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Regular Article

Perospirone in the treatment of patients with delirium

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Abstract Perospirone is a recently developed atypical antipsychotic with potent serotonin 5-HT_2 and dopamine D_2 antagonist activity. Other atypical antipsychotics including risperidone, quetiapine and olanzapine have been widely used for treatment, not only for schizophrenia symptoms but also for delirium, because of their low potential to induce extrapyramidal disturbances. In the present study the effectiveness and safety of perospirone in patients with delirium are described. Thirty-eight patients with DSM-IV delirium were given open-label perospirone. To evaluate the usefulness of perospirone, scores from 13 severity items of the Delirium Rating Scale-Revised-98 were assessed. Data were gathered from October 2003 to September 2004. Perospirone was effective in 86.8% (33/38) of patients, and the effect appeared within several days (5.1 ± 4.9 days). The initial dose was 6.5 ± 3.7 mg/day and maximum dose of perospirone was 10.0 ± 5.3 mg/day. There were no serious adverse effects. However, increased fatigue (15.2%), sleepiness (6.1%), akathisia (3.0%) and a decline in blood pressure (3.0%) were observed. It is proposed that perospirone may be another safe and effective atypical antipsychotic drug for the treatment of delirium symptoms in hospitalized patients. This is a preliminary open trial, and further randomized double-blind placebo-controlled tests are needed.

Key words atypical antipsychotics, delirium, perospirone.

INTRODUCTION

Delirium seen in hospitalized patients often produces a significant problem in the treatment and nursing of the underlying diseases.[1] It occurs in 10–30% of the hospitalized medically ill,[2] and it is associated with both increased mortality and longer hospitalization.

High-potency typical antipsychotics such as haloperidol are the drugs of first choice in the treatment of delirium. However, haloperidol is frequently associated with adverse effects, especially extrapyramidal symptoms (EPS) such as parkinsonism and dystonia.

Atypical antipsychotics, including risperidone,[3–10] quetiapine,[11–16] and olanzapine[12–20] have been widely used for treatment not only for schizophrenia symptoms but also delirium, because of their low potential to induce extrapyramidal disturbances.[21,22]

Perospirone is an atypical antipsychotic recently developed in Japan. It is an antagonist of serotonin 5-HT_2 and dopamine D_2 receptors, and also a partial agonist of the 5-HT_{1A} receptors.[23–26] In the present study we describe the effectiveness and safety of perospirone in patients with delirium.

METHODS

Patients

We examined patients from medical and surgical inpatients who were referred to psychiatrists at Tokyo Medical and Dental University between October 2003 and September 2004. Those able to satisfy the following requirements were enrolled as subjects in the study: (i) diagnostic criteria for delirium in the DSM-IV; (ii) oral

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perospirone; (iii) no history of substance abuse or any other mental disorders; and (iv) able to be thoroughly followed by the authors through the course of their delirium. A total of 38 patients met the inclusion criteria for this study.

Written informed consent to participate was obtained from patients and/or family members responsible for the patients before starting perospirone therapy.

Measurements

The severity of delirium was evaluated using the severity items of the Delirium Rating Scale-Revised-98 (DRS-R-98), which is a 16-item scale with 13 severity items and three diagnostic items.[27] The severity items represent symptoms that are rated on a scale of 0–3 points, and the maximum severity score is 39 points. We obtained permission to use this scale from Paula T. Trzepacz.

Procedures

The severity of delirium was evaluated in each patient using the DRS-R-98 before starting perospirone treatment. After the treatment, the patients were examined every day. The DRS-R-98 was rated based on the examination, charts and nursing records. The period to maximum effect was defined as the time to the minimum DRS-R-98 score. A marked improvement was defined as >50% reduction in the baseline severity score of DRS-R-98, a moderate improvement as 25–50% reduction, and no improvement as 0–25% change in the score. Adverse effects were also investigated during the study.

Perospirone treatment was usually started at 4 or 8 mg/day. Subsequent titration of the dosage was based on clinical decision. Perospirone was titrated upward until the maximum clinical effect was obtained or until intolerable adverse effects necessitated cessation. After the period to maximum effect, perospirone treatment was continued for 1 week and the study was terminated after confirming the absence of a marked change of symptoms. Thereafter perospirone was reduced or stopped according to the conditions of each case. Perospirone was administered orally once daily before bedtime. No concomitant psychotropic drugs were permitted during the study except for benzodiazepines.

During the first visit, medical and psychiatric histories were recorded. Electrocardiogram and laboratory studies were performed regularly. Blood pressure and heart rate were measured every day.

Statistical analysis

Estimates are reported as mean scores and standard errors (mean ± SE). Two-tailed paired *t*-test was used to analyze changes. $P < 0.05$ was considered significant.

RESULTS

Baseline characteristics of patients

Thirty-eight delirium patients were enrolled in the study. The mean ± SD age of the patients was 69.4 ± 10.1 years. The patients consisted of 31 men and seven women (Table 1).

The etiologies of their delirium were as follows: postoperative state, $n = 22$ (57.9%; cardiovascular disease, brain tumor, brain aneurysm, brain hemorrhage, stomach cancer, otolaryngological disease etc.); heart failure, $n = 4$ (10.5%); aortic dissection, $n = 2$ (5.3%); brain tumor, $n = 2$ (5.3%); brain infarction, $n = 2$ (5.3%); other disease, $n = 6$ (15.8%).

Prior to the study, 20 patients had undergone drug therapy for delirium but experienced no clinically beneficial effects. Sixteen of them had been given haloperidol injections. Haloperidol at a dose of 2.5–5.0 mg/day had been used temporarily in all patients, but not continuously. Mianserin was used for three patients, and chlorpromazine for one patient during a few days.

Effectiveness and drug treatment

Thirty-three patients (86.8%) were improved. Twenty-seven patients (71.1%) had a marked improvement, six (15.8%) a moderate improvement, and five (13.2%) showed no improvement (Table 2).

Thirty-three patients who showed marked or moderate improvement were investigated. The mean ± SD initial dose of perospirone was 6.5 ± 3.7 mg/day, and

Table 1. Patient characteristics

	n (%)
Age (years) (mean ± SD)	69.4 ± 10.1
Sex	
Male	31
Female	7
Diagnosis	
Postoperative state	22 (57.9)
Heart Failure	4 (10.5)
Aortic dissection	2 (5.3)
Brain tumor	2 (5.3)
Brain infarction	2 (5.3)
Other	6 (15.8)

Table 2. Global assessment for effect of perospirone

	n (%)
Marked improvement	27 (71.1)
Moderate improvement	6 (15.8)
No improvement	5 (13.2)

Table 3. Medication history and changes of the severity score of the DRS-R-98†

	Mean ± SD
Initial dose (mg/day)	6.5 ± 3.7
Maximum dose (mg/day)	10.0 ± 5.3
Day of maximal response	5.1 ± 4.9
DRS-R-98	
Before treatment	23.9 ± 7.6
After treatment	7.0 ± 6.0*

* $P < 0.05$, versus pretreatment.
†For 33 patients who showed marked or moderate improvement.
DRS-R-98, Delirium Rating Scale-Revised-98.

Table 4. Adverse effects

	n (%)
Increased fatigue	5 (15.2)
Sleepiness	2 (6.1)
Akathisia	1 (3.0)
Decline in blood pressure	1 (3.0)

the mean ± SD maximum dose was 10.0 ± 5.3 mg/day (Table 3).

The mean ± SD duration of treatment until the period to maximum effect was 5.1 ± 4.9 days. The mean of the severity score of DRS-R-98 was 23.9 ± 7.6 at baseline and 7.0 ± 6.0 after the period to maximum effect; the change from baseline indicated a statistically significant improvement ($P < 0.001$).

