Furthermore, our findings apparently showed that NTS induced compensatory as well as enhancing effects on the activities and emotional states of both sexes.

Pauk et al. [25] previously demonstrated that tactile stimulation with a camel hair brush during maternal deprivation for 2 h prevented the increase of serum corticosterone in response to maternal deprivation in infants rats, which is similar to the effect of NTS observed in the present study. Although further studies are needed to verify the efficacy of tactile stimulation, it is postulated that neonatal tactile stimulation may prevent the developmental disturbance of emotionality in response to early adversity. On the other hand, it is well known that maternal behavior plays an important role in physical and psychological development through mother–infant contact. For example, early adversity such as NI [3] or low maternal care [6] was reported to enhance activity of the HPA axis in response to stress in later life. In this context, it cannot be ruled out that the ability of NTS to reverse the effect of NI in these behavioral tests may be due to differences in maternal care for 23 h/day between the NI and NTS groups, since we did not measure maternal behaviors such as licking and grooming or arched-back nursing in this study.

In the contextual fear-conditioning test, whereas NTS led to a significant reduction in contextual freezing among both sexes as compared with sham-treatment, NTS reversed the NI-induced increase in contextual freezing in female but not male rats. The differential effect of NTS on contextual freezing in male and female rats may be due to differences in the effect of NI, since NI significantly enhanced contextual freezing in female but not male rats. The results of the present study are almost consistent with a previous study which showed that 1 h-neonatal isolation on PN days 2–9 enhances contextual fear in female Sprague–Dawley rats and impairs it in male Sprague–Dawley rats [11]. It has been suggested that contextual freezing behavior is fear and anxiety-related response, at least in part, mediated by the hippocampus [5,26]. We speculated, based on the results of these studies, that the intensity of hippocampal-dependent fear and anxiety responses in adulthood due to early adversity may differ between male and female rats.

There was no significant interaction between neonatal treatments and gender with respect to locomotor activities or rearing movements in the open-field test, anxiety-like behavior without head dippings in the elevated plus maze test, or pain sensitivity in the hot-plate test. In contrast, Rhees et al. [28] reported that females were more active than males in both the sham-treated and maternally separated groups. This difference may be due to differences in the experimental paradigms such as the breeding environment, age of animals, period of handling, etc.

It is noteworthy that the estrous cycle is an important factor for mediating stress responses in females. Unfortunately, in the present experiments, the behaviors of the female rats were not tested at any specified or consistent time in their estrous cycle. Rat behavior in an open-field test was reported to be independent of hormonal variations during the estrous cycle [33], and no significant difference in anxiety-like behavior was seen in the elevated plus maze test during proestrus and diestrus [23]. In contrast, female rats were reported to show higher % open arm times and % open arm entries during proestrus and estrus than during diestrus [22]. Furthermore, Severino reported that there was no significant interaction between neonatal handling and estrous cycle phases on the percentage of open arm time and percentage of open arm entries, but not total entries [31]. They reported that in diestrus the total number of arm entries in handled females was higher than in the nonhandled group in the same phase and also higher than in the handled females in estrus. Based on these findings, further studies examining the influence of the estrous cycle on anxiety-like behavior in the open arm test are required.

There are several limitations in this study that should be taken into consideration. First, the behavioral experiments in adulthood were undertaken during the light period but not the dark period of the dark/light cycle. As in our study, numerous studies examining the influence of neonatal manipulations on the adulthood behavioral responses to environmental stimuli, were performed during the light phase [2,13,24,28,30–32]. However, since the differences in the locomotor activity or anxiety-like behavior [7,8] between the light and dark phase were reported, assessment during both the light and dark phase is required to promote our understanding of behavioral responses. Secondly, the behaviors of the female rats were not tested at any specified or consistent time in their estrous cycle. Thirdly, although Kosten et al. [13] showed that 1 h of neonatal isolation during PN days 2–9 did not affect on estrous stage cyclicity, it cannot be ruled out that the neonatal handling may affect estrous stage cyclicity and may subsequently change the neuromodulatory role of gonadal steroids on the adulthood behavior.

In summary, the present study clearly indicated that whereas NI altered behavioral and emotional responses to environmental stimuli in later life, NTS could reverse the effect of NI and promote behavioral and emotional responses, to the same extent, in both sexes. In other words, an adequate tactile stimulation in early life plays an important role in the prevention of susceptibility to environmental stimuli induced by an early adverse experience. In addition, long-lasting behavioral and emotional changes, induced by an early adverse experience, differ somewhat between male and female rats in adulthood.

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Addition of risperidone to sertraline improves sertraline-resistant refractory depression without influencing plasma concentrations of sertraline and desmethylsertraline

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In the present study, we examined the efficacy of risperidone addition on sertraline-resistant depressed patients and the effects of risperidone on the metabolism of sertraline. Ten patients (M/F: 4/6, age: 54 ± 10 years) met the DSM-IV criteria for major depressive disorder enrolled the study. Hamilton Dating Scale for Depression (HAM-D) scores (mean ± SD) in all 10 patients significantly decreased from 19 ± 4 (before risperidone addition) to 11 ± 3 (4 weeks after risperidone addition). Plasma levels of sertraline and desmethylsertraline did not change after risperidone addition. Serum BDNF levels in responders to risperidone addition were changed from 8.1 ± 2.7 ng/ml (before risperidone addition) to 11.5 ± 0.9 ng/ml (4 weeks after risperidone addition); in contrast, those in nonresponders changed from 7.8 ± 2.2 ng/ml (before risperidone addition) to 7.9 ± 2.4 ng/ml (4 weeks after risperidone addition). These results suggest that the addition of risperidone to sertraline is effective and well tolerated for sertraline-resistant depressive patients, which is accompanied with the increase in serum BDNF levels in responders to the risperidone addition, and the addition of risperidone to sertraline does not seem to influence sertraline metabolism. Copyright © 2008 John Wiley & Sons, Ltd.

KEY WORDS — refractory depression; sertraline; desmethylsertraline; risperidone; BDNF

INTRODUCTION

Selective serotonin reuptake inhibitors (SSRIs) and serotonin noradrenaline reuptake inhibitors (SNRIs) are generally considered first-line treatments for depression. Despite a sufficient dose and duration of antidepressant treatment 30–40% of all depressive patients fail to respond (Trivedi *et al.*, 2006). Further approaches in the treatment of resistant depression may be to switch to another drug of the same class or to another class of antidepressants (Kelsey, 1997), or to augment the antidepressant with a further drug such as thyroid hormones (Joffe, 1998), lithium (Roullion and Gorwood, 1998), bupropion (Bodkin *et al.*, 1997), pindolol (Blier and Bergeron, 1998), or dexamethasone (Dinan *et al.*, 1997).

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-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 few extrapyramidal side effects (Yoshimura *et al.*, 2003, 2005). Furthermore, we have reported that risperidone is also effective in treating 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 systems, indicating the complex nature of the serotonergic system, have been described (Marsden, 1991). Furthermore, our previous findings

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indicate that the effects of risperidone on the noradrenergic system as well as on the serotonergic system might be related to its efficacy in treating negative symptoms of schizophrenia (Yoshimura *et al.*, 2000a, 2000b) and mood disorder (Yoshimura *et al.*, 2006). Taken together, the above results show that the effects of risperidone on three monoaminergic systems (dopamine, serotonin, noradrenaline) are associated with its clinical efficacy for schizophrenia and mood disorder.

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 reports suggest that risperidone is useful for treating refractory depression (Goto *et al.*, 2005; Papakostas *et al.*, 2007). Rapaport *et al.* (2006) investigated the long-term efficacy of risperidone augmentation of SSRI treatment for resistant depression. In 57 in- and outpatient centers in three countries, the authors conducted a three-phase study with 4–6 weeks of open-label citaloplam monotherapy, 4–6 weeks of open-label risperidone augmentation, and a 24-week double-blind, placebo-controlled discontinuation phase. A total of 489 patients with major depressive disorder and 1–3 documented treatment failure entered the citalopram monotherapy phase (20–60 mg/day). Patients with <50% reduction in Hamilton Rating Scale for Depression (HAM-D) scores entered the risperidone augmentation phase (0.25–2.0 mg/day). Patients with HAM-D $\leqq 7$ or CGI-S $\leqq 2$ were randomized to risperidone or placebo augmentation. Of the 386 nonresponders who entered the augmentation phase, 243 remitted and 241 entered the double-blind phase. Median time to relapse was 102 days with risperidone augmentation and 85 days with placebo; relapse rates were 53.3 and 54.6%, respectively. These results suggest that open-label risperidone augmentation substantially enhanced response in treatment-resistant patients, but the longer-term benefits of augmentation were not demonstrated in the study.

