Effect of Repetitive Transcranial Magnetic Stimulation and Intensive Occupational Therapy on Serum Levels of Brain-Derived Neurotrophic Factor in Patients after Stroke

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ABSTRACT

Purpose : The aim of this study was to clarify the effect of combined protocol with low-frequency repetitive transcranial magnetic stimulation (rTMS) and intensive occupational therapy (OT) on serum levels of brain-derived neurotrophic factor (BDNF) in patients with hemiparesis after stroke.

Subjects and Methods : The subjects were 21 patients with upper-limb hemiparesis after stroke (age : median, 69 years ; interquartile range, 63-74 years ; time after onset : median, 42 months ; interquartile range, 24-63 months). During the 15-day hospitalization, each patient received 22 sessions of 20 minutes of low-frequency rTMS over the nonlesional hemisphere and 120 minutes of OT. Serum levels of BDNF were measured with a microsphere-based multiplex immunoassay before the first and after the last treatment sessions. The Fugl-Meyer Assessment (FMA) and Wolf Motor Function Test (WMFT) were administered on the days of admission and discharge.

Results : Low-frequency rTMS and intensive OT resulted in a significant increase in serum levels of BDNF ($p < 0.005$), a significant increase in the FMA score ($p < 0.001$), and a significant decrease in the performance time of the WMFT ($p < 0.001$). The percent change in serum levels of BDNF weakly correlated with the percent increase in the FMA score ($R^2 = 0.295$, $p < 0.05$) and the percent decrease in the performance time of the WMFT ($R^2 = 0.264$, $p < 0.05$).

Conclusion : Motor functional improvement of the paretic upper limb with low-frequency rTMS and intensive OT might be achieved in part through up-regulation of BDNF.

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Key words : brain-derived neurotrophic factor, stroke, rehabilitation, upper-limb hemiparesis, transcranial magnetic stimulation, occupational therapy

INTRODUCTION

Brain-derived neurotrophic factor (BDNF), a member of the nerve growth factor family, plays an important role in the growth and maintenance of several neuronal systems[1,2]. The decrease in peripheral levels of BDNF is reportedly associated with age-related neuronal loss and the development of several neurodegenerative diseases, including Parkinson's disease and Alzheimer's disease, and psychiatric disorders, such as depression[3-6]. In addition, BDNF is involved in various forms of neuroplasticity, such as long-term potentiation, learning, and memory, in both the intact and damaged brain[7-9].

Recently, we developed a 15-day protocol of low-fre-

quency repetitive transcranial magnetic stimulation (rTMS) combined with intensive occuptional therapy (OT) for patients with upper-limb hemiparesis after stroke, with the aim of facilitating the beneficial plastic changes in the lesional hemisphere. The results of a pilot study showed motor functional improvement of the affected upper limb in most patients without any adverse effects[10,11]. Application of low-frequency (1 Hz or less) rTMS over the nonlesional motor cortex of patients with hemiparesis after stroke significantly improves the motor function of the affected upper limb[12]. Intensive OT, such as constraint-induced movement therapy, has also been reported to increase the neural activity of the lesional hemisphere and to improve the function of the paretic upper limb after stroke[13,14]. To date, however, no information is available about the effect of low-frequency rTMS applied over the nonlesional hemisphere with intensive OT on serum BDNF levels in patients after stroke. Furthermore, the relationship between changes in serum BDNF levels and motor functional recovery with therapeutic application of low-frequency rTMS with intensive OT remains to be clarified.

The purpose of the present study was to clarify the effect of low-frequency rTMS combined with intensive OT (low-frequency rTMS/OT) on serum BDNF levels in patients with hemiparesis after stroke and to investigate the relationship between changes in serum BDNF levels and motor functional improvement of the affected upper limb after low-frequency rTMS/OT.