Safety

No serious adverse effects were observed, and none of the patients discontinued perospirone due to adverse effects. However, five patients (15.2%) experienced increased fatigue and two (6.1%) experienced sleepiness, one (3.0%) akathisia and one (3.0%) a decline in blood pressure (Table 4). There were no consistent changes or clinically relevant abnormalities in electrocardiogram, laboratory studies or heart rate.

DISCUSSION

We investigated the therapeutic effectiveness and safety of perospirone in patients with delirium. The benzisothiazole derivative perospirone is an atypical antipsychotic agent available in Japan for the treatment of schizophrenia. Its pharmacologic profile is similar to that of risperidone, having a potent serotonin 5-HT_2 and dopamine D_2 antagonist (SDA) activity.[24]

Perospirone binds with high affinity to serotonin 5-HT_{2A} receptors. The affinity of perospirone for rat 5-HT_{2A} receptors was >100-fold higher than that of haloperidol. It is known that selective 5-HT_2 receptor antagonists increase slow-wave sleep. In patients with delirium, sleep disturbance is one of the most frequent symptoms, therefore it is possible that perospirone, like risperidone, has a beneficial effect on the quality of sleep and improves delirium by reducing sleep–wake-rhythm disturbances.[23]

Perospirone also binds with high affinity to dopamine D_2 receptors. The affinity of perospirone with D_2 receptors *in vitro* is similar to that of haloperidol. Therefore perospirone induces a sedative efficacy that is equivalent to haloperidol, with few EPS due to serotonin 5-HT_{2A} antagonist activity.[23]

The affinity of perospirone with H_1 receptors is similarly high. It would contribute sedative action.

The low affinity of perospirone with muscarinic M_1 receptors and α1-adrenergic receptors may be an advantage in the treatment of delirium, because action as an M1 receptor antagonist could exacerbate delirium by influencing cognitive function, and action as an α1 receptor antagonist could induce hypotension.

Sleepiness was not observed, except in two patients (6.1%). This is because the half-time of serum concentration ($T_{1/2}$) of perospirone is shorter than that of haloperidol and risperidone. Therefore perospirone is viable for elderly patients and medically ill patients who are easily sedated.

Perospirone also acts as a partial agonist of the 5-HT_{1A} receptors. It was found that 5-HT_{1A} receptor activation contributes to dopamine release in the prefrontal cortex, so that cognitive function is improved.[26] This feature of perospirone may induce amelioration in patients with delirium.

In Japan olanzapine and quetiapine, which are classified as multi-receptor targeting antagonist (MARTA), are forbidden for patients with diabetes. Diabetes is frequently observed among the patients with delirium, therefore it is difficult for us to use MARTA. Consequently we suggest that SDA are eligible as the first choice for patients with delirium, especially perospirone because its short $T_{1/2}$ makes it very safe.

This study indicates that perospirone is an effective and safe alternative to conventional antipsychotics such as haloperidol for the treatment of delirium. This trial is a preliminary open study, and further controlled studies will be needed to confirm our findings.

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Regular Article

Quantitative magnetic resonance spectroscopy of schizophrenia: Relationship between decreased *N*-acetylaspartate and frontal lobe dysfunction

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Abstract Numerous studies using proton magnetic resonance spectroscopy (^{1}H-MRS) have detected signal changes in schizophrenia. However, most studies investigated relative concentrations such as *N*-acetylaspartate/creatine plus phosphocreatine (NAA/Cre) and choline-containing compounds/creatine plus phosphocreatine (Cho/Cre), and individual metabolite concentrations have not been clarified. Using absolute quantification measurement of ^{1}H-MRS, the aim of the present paper was to demonstrate the changes in metabolite concentrations in the frontal lobe of patients with chronic schizophrenia. The ^{1}H-MRS was performed in the left frontal lobe in 14 patients with schizophrenia and in 13 healthy comparison subjects. Individual MRS peak concentration was quantified based on a frequency-domain fitting program: LCModel. The scores on the Positive and Negative Symptoms Scale and Wisconsin Card Sorting Test were used for clinical assessment. The NAA concentration was reduced in schizophrenic patients (average, 7.94 mmol/L, $t = 2.28$, $P < 0.05$) compared with healthy subjects (average = 8.45 mmol/L) while choline, creatine or NAA/Cre ratio did not show any differences. The reduction in NAA concentration had a significant correlation with the severity of negative symptoms ($r = -0.536$, $P < 0.05$) and poor performance in Wisconsin Card Sorting Test ($r = -0.544$, $P < 0.05$). Using quantitative MRS, decreased NAA concentration was confirmed in the left frontal lobe of schizophrenic patients and was demonstrated to be correlated with negative symptoms and cognitive dysfunction in schizophrenia.

Key words absolute quantitative method, left frontal lobe, negative symptoms, proton magnetic resonance spectroscopy, schizophrenia, Wisconsin Card Sorting Test.

INTRODUCTION

Proton magnetic resonance spectroscopy (^{1}H-MRS) is a non-invasive functional neurological measurement. The ^{1}H-MRS imaging detects signals arising from *N*-acetylaspartate (NAA), choline-containing compounds (Cho), and creatine plus phosphocreatine (Cre). The NAA signal is thought to represent neurons. *N*-acetylaspartate is located almost exclusively in neurons and its reduction has been considered not only as a marker for neuronal loss but also of the level of neuronal functioning including mitochondrial activity.[1] The creatine signal arises from the combination of creatine and phosphocreatine. The choline signal is mainly from choline, phosphocholine and glycerophosphocholine. Elevation of choline probably means increased membrane phospholipid turnover or demyelination, indicating conditions such as brain tumors or inflammation.[2]

Patients with schizophrenia often present negative symptoms such as flattened affect, alogia, avolition,

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and cognitive impairment, as well as positive symptoms such as hallucinations and delusions. In general, negative symptoms remain persistent, are relatively unresponsive to treatment,[3] and are believed to be caused by frontal lobe dysfunction. Previous studies have found a relationship between frontal lobe abnormalities and negative symptoms, suggesting the hypothesis of hypofrontality,[4] and another study reports an association between some negative symptoms and low left-frontal blood flow both at rest and during Wisconsin Card Sorting Test (WCST).[5] Although its specificity has been questioned, the WCST is thought to represent frontal lobe function, which involves working memory and the ability to adapt behavior based on performance feedback.[6] Patients with schizophrenia show greater impairment with this cognitive challenge.[7–9]

Magnetic resonance spectroscopy has been widely used in the field of psychiatry and clinical neuroscience, especially in providing important additional information about the epileptogenic focus.[10] Numerous MRS studies have documented alterations in metabolite signals of schizophrenic patients. Most of them analyzed MRS spectra by applying metabolic ratios, and they found decreased NAA/Cre ratio in frontal regions, suggesting that the NAA reduction was related to neuronal damage in schizophrenia.[11–20] However, some of the previous studies have found metabolite changes not only in NAA but also in choline and creatine.[15,17] A recent MRS study found no difference in NAA/Cre ratio but found reduced NAA/Cho ratio and elevated Cho/Cre ratio in the cingulate cortex in schizophrenia.[21] Considering these results, each metabolite concentration should be measured quantitatively based on absolute quantification. However, only a few MRS studies have investigated the absolute concentrations, and they have come up with inconsistent results.[22–24] It is probable that NAA reduction in schizophrenia reflects neuronal changes and relates to schizophrenic symptomathology, but most of the previous studies analyzing metabolite ratios failed to establish a significant correlation with symptom scores. Further, there have been few studies demonstrating a correlation between MRS signals and cognitive performance in the frontal lobe.