In the present study, we investigated the efficacy of risperidone addition on sertraline-resistant refractory depressed patients, and we also examined the effects of risperidone on the metabolism of sertraline.

SUBJECTS AND METHODS

Ten patients met the DSM-IV criteria (American Psychiatric Association, 1994) for major depressive disorder enrolled the study. Four were male and six were female, and their ages ranged from 38 to 71 (mean $\pm$ SD = 54 $\pm$ 10) years. All patients were physically healthy and free of current alcohol and/or drug abuse or comorbid anxiety or personality disorders. Drug resistance was defined as a failure to respond at least three course of a single antidepressant medication with adequate dose and duration (stage III definition from Thase and Rush, 1997). 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. The patients were treated with risperidone at doses ranging from 0.5 to 2.0 (mean $\pm$ SD = 1.0 $\pm$ 0.3) mg/day. All patients were treated with sertraline at doses ranging from 50 to 100 (mean $\pm$ SD = 87.5 $\pm$ 16.7) mg/day for at least 8 weeks, but their scores on the HAM-D (Hamilton, 1960) were 15 points or more and <50% reduction. The dosage of sertraline is considered to be low however upper limit of sertraline approved in Japan is 100 mg/day. Therefore, mean dosage of sertraline prescribed in the study was relatively high in Japanese standard. Risperidone was added to the ongoing sertraline. Only benzodiazepines were permitted as hypnotics, and the dosage was kept constant throughout the study period. The dosages of risperidone and sertraline varied among patients and, based on ethical considerations, were not fixed. However, doses of sertraline and risperidone were not altered during the co-medication period. The patients were evaluated regarding their clinical improvement and extrapyramidal symptoms using the HAM-D and Simpson and Angus Rating Scale (SAS) (Simpson and Angus, 1970), respectively, by two experienced psychiatrists (R.Y. and N.U.) The HAM-D was evaluated at before starting antidepressants (primary), before and every week after risperidone administration, and the SAS was evaluated before and every week after risperidone administration. Furthermore, blood levels of triglyceride and glucose and body weight were assessed before and 4 weeks after risperidone administration. The psychiatrists assessing the HAM-D and SAS were blind to the results of plasma levels of sertraline and desmethylsertraline or serum BDNF levels. 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 levels of sertraline and desmethylsertraline were analyzed by high-performance liquid chromatography (HPLC) accord-

ing to the method of Mandrioli *et al.* (2006). In short, solid-phase extraction was carried out using Varian BondElut C2 cartridges (100 mg, 1 ml) on a Varian VacElut apparatus. Aliquots of 50 µl of internal standard (clomipramine) were added to 250 µl of patient's plasma. The resulting mixture was diluted with 500 µl of water and loaded onto a previously conditioning C2 cartridge. The cartridges were equilibrated five times with 1 ml of methanol and then conditioned five times with 1 ml of water. After loading, the cartridges were washed twice with 1 ml of water, then with 1 ml of methanol/10 mM, pH 10 carbonate buffer (20:80, v/v), and finally with 50 µl of methanol. Elution was carried out with 1 ml of methanol. The elute was brought to dryness (rotary evaporator), redissolved in 250 µl of mobile phase (acetonitrile and a 12.3 mM, pH 3 phosphate buffer containing 0.1% triethylamine 35:65, v/v), and injected into the HPLC system.

Plasma risperidone and 9-hydroxyrisperidone were analyzed using HPLC according to Olesen and Linnet (1997). In brief, 1 ml plasma was mixed with 0.5 ml 0.6 M sodium carbonate/bicarbonate buffer (pH = 10) and 50 µl of haloperidol solution, with 3.76 mg/ml (10 mM) as an internal standard. Next 8 ml heptane-isoamyl alcohol (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 containing 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 mobile phase (40 mM ammonium acetate buffer pH 7—methanol; 100:900, v/v), of which 65 µl was injected into the HPLC.

The serum 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 h. The plates were incubated in a blocking buffer for 1 h at room temperature. The samples were diluted with assay buffer 100-times and the BDNF standards were kept at room temperature under conditions of horizontal shaking for 2 h, followed by washing with the appropriate washing buffer. The plates were incubated with antihuman BDNF polyclonal antibody at room temperature for 2 h and washed with the washing buffer. The plates were then incubated with anti-IgY antibody conjugated to horseradish peroxidase for 1 h 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 microplates reader. The standard curve was linear from 5 to 5000 pg/ml, and the detection limit was 5 pg/ml. The intra- and interassay coefficients of variation were 5 and 7%, respectively. The recovery rate of the exogenously added BDNF in the measured plasma samples exceeded 95%.

Statistical analysis

Statistical analyses were conducted by the Wilcoxon test to compare HAM-D scores, plasma levels of sertraline, desmethylsertraline, triglyceride, and glucose, serum BDNF levels, or body weight before and 4 weeks after risperidone addition. The level of significance for all analysis was set at $p < 0.05$.

RESULTS

All participants completed the study (completion rate was 100%). HAM-D scores (mean ± SD) in all 10 patients were 22 ± 6, 19 ± 4, and 11 ± 3 at before starting sertraline (primary), before risperidone addition, 4 weeks after risperidone addition, respectively. HAM-D scores in all 10 patients significantly decreased 4 weeks after risperidone addition ($z = -2.023$, $p = 0.005$) (Figure 1). Five of 10 (50%) became responders (50% or more improvement in HAM-D scores) and 1 of 10 patients (10%) became remission (HAM-D scores of under seven points) in 4 weeks after risperidone addition. Plasma levels of sertraline and desmethylsertraline did not change after risperidone addition (Table 1). Furthermore, the

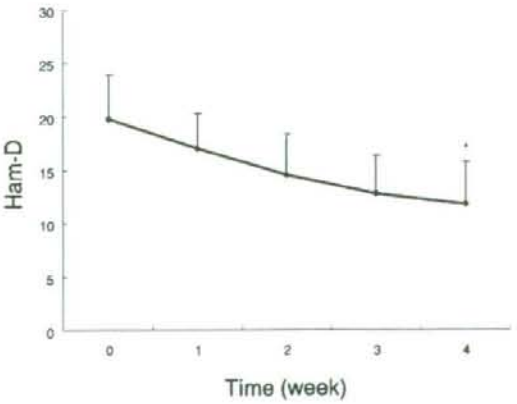

Figure 1. Changes in HAM-D scores after risperidone addition

Table 1. Plasma levels of sertraline and desmethylsertraline for each case

Case	Dose of ser (mg/day)	Dose of ris (mg/day)	Plasma ser level (B) (ng/ml)	Plasma dser level (B) (ng/ml)	Plasma ser level (4W) (ng/ml)	Plasma dser level (4W) (ng/ml)
1	100	0.5	44	49	39	35
2	100	1	58	62	50	51
3	75	0.5	39	35	48	44
4	100	1	51	45	44	49
5	50	1	16	16	23	20
6	75	1	22	31	14	20
7	100	2	39	42	33	35
8	75	1	29	32	33	30
9	100	1	49	53	52	55
10	100	1	61	58	54	59
Mean ± SD	87.5 ± 16.7	1.0 ± 0.38	40.8 ± 14.1	42.3 ± 13.3	39.0 ± 13.3	39.8 ± 13.0

ser, sertraline; dser, desmethylsertraline; rs, risperidone; B, before risperidone addition; 4W, 4 weeks after risperidone addition.

plasma level of active moiety of risperidone (risperidone plus 9-hydroxyrisperidone) at 4 weeks after risperidone administration was 17.7 ± 5.0 ng/ml. Serum BDNF levels in all patients slightly increased 4 weeks after risperidone addition (before risperidone addition: 7.9 ± 2.3 ng/ml, 4 weeks after risperidone addition: 9.7 ± 2.5 ng/ml), however the increase was not statistically significant ($z = -1.580$, $p = 0.1141$). Serum BDNF levels in responders to risperidone addition were changed from 8.1 ± 2.7 ng/ml (before risperidone addition) to 11.5 ± 0.9 ng/ml (4 weeks after risperidone addition) ($z = -2.023$, $p = 0.0431$); in contrast, those in nonresponders changed from 7.8 ± 2.2 ng/ml (before risperidone addition) to 7.9 ± 2.4 ng/ml (4 weeks after risperidone addition) (Figure 2). The SAS scores were not significantly different between before and 4 weeks after risperidone addition (before: 0.2 ± 0.4, 4 weeks after risperidone addition: 0.4 ± 0.4). In addition, blood levels in triglyceride and glucose and body weight were not significantly changed 4 weeks after risperidone addition.