Materials and Methods

Patients

The study protocol was approved by the ethics committee of Shimizu Hospital, Tottori, Japan, and informed consent was obtained from all patients. This study was performed in compliance with the Declaration of Helsinki. All patients met the following inclusion criteria : 1) upper-limb hemiparesis categorized as Brunnstrom stage 3 to 5 for the hands and fingers (ability, at least subjectively, to flex the fingers with or without extension) ; 2) age at intervention of 18 to 80 years ; 3) interval between onset of stroke and the intervention of more than 12 months ; 4) history of a single symptomatic stroke only (no bilateral cerebrovascular lesion) ; 5) no cognitive impairment, as indicated by a pretreatment Mini-Mental State Examination

score of more than 26 ; 6) clinically confirmed plateau state of motor functional recovery, as indicated by no increase in the Fugl-Meyer Assessment (FMA) score on monthly evaluation by occupational therapists from our department in the most recent 3 months despite conventional OT ; 7) no active physical or mental illness requiring medical management ; 8) no history of symptomatic seizure ; 9) no documentation of epileptic discharge on pretreatment electroencephalography ; and 10) no contraindications for rTMS, according to the guidelines suggested by Wasserman[15] (e.g., cardiac pacemakers, intracranial implants, implanted medication pumps, pregnancy). As an in-patient intervention, each patient received 22 treatment sessions of low-frequency rTMS/OT during the 15-day hospitalization (Fig. 1).

Application of low-frequency rTMS

Two treatment sessions of low-frequency rTMS were provided daily, excluding the days of admission and discharge and Sundays. A 70-mm figure-8 coil attached to a magnetic stimulator (MagPro R30, MagVenture A/S, Farum, Denmark) was used for low-frequency rTMS. The 1-Hz rTMS was delivered to the skull of the nonlesional hemisphere at the site that elicited the largest motor-evoked potentials in the first dorsal interosseous muscle of the unaffected upper limb (confirmed with surface electromyography). One session of low-frequency rTMS consisted of 1,200 pulses and lasted 20 minutes. The intensity of stimulation was set at 90% of the motor threshold of the first dorsal interosseous muscle, which was defined as the lowest intensity of stimulation that could produce motor-evoked potentials.

Rehabilitation program of occupational therapy

All patients received 120 minutes of OT daily, consisting of 60 minutes of one-to-one training and 60 minutes of self-training[10,11]. In the one-to-one training provided by occupational therapists, the main components were shaping techniques (e.g., reaching forward to move a cup from one place to another and writing letters and drawing pictures with a pencil) and repetitive task practice techniques (e.g., squeezing and molding clay and pinching small coins). The one-to-one training was tailored to the motor function of the affected upper limb and the patient's lifestyle (e.g., occupation, interest, and household work). The tasks for self-training were similar to those applied in the one-to-one training. The problems associated with the tasks in

	Thursday	Friday	Saturday	Sunday	Monday-Saturday	Sunday	Monday-Wednesday	Thursday
Morning	Admission	BDNF measurement (baseline)		No treatment		No treatment		BDNF measurement (follow-up)
		Low-frequency rTMS (20 min)			Low-frequency rTMS (20 min)		Low-frequency rTMS (20 min)	Post therapy evaluation
		One-to-one training (60 min)			One-to-one training (60 min)		One-to-one training (60 min)	
		Self-exercise (60 min)			Self-exercise (60 min)		Self-exercise (60 min)	
Afternoon	Pre therapy evaluation	Low-frequency rTMS (20 min)			Low-frequency rTMS (20 min)		Low-frequency rTMS (20 min)	Discharge
		One-to-one training (60 min)			One-to-one training (60 min)		One-to-one training (60 min)	
		Self-exercise (60 min)			Self-exercise (60 min)		Self-exercise (60 min)	

Fig. 1. The 15-day protocol of combination treatment of low-frequency rTMS and intensive OT (example for a patient admitted on Thursday). Blood samples for measurement of serum BDNF was drawn in the morning on the second day and on the 15th day of hospitalization.

self-training session were aggressively approached in the following session by the therapists.