Metabolite concentrations for NAA, Cho, and Cre were estimated using a frequency-domain fitting program: LCModel.[25] The LCModel estimation is based on a comparison of *in vivo* spectra to *in vitro* spectra. This basis set is a complete model of spectra acquired from a set of metabolite solutions under the same experimental conditions. Because it analyzes the *in vivo* spectrum as a linear combination of *in vitro* spectra, two metabolites with overlapping peaks can be separated if their spectra are sufficiently different. This program enables estimation of absolute concentrations in institutional units by correcting for the transmission gains.

In the present study, using ^{1}H-MRS analysis based on the LCModel, we aimed to clarify the changes in individual metabolite concentrations in the frontal lobe of patients with chronic schizophrenia and to investigate correlations of MRS signal alterations with symptomathology and cognitive dysfunction.

METHODS

Subjects

We studied 14 patients with schizophrenia diagnosed according to DSM-IV criteria. They consisted of four women and 10 men (all right-handed) with a mean age of 29.4 ± 4.1 years. They were recruited from outpatients of Asai Hospital, Chiba, Japan. All the patients were receiving a stable dose of antipsychotic medication at the time of scanning (Table 1). The average dose of medication was 9.5 mg/day (haloperidol-equivalent). The mean duration of illness was 9.4 ± 3.7 years. The patients had no history of substantial medical illness, head injury, neurological disorder, any other psychiatric disorder, or clinically significant alcohol or substance abuse. They had no atrophy of the brain on MR imaging, which was judged by two experienced specialists, a radiologist and a neuropsychiatrist. The healthy control subjects were three women and 10 men (all right-handed) with a mean age of 29.5 ± 4.1 years. The same exclusion criteria as for the patients were applied. Patient characteristics and treatment are summarized in Table 1. In demographic characteristics, no significant difference was found in the distribution of age or sex between schizophrenia patients and healthy control subjects (Table 1).

Informed consent

Before participating, an explanation of the risks and benefits of the MR procedure was given to all subjects, and all gave their written informed consent. This study was approved by the ethic committee of the hospital.

Clinical assessments

Before the MR scanning, the psychopathology and frontal lobe function of the patients were assessed. Psychopathology was evaluated by two experienced psychiatrists (TS, YT) using the Positive and Negative Symptoms Scale (PANSS).[26] They then reviewed the ratings after the interviews, and disagreements were resolved by consensus; the consensus ratings were used in the present study. The symptom scores were calcu-

Table 1. Patient information

Subject	Sex	Age (years)	Drug	Onset age (years)	Duration of illness (years)	Handedness
1	M	34	HPD18 CP125	28	6	Right
2	F	22	QUE300 Ola20	12	10	Right
3	F	34	HPD10	27	7	Right
4	M	30	LP150	21	9	Right
5	M	26	QUE600	18	8	Right
6	F	32	RIS2	28	8	Right
7	M	23	OLA5	15	8	Right
8	M	30	RIS4	18	12	Right
9	M	30	RUE150	15	15	Right
10	M	30	RIS2	22	8	Right
11	F	33	QUE300	21	12	Right
12	M	24	RIS6	18	6	Right
13	M	34	RIS2	29	5	Right
14	M	30	OLA15	22	8	Right

The number next to the drug name represents the drug dose (mg).
CP, chlorpromazine; HPD, holoperidol; LP, lovomepromazine; Ola, olanzapine; Que, queriapine; RIS, risperidone.

lated as sum, positive, negative, and general subscores of PANSS.

Frontal lobe function was assessed by the WCST. This test evaluates abstract problem-solving strategies, in which patients have to sort cards according to color, form and, number, switching their approach as unannounced shifts in the sorting principle occur, and the number of perseveration errors (p-errors) is counted. A larger number of p-errors suggests poorer frontal lobe function.[27]

Magnetic resonance spectroscopy

A Signa MR system (General Electric Medical Systems, Milwaukee, WI) operated at 1.5 T was used in this study. The ^{1}H-MRS was performed with a bird-cage-type coil. The volumes of interest (VOI) were set at the left frontal lobe including Brodmann area 9 under the guidance of proton scout images, with their size being $1.5 \times 1.5 \times 1.5$ cm so as to minimize the amount of cerebrospinal fluid (CSF) contained in the VOI. We visually checked T1 and T2 imaging and located VOI to avoid CSF. Repetition time (TR) was 3000 ms, and echo time (TE) was 30 ms. A volume selective spin-echo sequence (Point-resolved spectroscopy, PRESS) was used. There were 1024 data points, and the bandwidth was 2000 Hz. Scan average was 128 times and scan time was 7 min. Global and local shimming was performed before the ^{1}H-MRS sequence. To evaluate the structural brain changes, approximately 90 axial MR images, covering the entire brain, were taken for each subject. An inversion recovery pulse sequence (TR, 2500 ms; TE, 20 ms; inversion time, 300 ms) was used. Matrix size was 205×256, and field of view was 230 mm. Slice thickness was 2 mm without slice gaps. Total scan time was approximately 20 min.

Metabolite concentrations for NAA, total creatine (phosphocreatine), and choline were estimated using LCModel. We used a basis set for TE of 30 ms (General Electric Medical Systems). At the same time, we analyzed the spectrum from a phantom containing 50 mmol/L NAA with LCModel to calibrate for absolute concentrations.

The basis set also contains alanine, asparagines, γ-aminobutyric acid, glutamine, glutamate, inositol, lactate, and taurine, but signals from those metabolites were too uncertain and scattered so we did not take them into account. Fitting quality of each spectrum was shown as percent SD. Spectra with SD >20% were rejected from analysis.

Statistical analysis

For comparison of the mean values of the metabolite concentrations between patients and control subjects, Student's t-test was used. Correlation between the metabolite concentrations and each of the clinical parameters (PANSS score: positive, negative, general; WCST score: no. p-errors) were evaluated using Spearman's correlation test. Statistical significance was set at $P < 0.05$.

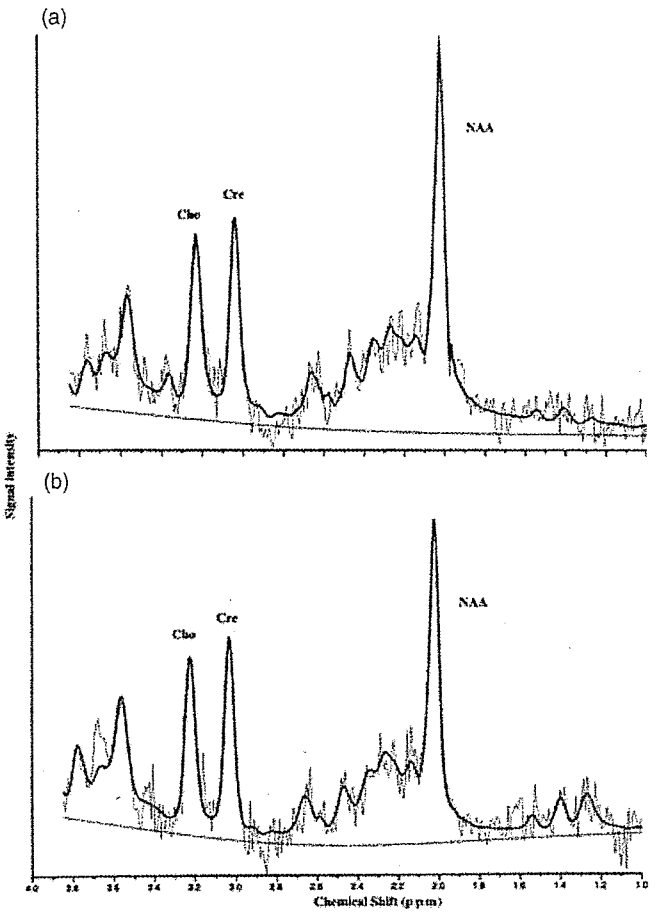

Figure 1. A typical ^{1}H spectrum acquired from the left frontal lobe of (a) a control subject and (b) a schizophrenic patient. It shows strong sharp resonances from *N*-acetylaspartate (NAA) at 2.0 p.p.m., creatine/phosphocreatine at 3.0 p.p.m., and choline compound at 3.2 p.p.m., with average line width of approximately 7 Hz.