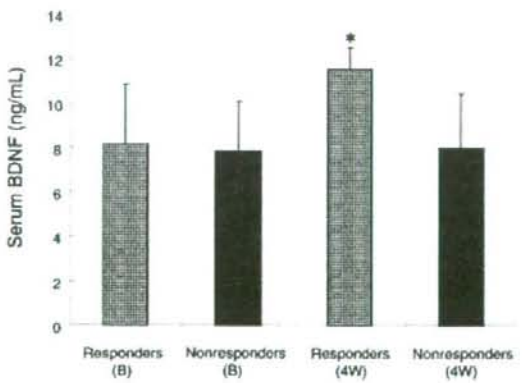

Figure 2. Effects of risperidone addition to sertraline on serum BDNF: B, before risperidone addition to sertraline; 4W, 4 weeks after risperidone addition to sertraline; responders, patients whose HAM-D scores decreased 50% or more; nonresponders, patients whose HAM-D scores decreased below 50%; $^*p = 0.043$, compared with B

DISCUSSION

Addition of a low dose of risperidone to sertraline was effective and well tolerated for patients with sertraline-resistant refractory depression. We have previously demonstrated that risperidone addition to antidepressants, such as SSRIs and tricyclic antidepressants, is effective for the treatment of psychotic depression, a subtype of refractory depression. In that study, we also found that plasma levels of homovanillic acid (HVA), a major metabolite of dopamine, in responders are higher than those in nonresponders. Furthermore, there is a negative correlation between changes in plasma HVA levels and per cent improvement on HAM-D scores in patients with psychotic depression. Taken together, the findings suggest that the influence of risperidone on the dopaminergic system might play an important role in its efficacy in psychotic depression. Rapaport *et al.* (2006) investigated the long-term efficacy of risperidone augmentation of SSRI treatment for resistant depression. In 57 in- and outpatient centers in three countries, the authors conducted a three-phase study with 4–6 weeks of open-label citaloplam monotherapy, 4–6 weeks of open-label risperidone augmentation, and a 24-week double-blind, placebo-controlled discontinuation phase. A total of 489 patients with major depressive disorder and 1–3 documented treatment failure entered the citalopram monotherapy phase. Patients

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with <50% reduction in HAM-D scores entered the risperidone augmentation phase (0.25–2.0 mg/day). Patients with HAM-D ≤ 7 or CGI ≤ 2 were randomized to risperidone or placebo augmentation. The primary outcome was time to relapse during the double-blind phase. During citalopram therapy, 434 patients had <50% HAM-D reduction; 299 (68.9%) were fully nonresponsive and 135 were partially nonresponsive. Of the 386 nonresponders who entered the augmentation phase, 243 remitted and 241 entered the double-blind phase. Median time to relapse was 102 days with risperidone augmentation and 85 days with placebo. Mahmound *et al.* (2007) have reported that risperidone augmentation was associated with improvements in clinician-rated depressive symptoms and patients' perception of those symptoms, reduced disability, and increased response and remission rate in patients with suboptimal responses to standard antidepressant monotherapy. The response rate in the present study was 50%, which is a little higher than that of Mahmund's study (35.6%). The reason for this discrepancy might be related to the study design. Our study was an open-labeled, while that of Mahmund *et al.* was a randomized controlled study. In any cases, further studies should be needed to confirm the benefits of risperidone augmentation to antidepressants in refractory depression. A recent meta-analysis by Papakostas *et al.* (2007) demonstrated that augmentation of antidepressant with atypical antipsychotic drugs including olanzapine, risperidone, and quetiapine, for treatment-resistant depression is definitely effective based on both the response rate and remission rate.

Another important finding in the present study was that risperidone addition did not influence plasma levels of sertraline and its metabolite, desmethylsertraline. The major metabolic pathway of sertraline is the demethylation to form desmethylsertraline, a reaction primarily mediated by cytochrome P450 (cyp) 3A4. However, other cyp enzymes, including cyp2D6, 2C9, and 2C19, also play a significant role in mediating demethylation (Greenblatt *et al.*, 1999; Wang *et al.*, 2001). In contrast, risperidone is extensively metabolized primarily via 9-hydroxylation, yielding an active metabolite 9-hydroxyrisperidone. *In vivo* and *in vitro* studies have indicated that cyp 2D6 and, to a lesser extent cyp3A4 are involved in the 9-hydroxylation of risperidone. Therefore, it is plausible that the addition of risperidone to sertraline alters metabolization from sertraline to desmethylsertraline. Indeed, Wang *et al.* (2006) recently reported that risperidone administration to sertraline slightly shortened the absorption T1/2 and slightly decreased the Tmax of sertraline in plasma compared to sertraline administration alone. In the brain, risperidone coadministration greatly increased the Tmax of sertraline and remarkably shortened the T1/2 in brain. The authors proposed that these findings suggest that sertraline and/or desmethylsertraline interacted at sites of the blood brain barrier to increase the brain entry of risperidone and 9-hydroxyrisperidone. Both risperidone and 9-hydroxyrisperidone are substrates of p-glycoprotein. Therefore, the increment of brain risperidone and 9-hydroxyrisperidone by coadministered sertraline suggests an inhibitory effect on p-glycoprotein in blood brain barrier by sertraline and/or desmethylsertraline. It is possible that risperidone, as an inhibitor of p-glycoprotein, could decrease the efflux of sertraline and its metabolite out of the brain by p-glycoprotein inhibition thereby increasing the central nervous system accumulation of antidepressant leading to greater therapeutic effects (Wang *et al.*, 2006). As this interaction occurs at the level of the blood brain barrier, it is not necessarily detectable by measuring plasma drug concentrations alone.

However, the addition of risperidone to sertraline did not influence the metabolism of sertraline. The dose of risperidone was small, possibly because cyp2D6 might not play a major role in sertraline metabolism, or because the inhibitory effects of risperidone on cyp2D6 might not be potent. The average plasma sertraline levels in all patients were approximately 40 ng/ml before risperidone addition. Furthermore, no difference was found in plasma sertraline levels between the responders (37.0 ± 10.3 ng/ml) and the nonresponders (44.6 ± 16.2 ng/ml). Desmethylsertraline, an active metabolite of sertraline is up to 25 times weaker than sertraline *in vitro*, producing only with parent drug, desmethylsertraline being considered substantially less active than the parent compound playing a negligible role in its clinical activity. Although the biological activity in desmethylsertraline does not seem to be associated with the clinical efficacy of sertraline, plasma desmethylsertraline level also did not differ between the responders (39.8 ± 7.1 ng/ml) and the nonresponders (44.8 ± 17.1 ng/ml). Some studies have found that therapeutic drug monitoring may be useful for dose optimization as serum concentrations of 25–50 ng/ml appear to be adequate for efficacy (Lundmark and Reis, 2000; Mauri *et al.*, 2003). In contrast, Hong Ng *et al.* (2006) have indicated no visible relationship between plasma sertraline levels and its clinical efficacy. Nonetheless, it remains unclear whether there is a link between plasma sertraline levels and the clinical efficacy of sertraline.

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It has been reported that sertraline moderately inhibits cyp2D6 (Greenblatt *et al.*, 1998) and its inhibitory effects of cyp 2D6 and other cyp isoenzymes are dose-related fashion (Spina *et al.*, 2004). The major enzyme of risperidone to 9-hydroxyrisperidone is cyp2D6, and to some extent cyp3A4 (Fang *et al.*, 1999). Taking these findings into account, it is plausible to assume that sertraline increases plasma risperidone levels, which leads to the emergence of extrapyramidal symptoms. Actually, we have previously demonstrated a positive correlation was found between plasma levels of risperidone and 9-hydrtoxyrisperidone and the emergence of extrapyramidal symptoms in Japanese populations (Yoshimura *et al.*, 2001). In the present study, coadministration of sertraline with risperidone did not deteriorate extrapyramidal symptoms, because the dose of risperidone was small, and the plasma levels of active moiety of risperidone was low level. Otherwise, larger dosage of sertraline might be needed to increase plasma levels of active moiety of risperidone. Since sertraline itself has been reported to cause extrapyramidal symptoms (Lambert *et al.*, 1998), clinicians should be aware of the possibility of extrapyramidal symptoms developing when sertraline and risperidone are coadministered.