Measurement of serum BDNF levels

A microsphere-based multiplex immunoassay (Milliplex MAP kit, Millipore, Bedford, MA, USA) was used to measure serum concentrations of BDNF. Fasting blood samples were drawn in the morning on the second day of hospitalization (before the first treatment session) to measure baseline data and on the day of discharge (after the last treatment session) to obtain follow-up data (Fig. 1). The BDNF levels were measured according to manufacturer's instructions. Briefly, the serum samples were prepared in a threefold dilution with assay buffer. A sample was added to the appropriate wells after the addition of antibody-coated fluorescent beads. The reaction mixture was then incubated with a streptavidin-phycoerythrin conjugate after the addition of a biotinylated detection antibody. Finally, a bead-based liquid hybridization assay (Luminex® 200™ System, Luminex Corp., Austin, TX, USA) and a high-speed digital-signal processor were used to identify individual microspheres and to quantify the results of the bioassay on the basis of fluorescent reporter signals. The raw data were analyzed with a curve-fitting software program (DNA-SIS® Plex, version 2.5, Hitachi Solutions, Hitachi, Tokyo, Japan), on the basis of a standard curve of concentrations from 12 to 50 ng/ml generated by serial dilution of a reconstituted standard. The assay was run in duplicate for each serum sample.

Evaluation of motor function in the affected upper limb

The motor function in the affected upper limb was evaluated on the days of admission and discharge by means of the FMA and the Wolf Motor Function Test (WMFT). Both measures are associated with high interrater and test-retest reliabilities[16-19]. The FMA is a performance-based quantitative measure comprising 33 items to evaluate upper-limb motor impairment. Because each item is rated on a 3-point ordinal scale (0 = cannot perform, 1 = can perform partially, and 2 = can perform fully), the maximum score for upper-limb motor performance is 66. The WMFT includes 15 timed tasks to evaluate upper-limb motor function. The mean performance time of 15 timed tasks was calculated. When the task was not completed within 120 seconds, the performance time of the task was recorded as 120 seconds. For WMFT performance time, the natural

logarithm of the mean performance time of 15 timed tasks was calculated (as WMFT log performance time) to normalize the skewed distribution of the data, as applied in the analysis for the Extremity Constraint Induced Therapy Evaluation trial[20]. These evaluations were performed by an occupational therapist who provided no training to ensure blindness in outcome evaluation.

Statistical analysis

Changes with the intervention in serum BDNF levels, the FMA score, and the WMFT log performance time were analyzed with the signed Wilcoxon's rank-sum test for paired samples. The percent changes in the FMA score and the WMFT log performance time compared with baseline values were calculated. The relationship between the change in serum BDNF levels and changes in the 2 applied measures were analyzed with the Pearson correlation test. All statistical analyses were performed with the SPSS software package, version 17.0 (SPSS, Chicago, IL, USA). A p value less than 0.05 was considered to be statistically significant.

RESULTS

The subjects were 21 patients with upper-limb hemiparesis after stroke. All patients were admitted to Shimizu Hospital from May 1 through July 31, 2011, to receive low-frequency rTMS/OT for 15 days. The baseline characteristics of the patients are shown in Table 1. The median age at admission was 69 years (interquartile range [IQR],

63-74 years). The interval from onset of stroke to intervention ranged from 13 to 126 (median, 42 months ; IQR, 24-63 months). The patients included 7 patients with intracerebral hemorrhage and 14 patients with cerebral infarction (cortical in 4 patients and subcortical in 10 patients). The baseline severity of upper-limb hemiparesis was categorized as a Brunnstrom stage for hands and fingers of 3 in 5 patients, 4 in 11 patients, and 5 in 5 patients. All patients completed the 15-day low-frequency rTMS/OT protocol without showing any adverse effects. The 15-day course of in-hospital low-frequency rTMS/OT resulted in a significant increase in serum BDNF levels (baseline : median, 19.3 ng/ml ; IQR, 17.0-24.9 ng/ml ; discharge : median, 22.8 ng/ml, IQR 19.6-28.4 ng/ml, $p < 0.005$, Fig. 2-a). The mean percent change in serum BDNF level was 17.4% ± 25.2%. Both measures of motor function showed improvement of the function of the affected upper limb after low-frequency rTMS/OT, with a significant increase in the FMA score (baseline : median, 40 points, IQR, 31-51 points ; discharge : median, 53 points ; IQR, 36-58 points, $p < 0.001$, Fig. 2-b) and a significant shortening of the WMFT log performance time (baseline : median, 3.5 ; IQR, 1.9-4.8 ; discharge : median, 2.8 ; IQR, 1.7-3.9 ; $p < 0.001$, Fig. 2-c). The percent change in serum BDNF levels was only weakly correlated with the percent change in the FMA score ($R^2 = 0.295$, $p < 0.05$, Fig. 3-a). Furthermore, there was only weak negative correlation between the percent change in serum BDNF level and percent change in the WMFT log performance time ($R^2 = 0.264$, $p < 0.05$, Fig. 3-b).

