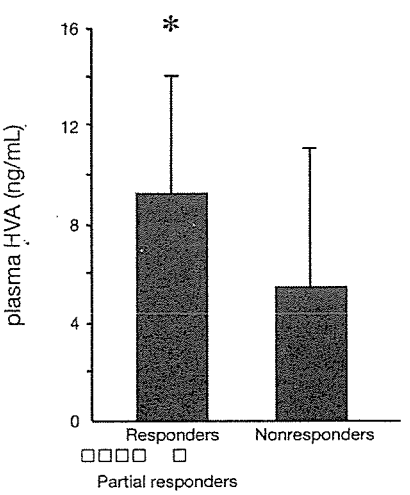

Fig. 2 Plasma levels of HVA before rTMS. Data are presented as the means ± SD. Patients with at least a 25% decrease in their Ham-D score are classified as responders, and the remaining patients as nonresponders. * $P < 0.01$, compared with nonresponders. Plasma levels of HVA were analyzed at one experiment in duplicate.

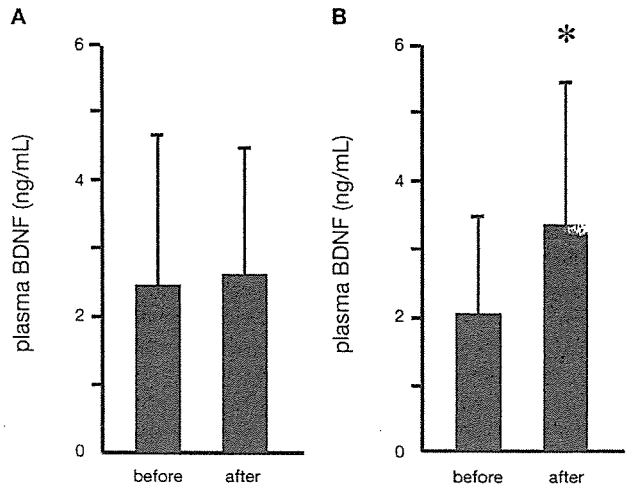

Fig. 3 (A)Plasma levels of BDNF before and 2 weeks after rTMS in non-responders. (B) Plasma levels of BDNF before and 2 weeks after rTMS in responders plus partial responders. Data are presented as the means ± SD. * $P < 0.05$, compared with before rTMS. Plasma levels of BDNF were assayed at one experiment in duplicate.

gion [46]. However, the exact mechanism by which rTMS treatment alters plasma MHPG remains unknown. As respective indicators of central noradrenergic and dopaminergic neuron activity, plasma MHPG and HVA should be used with caution, due to the fact that they only partially reflect activity in the brain. It has been hypothesized that only one third of the plasma MHPG and 30–50% of the plasma HVA are derived from the brain [52]. However, measuring plasma levels of catecholamine metabolites has the advantage of allowing more frequent sampling intervals than would be possible with urinary measures; in addition, the plasma measurement procedure is more convenient and comfortable for patients than the CSF measurement procedure. Thus, we have used plasma measures in a previous study [36,43,47,51].

We also found that rTMS treatment increased the plasma BDNF levels in responders plus partial responders, but not in nonresponders. In addition, a trend of association was found between the changes in the Ham-D scores and those in the plasma BDNF levels in all patients. These results suggest that the improvement of depressive symptoms by rTMS is in part related to the increase of plasma BDNF. Muller et al. [35] reported that rTMS increased BDNF mRNA and BDNF protein in the hippocampus as well as in the parietal and periform cortex. However, Jacobsen and Mønk [23] performed parallel measurements of BDNF mRNA and protein expression in the frontal cortex and hippocampus of the rat after chronic treatment with ECT, lithium, desipramine, or escitalopram. In their results, ECT increased BDNF mRNA and protein in the hippocampus and BDNF protein in the frontal cortex. Desipramine, a tricyclic antidepressant moderately increased BDNF mRNA in the dentate gyrus but did not change BDNF protein in either region. Escitalopram, a selective serotonin reuptake inhibitor did not affect BDNF mRNA, but decreased BDNF protein in the frontal cortex and hippocampus. Lithium increased the BDNF protein expression in the hippocampus and frontal cortex, but overall decreased BDNF mRNA. Taken together, it is difficult to conclude that the increased expression of BDNF mRNA and protein is a common action of antidepressant drug treatment and ECT. Karege et al. [24] reported that the serum BDNF levels were significantly decreased in antidepressant-free depressed patients, and that the serum BDNF levels were negatively correlated with the Montgomery-Asberg Depression Rating Scale. Shimizu et al. [42] also demonstrated that serum BDNF was significantly lower in an antidepressant-naïve group than in either a treated or in a control group, and that there was a significant negative correlation between serum BDNF and Ham-D scores in all patients. Furthermore, they reported preliminary findings that decreased serum BDNF levels in antidepressant-naïve patients recovered to normal levels in association with lower Ham-D scores after treatment with antidepressant medication. Moreover, Lang et al. [29] demonstrated that decreased serum BDNF levels were observed in healthy volunteers with neuroticism, and depression-related personality traits. The authors also speculated that BDNF levels have some influence on central serotonergic activity. Angelucci et al. [2] reported that daily low-frequency (1 Hz) rTMS motor cortex stimulation for 8 days is associated with a progressive reduction of the BDNF plasma levels in healthy subjects, but has no effect on the BDNF plasma levels in amyotrophic lateral sclerosis patients. On the other hand, high frequency (20 Hz) rTMS demonstrated a transient decrease in plasma BDNF levels. The authors speculated that this effect was due to the loss of motor cortex pyramidal cells. Although BDNF is highly concentrated in the brain, it is also present in the plasma and serum. The source of circulating BDNF remains unknown. Platelets, brain neurons, and vascular endothelial cells are considered candidate sources. Previously, it was reported that BDNF could cross the blood-brain barrier [37], and that BDNF levels in the brain and serum underwent similar changes during the maturation and aging process in rats [25], indicating that plasma BDNF levels might in part reflect the BDNF levels in the brain. In contrast to this, Radka et al. [39] reported that the BDNF detected in human plasma was derived from platelet degranulation, and

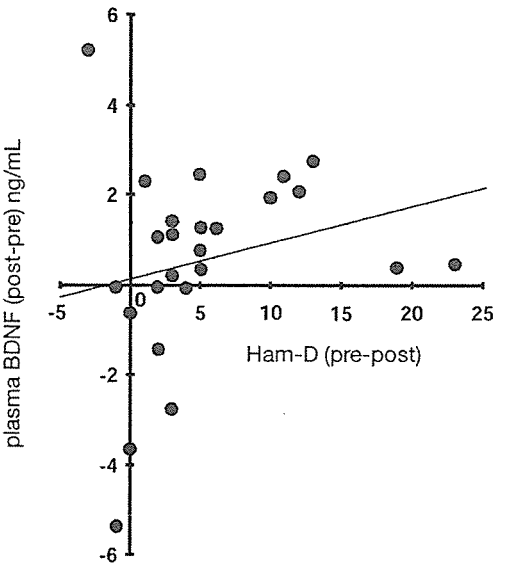

Fig. 4 Changes in plasma levels of BDNF and Ham-D scores. before: before rTMS, after: 2 weeks after rTMS. Data are presented as the means ± SD. Plasma levels of BDNF were assayed at one experiment in duplicate