RESULTS

Reduction in metabolite concentrations in schizophrenia

A typical ^{1}H spectrum acquired from the left frontal lobe of a control subject had sharp resonances from NAA at 2.0 p.p.m., Cre at 3.0 p.p.m., and Cho at 3.2 p.p.m., with an average line width of approximately 7 Hz (Fig. 1a). Schizophrenia patients had significant reduction in NAA concentration compared with healthy controls ($t = 2.28$, $P < 0.05$, Figs 1b,2a), while there was no significant difference in either choline concentration or creatine concentrations (Table 2). The NAA/Cre ratios were 1.61 ± 0.23 and 1.71 ± 0.19 in schizophrenia patients and control subjects, respectively, showing a slight but not statistically significant decrease in the patients.

Table 2. Concentration of *N*-acetylaspartate, choline and creatine in frontal lobe

	NAA (mmol/L)	Cho (mmol/L)	Cre (mmol/L)
Control	8.45 ± 0.59	1.42 ± 0.20	4.98 ± 0.70
Schizophrenia	7.94 ± 0.60	1.39 ± 0.21	4.96 ± 0.56
Statistics†	$P < 0.032$	NS	NS

†Independent *t*-tests; NS: $P > 0.05$.
Absolute values are means ± SDs.
Cho, choline; Cre, creatine; NAA, *N*-acetylaspartate.

Correlations between NAA concentration and the PANSS subscale and WCST

For the schizophrenia patients, a significant negative correlation was observed between NAA concentration and the PANSS subscale for negative symptoms ($r = -0.536$, $P < 0.05$, Fig. 2b). Furthermore, there was a significant negative correlation between NAA concentration and the number of *p*-errors in the WCST ($r = -0.544$, $P < 0.05$, Fig. 2c). No other clinical measurements (PANSS positive or general symptoms, duration of illness, dose of antipsychoticcs) had any correlation with NAA concentration.

DISCUSSION

The present study shows that the NAA concentration in the left frontal lobe was significantly lower in patients with schizophrenia in comparison with healthy controls, while choline and creatine concentrations did not differ between the two groups. Many studies have reported metabolite ratio change in schizophrenia, but few studies have measured the absolute metabolite concentrations (Table 3). In the present study we did not find any reduction in NAA/Cre ratio in schizophrenia patients, but we did find a statistically significant decrease in the absolute concentration of NAA. This suggests that quantitative measurement could reflect metabolite changes with greater sensitivity than metabolite ratios. The present results were contradictory to some previous results. The findings of no difference in NAA/Cre ratio but reduced NAA/Cho in schizophrenia patients have suggested that the reduction in NAA/Cho might be caused by increased Cho rather than decreased NAA.[21] Using LCModel, which allows the measurement of each MRS peak concentration quantitatively, we demonstrated that the abnormality in the MRS signal in schizophrenia is attributable not to an increase in choline or creatine but rather to a decrease in NAA itself. The NAA

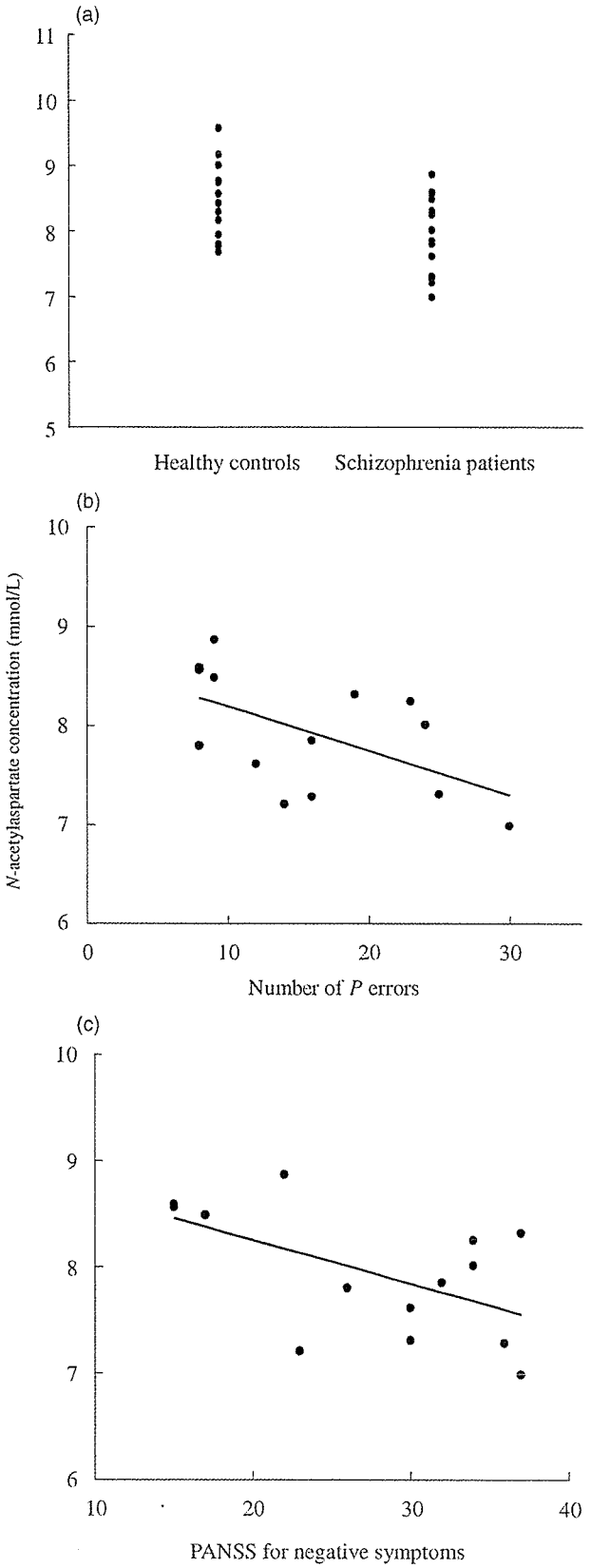

Figure 2. Metabolite concentrations for *N*-acetylaspartate (NAA) in schizophrenia patients and healthy controls. (a) The NAA concentration was significantly lower in patients compared with healthy controls (7.94 mmol/L, 8.45 mmol/L on average, respectively). (b) The NAA concentrations vs perseveration errors (*p*-errors) in Wisconsin Card Sorting Test (WCST) and (c) correlation of NAA concentrations vs severity in negative symptoms. Both negative symptom scores and *p*-errors were significantly correlated with NAA concentrations ($r = -0.536$, $P < 0.05$; $r = -0.544$, $P < 0.05$, respectively). The lines represent the expected values in NAA concentrations to the negative symptom scores or *p*-errors obtained by linear regression.

reduction is thought to result from neuronal losses in the brain of schizophrenia patients. Considering the postmortem human data that loss of neurophils and soma size is characteristic of schizophrenia,[28] it is likely that NAA reduction reflects decreased neurophils and soma size in schizophrenia.