We have recently reported that serum BDNF levels in patients with responders to paroxetine or milnacipran were raised at 8 weeks after their treatment, while those in nonresponders were not. These results suggest that serum BDNF levels can be considered state markers in depression. In the present study, a significant increase was found in serum BDNF levels after risperidone addition in responders, while these levels were not altered in nonresponders. Basically, the present results regarding serum BDNF levels are in accordance with those of our previous study (Yoshimura *et al.*, 2007). Interestingly, serum BDNF levels in responders to risperidone addition were raised at 4 weeks, but not 8 weeks after risperidone addition, suggesting that the effects of risperidone combined with sertraline on serum BDNF levels might be more potent than those of each drug. The source of circulating BDNF remains unknown. Platelet, brain neurons, and vascular endothelial cells are currently considered to be putative sources. It has been demonstrated that BDNF crosses the blood–brain barrier (Pan *et al.*, 1998) and that BDNF levels in the brain and serum have been shown to undergo similar changes during the maturation and aging process in rats (Karege *et al.*, 2002). These results indicate that blood BDNF levels might in part reflect the BDNF levels in the brain. Nonetheless, it remains unclear to what extent peripheral levels reflect brain BDNF levels.

In conclusion, the addition of risperidone to sertraline is effective and well tolerated for sertraline-resistant refractory depressive patients. Addition of risperidone to sertraline does not seem to influence sertraline metabolism. In addition, the serum BDNF levels were increased in responders to risperidone, while these levels were not altered in nonresponders. The limitations of this study, including the small number of patients and the open setting should be considered. Therefore, further studies must be performed to confirm our preliminary findings.

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Efficacy of electroconvulsive therapy is associated with changing blood levels of homovanillic acid and brain-derived neurotrophic factor (BDNF) in refractory depressed patients: A pilot study

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ABSTRACT

Electroconvulsive therapy (ECT) is effective for patients with antidepressant medication-resistant depression. However, the mechanisms of ECT's effectiveness for treating depression are not fully understood. We therefore investigated ECT's effects on blood levels of brain-derived neurotrophic factor (BDNF), catecholamine metabolites, and nitric oxide (NO) in 18 treatment-refractory depressed patients. Serum BDNF levels increased significantly following ECT in responders to ECT (before ECT: 8.0±9.7 ng/mL; five weeks after start of ECT: 15.1±11.1 ng/mL), whereas BDNF levels in non-responders were unchanged (before ECT: 11.5±11.0 ng/mL; five weeks after start of ECT: 9.4±7.5 ng/mL). Furthermore, the plasma HVA levels, but not MHPG levels, were significantly reduced after ECT (before ECT: 8.5±1.9 ng/mL; five weeks after start of ECT: 5.8±2.2 ng/mL). This latter finding occurred in parallel with the improvement of depressive symptoms in all patients. These results suggest that the mechanisms underlying ECT's effect on refractory depression may be related to dopaminergic neurons and BDNF.

1. Introduction

Depression, like anxiety disorder, is one of the most common psychiatric disorders, with a lifetime prevalence estimated to be between 1.5% and 19% (Weissman et al., 1996). Approximately half of the depressed patients experience a chronic course and up to 20% of that half show insufficient responses to antidepressant medication (Hussain and Cochrane, 2004). In other words, despite the administration of several different antidepressants, a considerable number of depressive patients do not adequately respond to the antidepressant therapy or others do not tolerate the side effects of antidepressants. Unlike antidepressant treatment, electroconvulsive therapy (ECT) has a shorter onset latency and has been used for patients with serious or treatment-refractory depression (Burt et al., 2002). ECT is one of the eligible strategies for treatment-refractory depression (UK ECT Review Group, 2003).

Brain-derived neurotrophic factor (BDNF), a major neurotrophic factor, has been found to play a critical role in long-term potentiation, a cellular mechanism of learning and memory, suggesting that it can influence neuroplasticity (Figurov et al., 2006). BDNF is also needed for the survival and guidance of neurons during development as well as the survival and function of neurons during adulthood (Duman et al., 2000). There is growing evidence that BDNF may have a crucial role in mental disorders such as depression (Durman et al., 1997; Dwivedi et al., 2003) and schizophrenia (Shoval and Weizman, 2005). Karege et al. (2000a) have shown that serum BDNF levels of drug-free patients are lower than those of controls, and Shimizu et al. (2003) found that serum BDNF levels of treated depressed patients do not differ from control levels. Aydemir et al. (2005) reported that serum BDNF levels are lower in depressed patients than in controls, and that treatment with antidepressant drugs for 12 weeks increases serum BDNF to control levels. Gonul et al. (2005) also reported that eight weeks of treatment with each of several antidepressant drugs significantly increases serum BDNF to control levels. These results indicate that antidepressant drugs increase serum BDNF levels in depressed patients.

Preclinical studies have demonstrated that ECT produces a robust increase in BDNF mRNA (Durman et al., 1997; Nibuya et al., 1995) and BDNF protein (Altar et al., 2004) in different rat brain areas. Some reports have demonstrated that ECT increases serum or plasma levels of BDNF (Marano et al., 2007). In contrast, another report has shown that ECT does not increase serum BDNF levels (Gronli et al., 2007). Because of these conflicting results, it remains to open question as to whether or not ECT could affect the peripheral levels of BDNF in depressed patients.

ECT does not appear to cause consistent changes in cerebrospinal fluid (CSF), plasma, or urinary levels of the major monoamine metabolites.

Abbreviations: ECT, electroconvulsive therapy; HVA, homovanillic acid; MHPG, 3-methoxy-4-hydroxyphenylglycol; BDNF, brain-derived neurotrophic factor; NO, nitric oxide.

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Some studies have reported either an increase or no change after ECT in homovanillic acid (HVA), a major metabolite of dopamine, 3-methoxy-4-hydroxyphenylglycol (MHPG), a major metabolite of noradrenaline, or 5-hydroxyindoleacetic acid (5-HIAA), a major metabolite of serotonin (Jori et al., 1975; Abrams et al., 1976; Linnoila et al., 1984; Aberg-Wistedt et al., 1986; Devanand et al., 1989; Lykouras et al., 1990). Alternatively, others have reported reduced CSF levels of HVA, MHPG, and 5-HIAA following ECT (Harnryd et al., 1979; Lerer and Belmarker, 1982). Interestingly, crosstalk between BDNF synthesis and monoaminergic systems has been reported (Alter et al., 1992; Juric et al., 2006; Do et al., 2007; Paredes et al., 2007). For example, Paredes et al. (2007) found that BDNF evoked dopamine release in a dose dependent fashion in the rat hippocampus. Juric et al. (2006) also reported that BDNF increased dopamine synthesis in cultured neonatal rat astrocytes. Finally, Do et al. (2007) reported that BDNF upregulated the expression of D1 receptor in catecholaminergic cell lines. Recently, we also reported a negative correlation between serum BDNF levels and plasma MHPG levels in healthy hospital workers, suggesting that excessive noradrenaline might suppress BDNF synthesis and/or secretion (Mitoma et al., 2008).

It has been reported that nitric oxide (NO) is associated with the pathogenesis of depression. Plasma metabolites of NO (NOx) are lower in patients with depression, and treatment with antidepressants changes plasma NOx levels (Chrapko et al., 2004, 2006). Therefore, it is possible that ECT will also affect plasma NOx levels. Taken together, these findings suggest it is plausible that BDNF, catecholamines, and NO might play important roles in the pathogenesis of depression, and ECT should influence these factors (Linnoila et al., 1983; Rosen et al., 2003).

In the present study, we hypothesized that ECT could alter blood levels of BDNF, catecholamines, and NO, and that these alterations are associated with its clinical efficacy. To test this hypothesis, we investigated ECT's effects on serum BDNF levels, plasma levels of catecholamine metabolites, and NOx in patients with antidepressant-refractory depression.

1.1. Subjects and methods

This study included 18 inpatients (nine male, nine female) at our university hospital who met the DSM-IV-TR criteria for major depressive disorder or bipolar I disorder (depressive episode) and patients who scored at least a 15 on the 17-item version of the Hamilton Rating Scale for Depression (Ham-D). Five of the 18 patients had exhibited psychotic symptoms. The age of the subjects ranged from 31 to 78 years (mean ± SD = 60.6 ± 14.1). All patients were physically healthy and had no history of alcohol or drug abuse or co-morbid anxiety or personality disorders. The mean Mini Mental State examination was 27.8 ± 2.1. For each patient, an independent psychiatrist recommended ECT according to his or her clinical judgment based on the patient's drug resistance. Drug resistance was defined as a failure to respond to at least three courses of a single antidepressant medication with adequate dose and duration (stage III definition from Thase and Rush, 1997).