Table 1.   Clinical characteristics of studied patients ($n = 21$)

Age at admission, years, median (IQR)	69 (63-74)
Gender, *n* (%)	Females : 6 (29)
	Males : 15 (71)
Time after stroke onset, months, median (IQR)	42 (24-63)
Subtype of stroke, *n* (%)	Intracerebral hemorrhage : 7 (33)
	Cerebral cortical infarction : 4 (19)
	Subcortical infarction : 10 (48)
Side of upper limb hemiparesis, *n* (%)	Dominant hand : 8 (38)
	Non-dominant hand : 13 (62)
Brunnstrom stage for hand-fingers at admission, *n* (%)	Stage 3 : 5 (24)
	Stage 4 : 11 (52)
	Stage 5 : 5 (24)
Medical history, *n* (%)	Hypertension : 14 (67)
	Deabetes mellitus : 6 (29)
	Dyslipidemia : 5 (24)
	Cardiac disease : 5 (24)

(ng/ml)
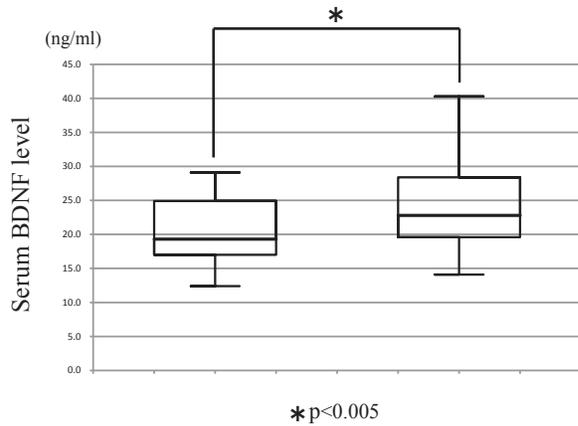
* p<0.005

Fig. 2-a.　Serum BDNF levels at baseline and discharge.　Data are medians (IQR).　*$p < 0.005$.
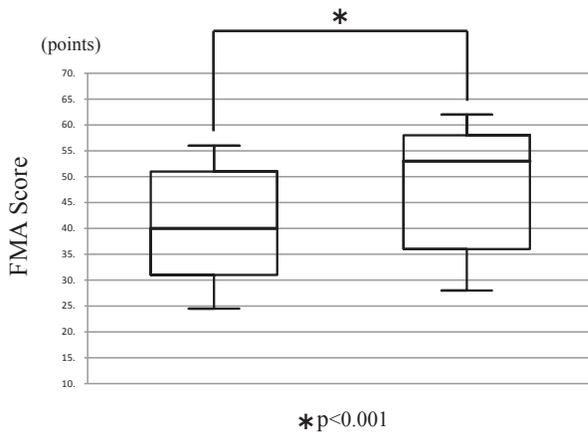
(points)

* p<0.001

Fig. 2-b.　The FMA scores measured at baseline and discharge. Data are medians (IQR).　*$p < 0.001$.
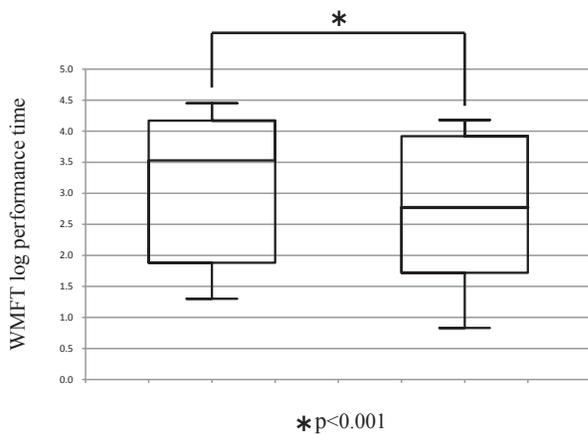