There is a possibility that reduction in NAA could be involved in the pathology of schizophrenia. As an endogenous peptide, *N*-acetyl aspartylglutamate (NAAG) act as an *N*-methyl-*D*-aspartic acid (NMDA) receptor antagonist, and low NAAG level could increase glutamate release. *N*-acetyl aspartylglutamate exists in cortical and hippocampal pyramidal neurons, is synthesized from NAA and glutamate, and the amount of NAA may limit NAAG synthesis.[29]

Previous studies showed remarkable deficits in different frontal lobe functions in schizophrenia.[30–33] Positron emission tomography (PET) studies have revealed decreased activation in the frontal lobes of schizophrenia patients during cognitive tasks.[34] However, only a few studies demonstrated correlations of MRS signal changes with cognitive dysfunction in schizophrenia. One MRS study reported that the NAA/Cho ratio correlated with impairment in procedural learning in neuroleptic-naive first-episode schizophrenia patients.[35] Another study reported that the NAA/Cho ratio correlated with activation in the dorsolateral prefrontal cortex.[36] However, there has been no MRS study to investigate the correlation of absolute metabolite concentrations with cognitive dysfunction. In the present study we confirmed that the NAA concentration in the left frontal lobe of schizophrenia patients correlated negatively with the number of *p*-errors in WCST, which represents the severity of frontal lobe dysfunction. The NAA concentration also correlated with the severity of negative symptoms.

The present study has several limitations. First, many previous studies have shown reduced brain volume and larger CSF volumes in schizophrenia.[37] The reduction

Table 3. Studies of proton spectroscopy in schizophrenia

Study	Subjects (patients/control)	Site	TE (ms)	Voxel (mL)	Method	Results	Relationship with symptoms
Buckley *et al.* (1994)	28/20	Frontal lobe	68	11	Quantification	NAA reduction	–
Choe *et al.* (1994)[11]	10/10	Frontal lobe	30	8	Metabolite ratio	NNA/Cre reduction	–
Bertolino *et al.* (1996)[13]	10/10	DLPFR	272	1.4	Metabolite ratio	NNA/Cre reduction	–
Choe *et al.* (1996)[12]	34/20	Frontal lobe	20	8	Metabolite ratio	No significant difference	Correlation between decrease in GABA + Glu and BPRS
Deicken *et al.* (1997)[20]	24/15	Frontal lobe	135	1.3	Qquantification	NAA reduction	–
Bertolino *et al.* (1998)[14]	12/12	DLPFR	272	1.4	Metabolite ratio	NAA/Cre reduction	–
Brooks *et al.* (1998)[15]	16/12	Frontal lobe	136	8	Metabolite ratio	NAA/Cre reduction	–
Heimberg *et al.* (1998)[38]	13/14	Frontal lobe	30	8	Metabolite ratio	NAA/Cre reduction	–
Cecil *et al.* (1999)[17]	8/14	DLPFR	21	8	Metabolite ratio	NAA/Cre reduction	–
Callicott *et al.* (2000)[18]	36/73	DLPFR	272	1.4	Metabolite ratio	NAA/Cre reduction	Correlation between decrease in NAA and dysfunction in fMRI
Ende *et al.* (2000)[22]	19/16	Anterior cingulate	135	2.4	Quantification	NAA reduction	–
Delamillieure *et al.* (2000)[19]	22/21	Medial prefrontal	30	8	Metabolite ratio	NAA/Cre reduction	–
Bustillo *et al.* (2002)[23]	10/10	Frontal lobe	40	12.6	Quantification	NAA decreased with time	Changes in symptom improvement did not correlated with NAA changes
Yamasue *et al.* (2002)[21]	15/13	Anterior cingulate	35	3.4	Metabolite ratio	NAA/Cho reduction	Negative correlation between NAA/Cho ratio and severity of blunted affect
Sigmundsson *et al.* (2003)[24]	25/26	DLPFR	136	2	Quantification	no significant difference	Negative correlation between severity of symptoms and NAA concentration
Present study	14/13	Frontal lobe	30	3.37	Quantification	NAA reduction	Negative correlation between NAA concentration and severity of negative symptom and poor performance in WCST

BPRS, Brief Psychiatric Scale; Cho, choline; Cre, creatine; DLPFT, dorsolateral prefrontal resion; fMRI, functional magnetic resonance imaging; GABA, γ-aminobutyric acid; Glu, glutamate; NAA, *N*-acetylaspartate; TE, echo time; WCST, Wisconsin Card Sorting Test.

of NAA could result from CSF volume enlargement. However, we did not find any morphological significant difference between patients and controls. Second, all the patients in the present study were receiving antipsychotics. One study showed normal NAA level in the frontal lobe of drug-free schizophrenia patients.[11] Three previous studies show decreased NAA levels in patients with typical antipsychotic treatment compared to atypical groups.[22,38,39] Considering these reports, we cannot exclude the possibility that antipsychotic medications may partly be responsible for the reduction in NAA. Ten of 14 patients in the present study were receiving atypical antipsychotics. In addition, the dose of antipsychotics did not show any relationship between NAA concentrations. Thus, it is unlikely that the observed NAA reduction can be ascribed to medication. However, further studies with drug-naive and drug-free patients will be necessary to clarify the effect of antipsychotics on NAA concentrations.

Multivariate analysis was not used in the present study due to the small number of subjects. It may weaken the reliability of this work. Further studies will be needed to confirm the present results.

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Research report

Effects of repetitive transcranial magnetic stimulation on [^{11}C]raclopride binding and cognitive function in patients with depression

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Abstract

Background: Several studies have demonstrated that repetitive transcranial magnetic stimulation (rTMS) elicits moderate antidepressant effects. Several previous studies suggested that the dopaminergic system might be related to this therapeutic action of rTMS. We attempted to determine the effects of chronic rTMS on central dopaminergic function in depression using positron emission tomography (PET) with [^{11}C]raclopride.

Methods: Nine patients with depression were treated with 10 daily sessions of rTMS (10 Hz, 5 s train, 20 trains at 100% motor threshold per session) over the left dorsolateral prefrontal cortex (DLPFC). Each patient underwent two [^{11}C]raclopride PET scans and neuropsychological tests — before rTMS and 1 day after rTMS.

Results: In five patients, the Hamilton Rating Scale for Depression (HRSD) significantly decreased. Patients showed significant improvement in verbal memory following rTMS. There were no changes in [^{11}C]raclopride binding in the caudate nucleus and putamen after rTMS treatment.

Limitations: Our sample size was limited, and our study was an open trial lacking sham-treated controls.

Conclusion: This study suggests that rTMS may be effective for the treatment of depression and also may improve verbal memory function. We observed no changes in [^{11}C]raclopride binding, suggesting that there was no measurable increase in the release of dopamine at the second PET scan. Several animal studies and healthy human studies have indicated that dopamine can be released soon after acute rTMS. Our results suggest that release of striatal dopamine induced by rTMS may be only transient, or that dopamine release may be attenuated following chronic rTMS.

Keywords: Depression; Repetitive transcranial magnetic stimulation; Dopamine; Positron emission tomography; [^{11}C]raclopride; Neuropsychological function

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1. Introduction

Repetitive transcranial magnetic stimulation (rTMS) is a non-invasive therapeutic tool for neuropsychiatric disorders, such as depression, schizophrenia, obsessive–compulsive disorder, and Parkinson's disease (Fitzgerald et al., 2002; George et al., 2002). Repetitive TMS applied to the left dorsolateral prefrontal cortex (DLPFC) is reported to have modest antidepressive effects (Gershon et al., 2003; Loo and Mitchell, 2005). Although its precise mechanisms are still unknown, the involvement of the dopaminergic system has been suggested in elucidating the therapeutic mechanisms of rTMS. Several animal studies using microdialysis and positron emission tomography (PET) indicated that rTMS over the frontal cortex has modulating effects on the dopaminergic system (Keck et al., 2002; Ohnishi et al., 2004; Kanno et al., 2004). In healthy human studies using PET, it was indicated that rTMS applied to the frontal cortex induced the release of endogeneous dopamine in the striatum (Strafella et al., 2001, 2003). However, the effects on the dopaminergic system by rTMS in patients with depression have not been studied.