A medical history and a physical examination together with blood and urine examinations, electrocardiogram, cerebral computed tomography scan, and a chest film were used to screen each patient's general medical conditions. Premedication included atropine sulphate (0.5 mg i.v.), propofol (1.0 mg/kg i.v.), vecronium (0.5–1.0 mg i.v.), and succinylcholine (1.0 mg/kg i.v.) for each subject. ECT was performed between 7:00 and 9:00 a.m. using a Thymatron TM DG (Somatics, Inc., Lake Bluff, IL, USA) with standard settings (Abrams et al., 1989) and a bipolar brief pulse square wave. The patients were given bilateral ECT. Two stimulus electrodes were placed over the left and right front-temporal scalp. ECT conditions were same for all patients (charge delivered max 504 mC, current 0.9A, frequency 10–70 Hz, pulse width 0.5 ms, duration max 8 s). During ECT, motor convulsions, electro-encephalogram, induced tachycardia and, if necessary, electromyogram were monitored. ECT was given 12 times (three times a week for four weeks). Patients were placed on drug treatment for at least one week before ECT and drug treatment was maintained during the entire study period. The antidepressants used and number of subjects being treated with each were paroxetine ($n=4$), milnacipran ($n=4$), clomipramine ($n=3$), sertraline ($n=3$), imipramine ($n=2$), amitriptyline ($n=1$) and sulpiride ($n=1$).

All blood samples were taken at 7:00 am before breakfast (at least 12 h after the last medication) before and one week after finishing 12 ECT sessions (i.e., five weeks after starting ECT). After the patient had been lying at rest overnight, 15 mL of venous blood was drawn with the patient in the supine position. The plasma and serum samples were quickly separated in a centrifuge (2000 g, 10 min, 4 °C) and stored at −80 °C until assay. The serum 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 h. The plates were incubated in a blocking buffer for 1 h at room temperature. The samples diluted with assay buffer 100-times and the BDNF standards were kept at room temperature on a horizontal shaker for 2 h, followed by washing with the appropriate washing buffer. The plates were incubated with antihuman BDNF polyclonal antibody at room temperature for 2 h and washed with the washing buffer. The plates were then incubated with anti-IgY antibody conjugated to horseradish peroxidase for 1 h at room temperature, then 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. The standard curve was linear from 5 pg/mL to 5000 pg/mL, and the detection limit was 5 pg/mL. The intra- and inter-assay coefficients of variation were 5% and 7%, respectively. The recovery rate of the exogenously added BDNF in the measured plasma samples exceeded 95%.

The plasma homovanillic acid (HVA) levels were analyzed 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 was added with 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 slowly through the cartridge under a mild vacuum (15 mm Hg), and the filtrate was collected. The cartridge was then 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 the supernatant was injected into the HPLC. The intra- and inter-assay coefficients of variation were 6% and 8%, respectively. The recovery rate was more than 80%.

The plasma 3-methoxy-4-hydroxyphenylglycol (MHPG) levels were analyzed according to the method of Minegishi and Ishizaki (1984). In brief, the 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 with 1 mL capacity disposable syringe. The columns, which were inserted into a vacuum chamber connected to an aspirator, were prepared by washing with a 1 mL methanol followed by 1 mL of water. After the addition of 50 μL of vanilly alcohol (internal standard equivalent to 5 ng/mL) to 1 mL of plasma, samples were applied to and passed through the columns, followed by 0.75 mL of water to rinse off both residual samples and to easily elute 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 intra- and inter-assay coefficients of variation were 4% and 8%, respectively. The recovery rate was more than 80%.

Plasma NOx was measured by the Griess reaction as the nitrate concentration after nitrate reduction to nitrite (Fiddler, 1977). In brief, 50 μl of 1% sulfanilamide was added to the sample first, incubated for

Table 1
Blood levels of catecholamine metabolites, BDNF, and NOx before ECT, responders: patients whose scores of Ham-D decreased by 50% or more, non-responders: patients whose scores of Ham-D decreased by less than 50%, the values are mean ± SD

	Responders (n=12)	Non-responders (n=6)
Sex	6/6	3/3
Age	58.6±13.9	62.4±15.1
Ham-D	25.0±8.2	20.6±4.4
MHPG (before) (ng/mL)	8.3±3.8	7.6±6.2
HVA (before) (ng/mL)	9.7±2.0*	6.1±2.1
BDNF (before) (ng/mL)	7.9±9.9	11.5±11.0
NOx (before) (μM)	30.7±18.1	54.6±40.5

* p=0.018, compared with non-responders.

5–10 min, and 50 μl of 0.1% *N*-1-naphthylethylendiamine dihydrochloride was then added. The reaction was performed at room temperature for 5–10 min, and absorbance at 540 nm was measured using nitrite solution as a standard. Levels of plasma NOx are reported in micromoles per liter. The intra- and inter-assay coefficients of variation were 2% and 3%, respectively. All the measurements were performed in duplicate or triplicate for each experiment.

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.

1.2. Statistical analysis

Statistical analysis was performed by the use of the Wilcoxon test or the Mann–Whitney *U*-test to investigate changes in Ham-D scores, as well as changes in MHPG, HVA, NOx, and BDNF levels. The relationship between two variables was examined using Spearman's correlation coefficients. The level of significance was set at $p<0.05$.

2. Results

The Ham-D score was significantly decreased after the 12 ECT sessions (before ECT: 23.1±4.5; five weeks after start of ECT: 10.3±3.4) ($p<0.001$). We defined the responders as the patients whose Ham-D scores decreased by 50% or more, and the non-responders as those whose scores decreased less than 50%. Twelve of 18 (67%) were responders (five weeks after start of ECT). Six of 18 (33%) showed Ham-D scores of under 7 points (remissional state) at five weeks after start of ECT.

Compared to non-responders, responders had significantly higher baseline (before ECT) plasma levels of HVA, but not MHPG (Table 1).

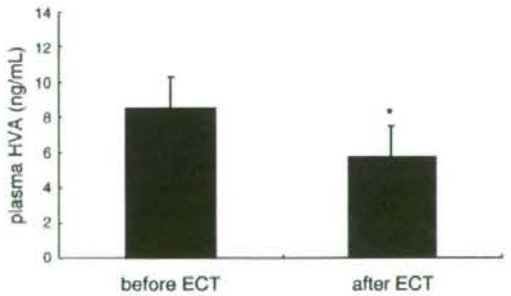

Fig. 1. Changes in plasma HVA levels before and after (i.e., five weeks after starting) ECT treatment (n=18). *p=0.008, compared with before ECT.

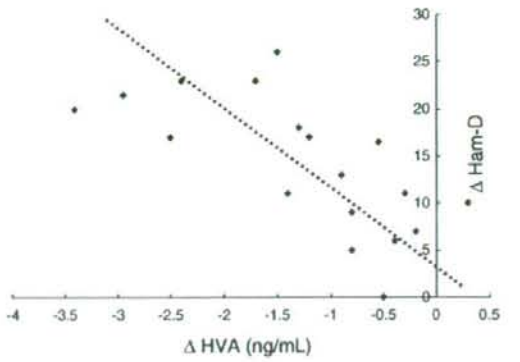

Fig. 2. Negative association between the changes in Ham-D and the changes in plasma HVA levels before and five weeks after start of ECT (n=18). rho=−0.620, p=0.0052.

Plasma HVA level was significantly decreased one week after finishing ECT treatment (five weeks after start of ECT) (before ECT: 8.5±1.9 ng/mL; five weeks after start of ECT: 5.8±2.2 ng/mL; p=0.008) (Fig. 1). However, no difference in plasma MHPG levels was found between before and after ECT treatment (before ECT: 8.1±4.8 ng/mL; five weeks after start of ECT: 6.5±5.1 ng/mL). A negative correlation was found between the changes in plasma HVA levels and the changes in Ham-D scores before and after ECT treatment (rho=−0.620, p=0.0052) (Fig. 2).