In the present study, we examined whether rTMS on the left DLPFC induces dopamine release in the striatum of patients with depression. We performed PET studies using the dopamine receptor ligand [^{11}C]raclopride, which can be used to estimate both the density of D_2 dopamine receptors and changes in concentration of extracellular dopamine (Endres et al., 1997). It has been reported that rTMS, unlike electroconvulsive therapy (ECT), does not have any substantial cognitive side-effects. In this study, we also sought to monitor neurocognitive aspects in patients with major depression before and after rTMS.

2. Method

2.1. Subjects

Nine patients with depression referred to Tokyo Medical and Dental University Hospital participated in this study. The patient sample consisted of 5 women and 4 men aged 36.4±6.1 years (mean±SD). All patients met the criteria of a DSM-IV diagnosis of major depressive disorder. They were all right-handed. The patients underwent general medical and laboratory evaluation consisting of blood test, electrocardiogram (ECG), and chest X-rays to exclude somatic disorders. Organic brain disease was ruled out by brain computed tomography (CT) and electroencephalogram (EEG). Clinical characteristics of the patients are summarized in Table 1. The patients were resistant to or intolerant of drug treatment; three patients had not responded to pretreatment with at least 2 kinds of antidepressant drugs, equivalent to more than 150 mg/day of imipramine for more than 4 weeks, and 6 patients had been unable to tolerate at least two previous antidepressant trials because of side-effects. Three patients had taken atypical antipsychotics as augmentation agents in treatment-resistant depression. They had at least a 4-week washout period from their previous medication. Fluvoxamine and lorazepam were allowed during the washout period and were maintained at the same dosage level during the full study period. Seven patients had not been able to take fluvoxamine in sufficient dosage, more than 150 mg/day, because of side-effects such as sleepiness, anxiety, nausea. The healthy control sample for [^{11}C]raclopride PET study consisted of 5 women and 11 men, age-matched at

Table 1
Clinical characteristics of patients

Patient no.	Age (years)	Gender	Diagnosis (DSM-IV)	Episodes (D)	Duration of present episode (months)	Dose of fluvoxamine(mg)	HRSD preTMS	BDI preTMS
1	34	M	296.22	D1	34	100	17	17
2	34	F	296.22	D1	8	50	21	32
3	44	F	296.32	D3	6	0	21	16
4	34	M	296.22	D1	36	300	16	16
5	34	F	296.32	D2	5	75	16	26
6	40	F	296.32	D2	22	100	14	15
7	46	M	296.22	D1	36	175	20	15
8	36	M	296.22	D1	12	100	17	20
9	26	F	296.22	D1	13	75	15	29
Mean	36.4				19.1	108.3	17.4	20.7
SD	6.1				13.1	85.7	2.6	6.6

DSM-IV, Diagnostic and Statistical Manual of Mental Disorders, 4th edition; D, number of depressive episodes before the study; HRSD, Hamilton Rating Scale for Depression; BDI, Beck Depression Inventory; M, male; F, female.

Table 2
Neuropsychological assessments

	Patients with major depression (n=9)		Controls (n=14)
	1 day before rTMS	1 day after rTMS	
Age	36.4±6.1		34.9±8.7
Gender	f5, m4		f12, m2
Education level (years)	15.3±1.4		15.6±1.3
MMSE	29.1±1.1	29.1±0.6	29.9±0.5
WMS–R			
Paired words/related/immediate	10.8±1.5	11.1±1.6	10.9±1.7
Paired words/unrelated/immediate	3.2±3.4	8.6±2.5	8.1±2.9
Paired words/related/delayed	4±0	3.9±0.3	3.9±0.3
Paired words/unrelated/delayed	1.6±1.2	3.4±1.0	3.3±0.9
Visual reproduction/immediate/A	6.4±0.9	6.7±0.7	6.4±0.6
Visual reproduction/immediate/B	6.9±0.3	7±0	6.9±0.3
Visual reproduction/delayed/A	5.9±2.3	6.1±2.3	5.9±0.7
Visual reproduction/delayed/B	6.7±0.7	6±2.3	6.8±0.4
TMT-A	85.2±19.0	80.8±17.2	83.6±11.6
TMT-B	100.9±26.8	92.3±37.5	102.4±15.4
EMC	10.4±4.1	6.6±4.1	8.6±3.1

Data are mean±SD. f, female; m, male; MMSR, Mini-Mental State Exam; WMS–R, Weschsler Memory Scale–Revised; TMT, Trail Making Test; EMC, Everyday Memory Checklist.

36.1±7.4 years. The healthy control sample for neuropsychological tests consisted of 12 women and 2 men, matched by age and level of education (see Table 2). They were recruited from the surrounding community. Based on unstructured psychiatric screening interviews, they were free of current and past psychiatric disease.

This study was approved by the human ethics committees of Tokyo Medical and Dental University and the National Institute of Radiological Sciences, Chiba, Japan. After providing a complete explanation of the study, written informed consent was obtained from all subjects.

2.2. Procedure

2.2.1. rTMS procedure

Stimulation was performed with a Magstim Rapid System (Magstim Company Limited, Spring Gardens, Whitland, U.K.), using an eight-shaped coil. Each patient was treated with 10 sessions (5 times per week for 2 weeks). Each session consisted of 20 trains of 5 s duration separated by 25 s pauses. Stimulation was applied at 10 Hz frequency and at an intensity of 100% motor threshold (MT). Thus, total 1000 pulses were delivered per treatment-day. MT was determined before the first session using the visual method with the right first dorsal interosseous (FDI) muscle as the target muscle (Wassermann et al., 1996). MT was defined as the stimulus intensity that produced visibly observable right FDI muscle contractions at least 5 times out of 10 stimuli. rTMS was performed over the left dorsolateral prefrontal cortex (DLPFC). The point of left DLPFC was determined by moving the coil 5 cm anteriorly from the point of MT determination. The point of stimulation was marked with an indelible skin marker. MT and coil placement were rechecked after the 5th treatment, but neither MT nor coil placement differed from the original ones in any patient. During rTMS the patients wore earplugs to dampen loud noise from the discharging coil.

2.2.2. Clinical ratings and neuropsychological assessments

The clinical symptoms of all patients were assessed using the 17-item Hamilton Rating Scale for Depression (HRSD; Hamilton, 1960) and the Beck Depression Inventory (BDI; Beck and Beamesderfer, 1974). Cognitive effects were assessed with a neuropsychological test battery. (1) Mini-Mental State Examination (MMSE; Folstein et al., 1975) was used as a general screen for cognitive impairment. (2) Visual immediate and delayed memory functions were evaluated with parts of Wechsler Memory Scale–Revised, Japanese version (WMS–R; Wechsler, 1987; Sugishita, 2001), visual reproduction using Card A and Card B. (3) Verbal immediate and delayed memory functions were evaluated with parts of WMS–R, Japanese version, paired words. (4) Trail Making Test (TMT; Reitan, 1958) A and B was administered to measure visual

scanning, sequencing, psychomotor speed and additional set-shifting. The clinical symptoms and cognitive effects of all patients were assessed 1 day before and 1 day after a series of rTMS. Control subjects were assessed with the same neuropsychological test battery once. (5) The patients' everyday memory problems were self-evaluated with the Japanese version of the Everyday Memory Checklist (EMC) (Kazui et al., 2003).