Serum BDNF levels were not increased one week after finishing ECT treatment (five weeks after start of ECT) (before ECT: 11.0±11.2 ng/mL; 5 weeks after starting ECT: 11.0±9.8 ng/mL) in all depressed patients. There was a significant increase of serum BDNF levels in responders (before ECT: 8.0±9.7 ng/mL, five weeks after start of ECT: 15.1±11.1 ng/mL; p=0.025), whereas serum BDNF levels in non-responders did not change (before ECT: 11.5±11.0 ng/mL; five weeks after starting ECT: 9.4±7.5 ng/mL) (Fig. 3). However, no correlations were found between the changes in serum BDNF levels and the changes in either Ham-D scores, or plasma levels of HVA, or MHPG from before to after ECT. Moreover, serum BDNF levels before ECT were not different between responders and non-responders (Table 1).

ECT did not alter plasma NOx levels (before ECT: 42.5±35.7 μM; five weeks after start of ECT: 25.7±16.7 μM, p=0.1909). In addition, plasma NOx levels before ECT were not different between responders and non-responders (Table 1).

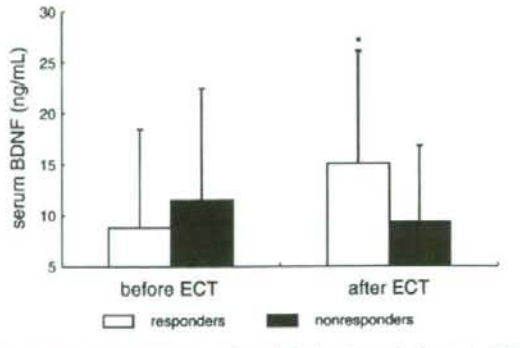

Fig. 3. Changes in serum BDNF levels before and after (i.e., five weeks after starting) ECT treatment in responders (n=12) and non-responders (n=6). Horizontal bar means median of the serum BDNF. *p=0.025, compared with before ECT.

3. Discussion

The present results reveal that ECT given to patients suffering from major depression increases serum BDNF levels in responders but not in non-responders. The present results are basically in accordance with previous reports that showed a regulation of peripheral BDNF levels in patients following ECT (Bocchio-Chiavetto et al., 2006; Marano et al., 2007). Thus, our results further support that this neurotrophin may play a role in the action of antidepressants (Karege et al., 2000a; Yoshimura et al., 2007). Marano et al. (2007) reported that 12 of 13 depressed patients who responded to ECT had an increase in plasma BDNF after their fourth ECT session. Bocchio-Chiavetto et al. (2006) demonstrated that serum BDNF levels were significantly increased one month after the end of ECT in antidepressant-refractory depressed patients. A longer duration of ECT may be needed to increase blood BDNF levels in refractory depressed patients. In the present study, we assessed the serum BDNF levels one week after finishing ECT (five weeks after starting ECT). It is possible that a longer treatment period is needed to elicit more robust increases in serum BDNF levels by ECT. In contrast to our findings, Gronli et al. (2007) found no significant changes in serum levels of nerve growth factor, BDNF, or neurotrophin-3 as a result of ECT. Nonetheless, it remains unclear how long it takes for ECT to increase peripheral BDNF levels. We recently demonstrated that eight weeks of treatment with paroxetine or milnacipran increased serum BDNF levels, whereas four weeks did not. In addition, responders to paroxetine or milnacipran had significantly higher serum BDNF levels after eight weeks of treatment compared to before treatment and to non-responders, in whom the levels were unchanged (Yoshimura et al., 2007). We have also reported that plasma BDNF levels were significantly increased in responders and partial responders, but not in non-responders, four weeks after repetitive transcranial magnetic stimulation (rTMS) treatment. In addition, we found an association between the changes in Ham-D scores and changes in plasma BDNF levels in all patients after rTMS treatment. These findings indicate that increased serum or plasma BDNF levels reflect improvement in the depressive state, regardless of the treatment type. Therefore, an increased serum or plasma BDNF level might be a biological state marker for recovering from depressive states via treatment with antidepressants, rTMS, or ECT. Moreover, rTMS or ECT most likely affects blood BDNF levels faster than antidepressants do.

The source of circulating BDNF remains unknown. Platelets, brain neurons, and vascular endothelial cells are currently considered to be putative sources. It was demonstrated that BDNF crosses the blood-brain barrier (Pan et al., 1998) and that BDNF levels in the brain and serum have been shown to undergo similar changes during the maturation and aging process in rats (Karege et al., 2002b). Furthermore, Lang et al. (2007) recently reported that serum BDNF concentrations reflect some aspects of neuronal plasticity, as indicated by the association of BDNF levels with those of *N*-acetylaspartate level in the cerebral cortex. These results indicate that blood BDNF levels might in part reflect the BDNF levels in the brain.

Another important finding of the present study was that ECT significantly reduced plasma HVA levels but not plasma MHPG levels, in parallel with the improvement of depressive symptoms. These results indicate that ECT's effects on dopaminergic neurons might be essentially associated with its clinical efficacy. We previously demonstrated that the levels of plasma MHPG, but not those of HVA, were significantly reduced after rTMS treatment, and that the change in plasma MHPG levels was negatively correlated with the change in scores of agitation. These findings suggest that noradrenergic neurons might be in part associated with the mechanisms for improving depressive state by rTMS (Yukimasa et al., 2006). Thus, it is possible that mechanisms of ECT's effect might be different from those of rTMS regarding the dynamics of catecholamines. In contrast, in several reports ECT increased, reduced, or did not alter cerebrospinal fluid or urinary monoamime metabolites levels (Jori et al., 1975; Harnryd et al., 1979; Lerer and Belmarker, 1982; Aberg-Wistedt et al., 1986; Lykouras et al., 1990). In addition, Linnoila et al. (1984) found no consistent change in plasma MHPG or plasma HVA, either acutely following seizure induction or at the end of the ECT course. Devanand et al. (1989) also failed to find significant ECT-induced acute or subacute changes in plasma MHPG or HVA. Taken together, these findings indicate that ECT's effect on catecholaminergic neurons remains controversial.

Depression is considered as a heterogeneous disorder consisting of various symptoms, which may be associated with the inconsistent results regarding ECT's effects on monoaminergic neurons. We previously demonstrated that depressed patients might be dichotomized into two groups; one characterized by anxiety, agitation, and/or hypochondriasis with high plasma MHPG levels, and the other by psychomotor retardation with low MHPG and/or HVA levels (Ueda et al., 2002; Shinkai et al., 2004). We also found a correlation between scores of agitation and/or anxiety in Ham-D and plasma levels of MHPG in 87 patients with major depressive disorder, indicating that depressed patients who predominantly exhibited agitation/anxiety were characterized by higher plasma MHPG levels (Yoshimura et al., 2004). In addition, we have also reported that patients with psychotic depression (depression with psychotic features) had higher plasma HVA levels, and combined treatment using antidepressants or mood stabilizers with atypical antipsychotic drugs decreased plasma HVA levels in parallel with improvement of depressive symptoms (Goto et al., 2006). Interestingly, in the present study, five of 18 patients were diagnosed as having major depressive episodes with psychotic features, all of whom responded to ECT. In regards to the association between BDNF and dopamine, Marano et al. (2007) reported that the presence of psychosis was associated with a greater percent increase in plasma BDNF, suggesting that patients with psychotic depression are particularly responsive to ECT. Taking those findings into account, it is plausible that hyper-dopaminergic activity might suppress the synthesis of BDNF, and ECT might affect the BDNF levels by reducing the dopaminergic activity. Indeed, Noh et al. (1999) demonstrated *in vitro* that both excessive and low catecholamine levels decrease neurosynthesis.

The ubiquitous free radical NO has a wide variety of roles in the central nervous system (Rosen et al., 2003). It acts as a neurotransmitter, a neuromodulator and an intraneuronal second-messenger, and both an anti- and pro-apoptotic activator (Rosen et al., 2003). Current research validates NO's ability to diffuse to adjacent neurons, thereby participating in non-synaptic diffusion neurotransmission. Several NO production factors have been implicated in the regulation of cerebral vascular tone at several levels. Endothelial NO (eNOS) is an important factor of cerebral blood flow. Decreased cerebral blood flow in the anterofrontal lobe as well as its recovery after ECT were reported in depressed patients (Navarro et al., 2004). Moreover, Chrapko et al. (2004) reported that the levels of both plasma NOx and platelet eNOS activity were significantly lower in subjects with major depressive disorder when compared to healthy controls. The authors also found that treatment with antidepressants increased these parameters in depressed patients (Chrapko et al., 2006). We thus speculated that ECT influenced the plasma NOx level. In the present study, however, we could not find an increase in this level after ECT, suggesting that NO might not be strongly involved in the mechanisms underlying ECT's effect.