2.2.3. PET procedure

Eight patients except patient #2 (Table 1) underwent [^{11}C]raclopride PET scans twice — before a series of rTMS and 1 day after the last session of rTMS. Each PET scan began more than 3 h after the patients took their last medication. Sixteen healthy volunteers underwent [^{11}C] raclopride PET scans once. The PET system ECAT EXACT HR+ (CTI-Siemens, Knoxville, TN) was used for all PET studies. The system provides 63 planes with a 15.5 cm axial field of view. After a transmission scan with a ^{68}Ge–^{68}Ga source, a bolus of [^{11}C]raclopride (patients before rTMS, 5.7±0.6 mCi; patients after rTMS, 5.6±0.2 mCi; controls, 5.8± 0.6 mCi) with high specific radioactivity (patients before rTMS, 113.1±30.4 GBq/μmol; patients after rTMS, 111.5±55.1 GBq/μmol; controls, 175.7± 50.2 GBq/μmol) was rapidly injected into the antecubital vein with a 20-ml saline flush. Radioactivity in the brain was measured by a series of scans for 90 min starting immediately after the injection. During image acquisition, subjects were instructed to lie quietly with their eyes closed and earplugs in place. Emission scans were reconstructed with a Hanning filter with a cut-off frequency of 0.4 [FWHM (full width at half maximum)= 7.5 mm].

Magnetic resonance (MR) images were acquired on Philips Gyroscan NT, 1.5 tesla. T1-weighted 1 mm-thick 3D images of the brain were obtained from each subject.

2.3. PET data analysis

Regions of interest (ROIs) were manually drawn on the transverse slices from each subject's MR images coregistered to the reconstructed PET images. ROIs were set to cover 3 adjacent slices for the right and left caudate nucleus, right and left putamen and cerebellar cortex. The sets of ROIs for each section were transferred to the corresponding PET images and time-activity curves were obtained. The time-activity curves of each region were analyzed using a simplified reference tissue compartment model in a least squares manner, in which the cerebellum was used as reference tissue (Lammertsma and Hume, 1996). This procedure revealed the binding potential (BP) value, which is defined as follows: $BP=B_{max}/K_d$, where B_{max} is the density of receptor and K_d is the dissociation constant. [^{11}C]raclopride and endogenous dopamine compete at the D_2 receptors. Endogenous dopamine release decreases [^{11}C]raclopride binding and BP decreases thereafter (Doudet and Holden, 2003).

2.4. Statistical analysis

2.4.1. Clinical data

Response to treatment was defined as a final HRSD less than 10 points or a 50% decrease in HRSD. Paired t test was used to statistically analyze the difference between HRSD and BDI scores 1 day before the first session and 1 day after the last session of rTMS. The same statistical procedure was used for each neuropsychological test. Unpaired t test was used to compare the score for patients with the score for healthy control subjects in each neuropsychological test. Values of $P<0.05$ were considered significant.

2.4.2. PET data

Statistical analysis of the difference between regional [^{11}C]raclopride BP obtained from patients before rTMS and from the healthy control subjects was performed by repeated measures analysis of variance (ANOVA) to find interaction between groups (patient group vs. control group) and places on which ROIs were drawn (right and left caudate nucleus, right and left putamen). Analysis of the difference between [^{11}C]raclopride BP in patients measured before rTMS and 1 day after the last session of rTMS was performed using two-way repeated ANOVA to find interaction between time (before and after) and regions (right and left caudate nucleus, right and left putamen). When we found any interaction, post hoc Bonferroni correction was used for multiple comparisons. Values of $P<0.05$ were considered significant.

3. Results

3.1. Clinical assessment

All patients tolerated rTMS without any complications. Five patients after some rTMS sessions complained about minor headaches that could be controlled with loxoprofen sodium. Changes in HRSD and BDI scores for all patients are shown in Fig. 1. Five out of nine patients responded to rTMS. Paired t test of HRSD

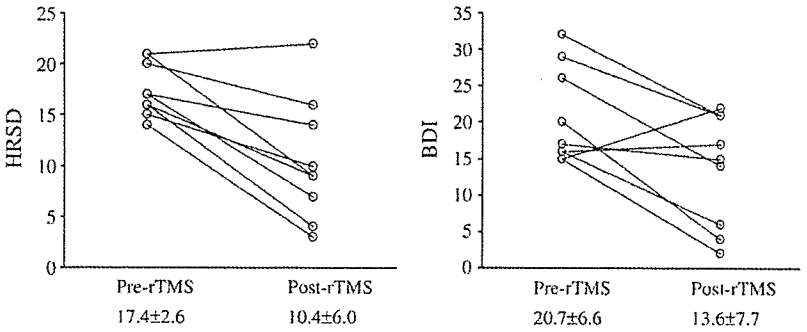

Fig. 1. Individual scores in the Hamilton Rating Scale for Depression (HRSD) and Beck Depression Inventory (BDI) before and after a 2-week course of repetitive transcranial magnetic stimulation (rTMS).

and BDI scores showed significant reduction in scores (HRSD, $P=0.0018$; BDI, $P=0.022$).

3.2. Neuropsychological assessment

The scores for patients 1 day before and 1 day after rTMS and those for the controls are shown in Table 2.

3.2.1. Difference between patients before rTMS and controls

For MMSE, paired words/related/immediate, paired words/related/delayed, visual reproduction, TMT, and EMC, no significant differences were observed by unpaired t test. Unpaired t test showed significant differences for paired words/unrelated/immediate and paired words/unrelated/delayed (immediate, $P=0.0012$; delayed, $P=0.0009$).

3.2.2. Difference between before rTMS and after rTMS in patients

For MMSE, paired words/related/immediate, paired words/related/delayed, visual reproduction and TMT, no significant differences were observed by paired t test. For paired words/unrelated/immediate, paired words/unrelated/delayed, and EMC, paired t test showed significant differences (paired words/unrelated/immediate, $P=0.001$; paired words/unrelated/delayed, $P=0.0045$; EMC, $P=0.0211$). There was no correlation between changes in HRSD scores and those in scores of paired words/unrelated/immediate and delayed (see Fig. 2; immediate, $r=0.198$; delayed, $r=-0.3$).

3.2.3. [^{11}C]raclopride binding

3.2.3.1. Difference between patients before rTMS and controls. Repeated measures ANOVA revealed no significant interactions between groups and regions [F (3, 20)=0.571, $P=0.641$] (Table 3).

3.2.3.2. Difference between before rTMS and after rTMS in patients. Two-way repeated ANOVA revealed significant interactions between time and regions [F (3, 5)=8.962, $P=0.019$], but post hoc Bonferroni correction showed no significant differences between [^{11}C]raclopride BP before and after rTMS in each ROI (right caudate nucleus, $P=0.217$; left caudate nucleus,

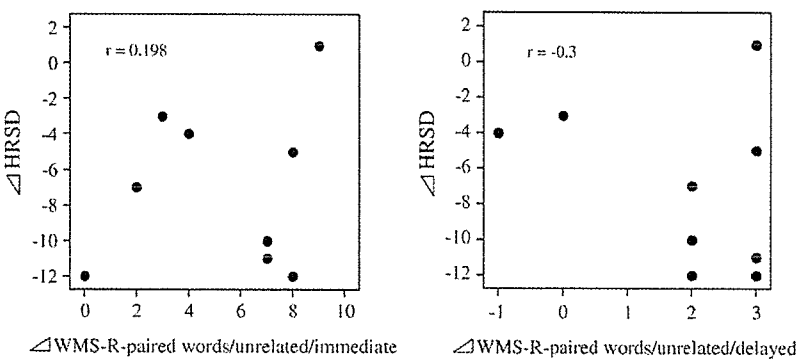

Fig. 2. The difference in scores of the Hamilton Rating Scale for Depression (HRSD) between pretreatment and post-treatment does not correlate with the difference in scores of the Wechsler Memory Scale–Revised (WMS–R)/paired words/unrelated both in immediate and delayed reproduction. ⊿ means the differences in scores between before rTMS and after rTMS. "r" indicates Pearson's correlation coefficient.