In conclusion, we found in all patients a significant decrease in plasma HVA levels, but not plasma MHPG levels or plasma NOx levels as well as a significant increase in serum BDNF levels in responders to ECT. However, in non-responders, ECT did not change serum BDNF levels. The present findings suggest that the mechanisms of ECT in treatment-refractory depression may be in part related to dopaminergic neurons and BDNF.

We are aware of the limitation of the present study. Our sample size was very small and heterogeneous, which prevented us from obtaining informative results for response to treatment. Plasma levels of

catecholamine metabolites, NOx and serum BDNF levels appear to be partially derived from central sources (Yoshimura et al., 2004, 2007). In other words, blood levels of catecholamine metabolites, BDNF, and NOx only partially reflect the activities of the neurons in the brain. Serum BDNF levels are approximately 100 to 250 times greater than platelet-poor plasma. Platelets are the major source of serum BDNF, as they sequester large quantities of it in order to release during clotting. Sources of circulating plasma BDNF include vascular endothelial cells as well as the brain (Lommatzsch et al., 2005). Platelets have a life span of approximately 10 days (Harker et al., 2000), whereas, plasma BDNF turnover is completed about every 6 min (Podusto et al., 1996). Therefore, because it is minimally affected by the amount stored in platelets, plasma BDNF is likely to be a more suitable index of brain BDNF levels (Marano et al., 2007). Nonetheless, it remains unclear to what extent peripheral levels reflect brain levels of BDNF.

The most serious limitation in the present study was that there was no control group without ECT (the sham controlled group). This makes it difficult to attribute the improvements of Ham-D to ECT rather than to a placebo response. In addition, all of the patients were taking antidepressants, and neurochemical changes are not incompatible with a placebo effect, as imaging findings in depressed patients have shown that the clinical improvement following placebo treatment was substantiated by regional metabolic changes in the cortical and subcortical regions. Thus, definitively attributing the behavioral or neurochemical changes to ECT is not possible until these results are replicated in a controlled fashion. In addition, we combined the use of antidepressants with ECT, and hence these drugs may have affected plasma levels of catecholamine metabolites and NOx as well as serum BDNF levels. Therefore, further study will be needed to confirm these preliminary findings.

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Definitions of recovery and outcomes of major depression: results from a 10-year follow-up

Furukawa TA, Fujita A, Harai H, Yoshimura R, Kitamura T, Takahashi K. Definitions of recovery and outcomes of major depression: results from a 10-year follow-up.

Objective: Consensus operational definitions for symptomatic remission and recovery of a major depressive episode have been proposed but only irregularly followed.
Method: We examined the predictive validity of different definitions of recovery in a multi-center 10-year follow-up study of an inception cohort of untreated unipolar major depressive episodes ($n = 95$). Time to recovery and time to recurrence after recovery were estimated by Kaplan–Meier survival analyses for alternative definitions requiring 2, 4, 6 or 12 months of remission to declare recovery.
Results: The median time to recovery was 3.0, 4.0, 4.0 and 12.0 months respectively. The index episode lasted longer than 24 months in 9.4%, 9.2%, 12.6% and 24.5%. The median time to subthreshold recurrence was 16.0, 32.0, 42.0 and 74.0 months.
Conclusion: Either 4- or 6-month duration of remission defined a change point before which the episode was continuous and after which the recurrence was reasonably unlikely.

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Key words: depressive disorder; diagnostic criteria; remission; recovery

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Significant outcomes

- Requiring two months of remission is probably too short to declare recovery because a subthreshold recurrence occurs in more than half of the cohort within a year and a half.
- If we require 4 or 6 months before we declare recovery, the median time to recovery is 4 months and that to subthreshold recurrence is nearly 3 years.
- Requiring 12 months of remission before declaring recovery would make the episode discontinuous yet long and inflate the rate of chronicity.

Limitations

- The sample size was relatively small and the confidence intervals were accordingly wide.
- This was a naturalistic study and the treatments were not controlled.
- Validity of alternative definitions of remission requires a separate study.

Introduction

Confusions and inconsistencies persist in the literature with regard to operational definitions of critical change points in the course of a major depressive episode, such as remission, recovery, relapse and recurrence. It was the US NIMH Collaborative Depression Study (CDS), a landmark long-term cohort study of patients with mood disorder, that first operationalized remission as a state with no more than one or two mild depressive criterion symptoms, and recovery as

eight or more weeks of remission (1). Some have followed this convention (2–5), while others have not (6–9). But none of these studies has provided empirical examination of the validity of competing definitions.

With regard to the official diagnostic criteria, the definitions in DSM-IV are in line with the CDS definitions because they declare 'recovery' if 'full remission', which is defined as a period of at least 2 months in which there are not significant symptoms of mania or depression, is attained between two mood episodes (10). The ICD-10 also appears to follow this tradition when it requires 'at least 2 months free from any significant mood symptoms' for depressive disorder to be recurrent (11). On the other hand, the DSM-III-R was somewhat aberrant because it required 'no significant signs or symptoms of the disturbance for at least 6 months' for an episode to be in full remission, while requiring '2 months of return to more or less usual functioning' for the disorder to be recurrent (12). The DSM-III did not have any explicit definitions for recurrence or remission (13).

This state of confusion is unfortunate not only for the psychiatric sciences because then findings cannot be compared and accumulated, but also for the psychiatric services because then the literature cannot inform the practitioners about how to judge recovery and declare the end of continuation treatment or about whether to recommend maintenance treatment based on knowledge of likelihood of recurrence.

It is important to note that, in this framework for long-term treatment of depression (14, 15), emphasis is placed on the symptomatic aspects of the course of a major depressive episode. This is in line with recent definitions of remission in psychotic disorders (16, 17) and anxiety disorders (18). However, there is growing tendency, especially with regard to schizophrenia and other serious mental disorders, to use the term 'recovery' in conjunction with quality and meaning of life in spite of continued symptoms (19). Whether we need to include functional or even broader normalization in the definition of recovery with regard to depression is being discussed and researched (20), but in this article, we follow the symptomatological orientation currently adopted in the mood disorder section of the DSM-IV (10) and ICD-10 (11).

Aims of the study

The present article therefore sets out to examine the differential predictive validity of the competing definitions of symptomatic recovery. We propose that a more valid definition of recovery should define a change point until which the syndrome is relatively continuous, but after which a return of the syndrome will become reasonably unlikely.

Material and methods

Data for this report come from the Group for Longitudinal Affective Disorders Study (GLADS), described in detail elsewhere (5, 21). Briefly, it is a multi-center collaborative naturalistic study of patients with heretofore untreated mood episodes who had presented to various psychiatric facilities all over Japan.

The 23 collaborating centers included psychiatric departments of 13 university hospitals and six general hospitals, three mental hospitals and one community mental health center. Participating psychiatrists at each center administered a semi-structured interview, called the Psychiatric Initial Screening for Affective Disorders (PISA) (22) to a representative subset of its first-visit patients to ascertain the patient's eligibility. The details of the predetermined rules on how to select a subset of first-visit patients were left to individual centers, depending on their human and logistic resources: some centers administered PISA to all their first-visit patients, others did so with those on a certain day of the week and still others did so with those seen by one or two collaborating psychiatrists only. The eligibility criteria were: i) depressive state or manic state; ii) having received no antidepressant or antipsychotic medication in the preceding 3 months; iii) aged 18 years or older; and iv) absence of conditions that would render detailed psychopathological assessment difficult. Out of all the eligible subjects, each participating center was expected to enter the first such patient every 1 or 2 months.

The study protocol was approved by the Ethics Committee of the National Center of Neurology and Psychiatry, Japan, as well as those of the participating centers. Written informed consent was obtained from all participants after full disclosure of the purposes and procedures of the study. The patients eligible for and consenting to the study were then interviewed within 1 week of entry by a psychiatrist using the entry version of the Comprehensive Assessment List for Affective Disorders (COALA) (23). The COALA consists of a series of semi-structured interviews that enable serial assessment of the cohort; these include the entry version, monthly follow-up

version, 6-monthly follow-up version and yearly follow-up version. The reliability of the PISA and COALA has been reported to be good to excellent (24). The cohort was followed up monthly until treatment termination, 6-monthly thereafter up to 2 years and then annually up to 10 years. At each assessment, the course of the illness was recorded for each month of the survey period in five grades of 5 = above diagnostic threshold for major depressive episode, 4 = between 5 and 3, 3 = asymptomatic or minimally symptomatic with at most two of nine diagnostic criteria symptoms of at most mild degree, 2 = between 3 and 1 and 1 = above diagnostic threshold for manic episode.

The present paper focuses on the course of the subset of the cohort who were diagnosed with major depressive disorder according to DSM-IV (10). We defined remission in accordance with the NIMH CDS as a state with no more than one or two mild criterion depressive symptoms (2). The CDS then defined recovery as consecutive two months of remission. Once recovery was declared, patients were considered to have fallen into a new mood episode (recurrence) when they met the DSM-IV criteria for major depressive episode, manic episode or hypomanic episode. In addition, if they did not yet meet the criteria for a major depressive episode but had more than two symptoms or had only one or two symptoms that were graver than mild degree for a month, they were considered to have fallen into a 'subthreshold' depressive episode. The duration of the well interval was counted after the end of the period required for judging recovery.

The CDS definition of recovery by 2 months of remission has been criticized for being too short (25, 26) and the consensus definitions proposed alternative definitions of recovery by 4 or 6 months of remission. The present paper examines the predictive validity of alternative duration requirements of 2, 4, 6 and 12 months of remission to define recovery. We hypothesized that a more valid definition of duration required for declaring recovery would:

i) not prolong the duration of the index episode too much, lest the episode contains too long well periods in itself;
ii) not increase the rates of chronicity (never attaining recovery) too much, lest we give an overly pessimistic impression that depression is a chronic or incurable disease;
iii) ensure that the time to recurrence is reasonably long, so that 'recovery' once declared can assure the patients that a return of symptoms is reasonably unlikely.

We used the SPSS for Windows 11.5 (27) to perform Kaplan–Meier analyses to depict survival curves of the major depressive episodes.

Results

A total of 1853 patients were screened at 23 participating centers between December 1992 and December 1995. A total of 466 patients suffered from broadly defined mood disorders, but either failed to meet the other entry criteria or declined consent and 126 entered the study. Of these, 95 met the DSM-IV criteria for major depressive disorder, either single episode (n = 67, 71%) or recurrent (n = 28, 29%). Fifty-six subjects (59%) were females, and the mean age was 44.3 (SD 15.2). The mean score for the 17-item Hamilton Rating Scale for Depression was 19.9 (SD 8.6) and 14 (15%) were inpatients upon study entry. The major depressive episode was superimposed on pre-existing dysthymia in five (5%). The median length of episode before study entry was 3.0 months (range: 0.5–48.0).

Table 1 gives the median duration of the index episode and rates of chronicity at 12, 24, 60 and 120 months, depending on the numbers of months of remission required to declare recovery. The table also gives the median time to recurrence of a full episode or a subthreshold episode after recovery

Table 1. Outcomes of major depressive episodes for different definitions of recovery

Definition of recovery	Follow-up rate (%)	Median duration of index episode (months)	Rates of chronicity (%)				Median length of well interval (months)	
			12-month	24-month	60-month	120-month	Until full episode recurrence	Until subthreshold recurrence
2 months of remission	90.5	3.0 (2.3–3.7)	16.4	9.4	5.4	3.6	103†	17.0 (1.9–32.1)
4 months of remission	89.5	4.0 (2.9–5.1)	21.9	9.2	6.2	4.1	>101‡	32.0 (1.6–62.4)
6 months of remission	88.4	4.0 (2.2–5.8)	29.9	12.6	6.3	4.2	113 (62–164)	47.0 (10.4–83.6)
12 months of remission	82.1	12.0 (7.9–16.1)	46.0	24.5	12.7	5.1	97†	74.0†

Numbers in parentheses represent 95% confidence intervals.
†95% confidence intervals could not be calculated.
‡The cumulative rate of relapse at the latest follow-up of 112 months was 53.2%, so that the median can be estimated to be close to 120.

was declared, according to each proposed definition.

We illustrate the actual numbers of patients reaching each critical change points or being lost before reaching one in the case of the operational definition of recovery requiring 6 months of remission. Of the original cohort of 95 patients, 84 patients reached recovery so defined, 10 patients were lost to follow-up before reaching recovery and one never experienced recovery over the entire 120 months of follow-up. Of these 84 who were judged recovered, 10 never had a recurrence until the end of the 120-month follow-up, 29 experienced a full episode recurrence, additional 11 experienced a subthreshold recurrence and one presented with a manic episode, 33 were lost to follow-up without ever recording any of these events. Because this was a naturalistic follow-up study and the treatment was not controlled, around the time of recovery, the patients were receiving on average 45.1 (SD 64.7, IQR 0–60) mg/day of imipramine equivalent and only 16 (19%) were on >75 mg/day.

Discussion

This is the first study to examine the predictive validity of different duration requirements of remission to achieve recovery in terms of the length of the index episode, rates of chronicity and the succeeding well interval until recurrence, based on a long-term naturalistic follow-up data. We found that different definitions can give up to fourfold differences in estimates of episode length and time to subthreshold recurrence but not in time to full recurrence.

Several recent studies have shown that subthreshold depression is associated with psychosocial disability and more severe future course of the illness and requires treatment (28, 29). A systematic review of continuing antidepressant treatment after acute phase treatment reported a consistent relative risk reduction of about 50% in relapse rates up to 3 years (30). For a representative patient in our cohort, then, even if recovery is achieved after 2 months of remission, continuing adequate antidepressant treatment for 1½ years would reduce the subthreshold recurrence rates from 50% to 25%; an average patient may then very well wish to stay on medication. On the other hand, if recovery is declared after 4 or 6 months of remission, one needs to be on medication for 3–4 years to reduce the subthreshold recurrence rates from 50% to 25%; many if not all the patients may choose to stop the medication.

Given our hypotheses regarding the predictive validity of the operational definition of recovery, requiring 12 months would include so much well time before recovery is declared as to draw an unnecessarily chronic picture for the index episode. Requiring only 2 months, on the other hand, would devaluate the significance of recovery because half of the patients so declared would experience a subthreshold recurrence within 1½ years. It is noteworthy that the time to full recurrence remained constant at about 100 months for the three definitions examined. However, given the clinical significance of subthreshold depression noted above, requiring 4- to 6-month remission before declaring recovery appears to be a reasonable definition, as it would not make the index episode unnecessarily chronic, yet assures a relatively low likelihood of subthreshold recurrence once recovery is declared and can provide some indication for ensuing treatments.

There are some possible weaknesses of the present study. First, the sample size was relatively small and 95% confidence intervals were sometimes incalculable or very wide, especially with regard to time to full episode recurrence. This may partly explain the apparent lack of differentiation among various definitions of recovery with regard to this variable. Secondly, this was a naturalistic study, in which we did not control the treatment, and the amount of treatment, actually provided was very low. During the continuation phase, 43% were not receiving any antidepressant therapy and a further 37% were on inadequate treatment with <75 mg/day of imipramine equivalent or <600 mg/day of lithium. Six months later, during the maintenance phase, the corresponding figures were 50% and 29% (31). Thirdly, our cohort consisted mainly of first episode patients and with less severe symptomatology, and this may have influenced estimates of time to recurrence and may be another reason for lack of difference in times to full episode recurrence among various definitions of recovery that we examined. However, Cox regression analyses did not reveal statistically significant influence of recurrent vs. single episode in the median durations of the index episode or the median lengths of the ensuing well intervals. It would be interesting to see analyses similar to ours with the data available from other long-term studies of more severe, recurrent cohorts. Finally, we could not examine the influence of different definitions of remission. One study clearly pointed to the importance of this definition because different symptomatological cut-offs to define remission resulted in an almost sevenfold increase in the length of a depressive episode (32). There are also

arguments for including more than symptomatic criteria to define remission (33). These problems need separate examination.

The greatest strength of the current study, on the other hand, is the high follow-up rates achieved through serial assessments of a prospective cohort for 10 years. Some drop-outs were inevitable and we adjusted for the censored cases through survival analyses. The current DSM-IV follows the NIMH CDS tradition and requires 2 months of no significant signs or symptoms of the disturbance before declaring 'full remission'. The naturalistic follow-up data given in the present report along with the functional and prognostic significance of subthreshold depression demonstrated in cross-sectional and longitudinal studies (28), and the experimental treatment data summarized in the meta-analysis (30) warrant reconsideration of the consensus definitions of remission, recovery, relapse and recurrence in major depression for the upcoming DSM-V.

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