Table 3
[^{11}C]raclopride binding potential in the striatum

Region	Controls (n=16)	Patients (n=8)	
		Before TMS	After TMS
R caudate	2.68±0.32	2.72±0.35	2.85±0.31
L caudate	2.77±0.33	2.76±0.30	2.78±0.25
R putamen	3.37±0.36	3.46±0.44	3.46±0.31
L putamen	3.36±0.45	3.52±0.32	3.47±0.28

Data are mean±SD.
TMS, transcranial magnetic stimulation; R, right; L, left.

P=0.873; right putamen, P=0.938; left putamen, P=0.607) (Table 3).

4. Discussion

4.1. Clinical aspects

Although the sample size is small, our clinical results indicate that rTMS over the left DLPFC improves mood in some patients with medication-resistant major depression with minimal side-effects. However, our study was an open trial and lacked a sham-treated control group. Thus, placebo effects were not able to be excluded. Further double-blind sham-controlled studies are required.

Patients before rTMS scored significantly lower than controls in verbal immediate and delayed memory (WMS–R/paired words/unrelated/immediate and delayed). These results are in accordance with previous studies showing partial memory impairment in patients with major depression (Landro et al., 2001; Macqueen et al., 2003).

Patients showed no deterioration in neuropsychological functions after 10 sessions of rTMS. Furthermore, there has been significant improvement on 3 tests: WMS–R/paired words/unrelated/immediate, delayed and EMC. A few earlier studies have also reported improved performance on several neuropsychological tasks in depressed patients after rTMS (Padberg et al., 1999; Loo et al., 2001). The improvement in EMC means that the patients were conscious of their memory function improvement.

We have to take some factors into consideration when interpreting our results in WMS–R/paired words/unrelated. First, it cannot be denied that the improvement in verbal memory might be partly due to practice effects because patients were tested with the same battery of tests twice. Secondly, it is uncertain whether cognitive improvement is associated with improvement in mood or rTMS has beneficial cognitive effects independent of its antidepressant efficacy. In this study, there was no correlation between changes in HRSD scores and those in scores of WMS–R/paired words/unrelated/immediate and delayed (see Fig. 2). This finding suggests the possibility that rTMS might independently improve parts of cognitive functions. Thus, rTMS over the left DLPFC may improve verbal memory function in patients with depression. Further controlled studies with larger study populations are necessary.

4.2. No differences in [^{11}C]raclopride BP between patients and healthy controls

We could not detect any significant differences in [^{11}C]raclopride BP between patients with major depression and control subjects. There have been a few studies that compared BP obtained with [^{123}I]iodobenzamide (IBZM) in depressed patients with that in age-matched healthy controls (D'haenen and Bossuyt, 1994; Ebert et al., 1994, 1996; Shah et al., 1997; Klimke et al., 1999; Parsey et al., 2001). [^{123}I]IBZM is a selective dopamine D_2 receptor antagonist that can compete with endogenous dopamine at D_2 dopamine receptors in a similar way to raclopride. Our results were consistent with the results of Ebert et al. (1994), Ebert et al. (1996), Klimke et al. (1999), and Parsey et al. (2001), who all found no significant differences in [^{123}I]IBZM BP in the striatum between depressed patients and controls. On the other hand, D'haenen and Bossuyt (1994) and Shah et al. (1997) reported higher [^{123}I]IBZM BP in the striatum in depressed patients than in controls, a finding suggestive of changes in dopaminergic function. Medication and clinical profiles of the patients in their studies are different from those in our study. This might possibly have contributed to the difference between their results and ours.

Our study had some limitations. Not only was our sample size limited, all of our patients had also been previously treated with tricyclic antidepressants and three patients had taken atypical antipsychotics, which might have influenced dopamine receptors. Our patients had also taken fluvoxamine during the study. However, in a previous measurement of dopamine D_2 receptor using [^{18}F]fluoroethyl spiperone (FESP) PET, no significant changes in [^{18}F]FESP binding were found in the basal ganglia of depressed responders to fluvoxamine (Moresco et al., 2000). Thus, it can be supposed that a therapeutic dose of fluvoxamine has only a minor effect on the dopaminergic system.

It is still not clear whether there are changes in dopamine function in the striatum in depressed patients or not. However, considering that not a few studies have

reported similar results to ours, it is more than likely that striatal D_2 receptor binding does not change in major depression.

4.3. No changes in [^{11}C]raclopride BP induced by rTMS

There was no significant difference between BP measured by [^{11}C]raclopride before and after rTMS in patients. Two PET studies previously reported that rTMS over the frontal cortex in healthy human subjects caused a reduction in [^{11}C]raclopride binding in the striatum (Strafella et al., 2001, 2003). Frequency and intensity of rTMS in their studies were almost the same as those in our study. However, we could not replicate their findings in patients with major depression. This discrepancy might be the result of several differences between their studies and ours. First, we started [^{11}C] raclopride PET scans about 24 h after the last session of rTMS, whereas they began them within 5 min of the completion of the rTMS session. Keck et al. (2002) described that intrastriatal release of dopamine was significantly elevated in rTMS-treated rats. They reported that the concentration of dopamine in the dorsal striatum reached its peak 120 min after rTMS and decreased thereafter. Thus, the possibility exists that the concentration of dopamine induced by rTMS in our study might have returned to baseline level (before rTMS) within 24 h, explaining why changes in [^{11}C] raclopride BP in patients after the last session of rTMS were not detected. Second, chronic (multiple treatments) rTMS was applied in our study, whereas acute (single treatment) rTMS was used in the studies of Strafella et al. In the study of Ben-Shachar et al. (1999), chronic rTMS on the rat brain did not induce any change in striatal concentrations of dopamine and its metabolites. The study of Hausmann et al. (2002) showed that chronic rTMS on the rat brain did not affect the expression of mesencephalic thyrosine hydroxylase, a dopamine-synthesizing enzyme. Similar phenomena have been observed in electroconvulsive shock (ECS), an animal model of ECT, studies. A few studies have reported that acute treatment with ECS caused significant changes in monoamine levels, which were attenuated or abolished following chronic treatment (Nomikos et al., 1991; Yoshida et al., 1997). Thus, it is possible that the release of dopamine induced by acute rTMS is transient, or that chronic rTMS, unlike acute rTMS, does not cause significant changes in striatal dopamine levels.

Other mechanisms, e.g., serotonergic and noradrenergic systems, might play a more important role in chronic rTMS. It has been reported that serotonergic or noradrenergic receptors were modulated after chronic rTMS in rat brains (Ben-Shachar et al., 1999; Gur et al., 2004). Further studies on other transmitter systems will be needed.

5. Conclusion

The present findings suggest that rTMS may be effective for the treatment of medication-resistant depression, and that rTMS may improve verbal memory function. However, we could not detect any changes in [^{11}C]raclopride binding in the striatum of patients with depression between before and after rTMS treatment.

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