* p<0.001

Fig. 2-c.　The WMFT log performance times at baseline and discharge.　Data are medians (IQR).　*$p < 0.001$.
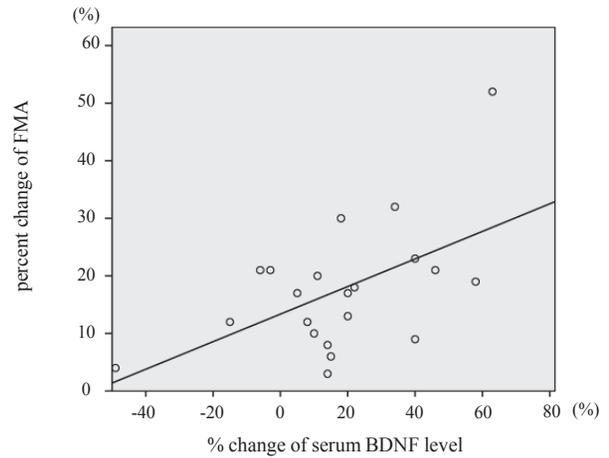

Fig. 3-a.　Relation between the percent change in serum BDNF levels and the percent change in the FMA scores. The relation between these 2 values was statistically significant ($R^2 = 0.295$, $p < 0.05$).
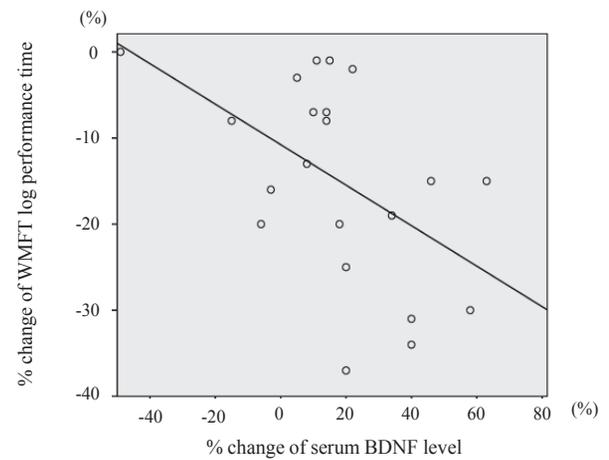

Fig. 3-b.　Relation between the percent changes in serum BDNF levels and the WMFT log performance time. $R^2 = 0.264$, $p < 0.05$.

DISCUSSION

　　Application of low-frequency rTMS/OT over 15 days resulted in a significant increase in serum BDNF levels in patients after stroke.　However, the increase in serum BDNF levels was only weakly correlated with the extent of motor functional recovery of the affected upper limb.　To our knowledge, this is the first study to investigate the effect of low-frequency rTMS/OT on serum BDNF levels in patients after stroke and to examine the relationship between changes in serum BDNF levels and motor functional improvement of the affected upper limb.

BDNF is produced and secreted within the central and peripheral nervous systems, and its biological effects are exerted upon many organ systems upon binding to its tyrosine kinase receptor and subsequent downstream activation of several signal-transduction pathways[1,2,7-9]. BDNF plays an important role in the plastic changes after stroke, leading to motor functional recovery. In the present study, we have shown that low-frequency rTMS/OT significantly increases serum BDNF levels in patients after stroke. Unfortunately, we did not dissect the effects of each arm of the intervention—low-frequency rTMS and intensive OT—on serum BDNF levels and motor function of the affected upper limb because both interventions were applied simultaneously. Furthermore, through the serial application of functional magnetic resonance imaging, our previous study demonstrated that the effect of low-frequency rTMS/OT on upper-limb motor function recovery after stroke is due to increased activation of the lesional hemisphere. In addition, we found that low-frequency rTMS/OT can produce beneficial functional reorganization in the lesional hemisphere[21].

Therefore, both rTMS and intensive OT can affect BDNF levels in the serum and in the brain. So far, there are no clinical data regarding the effect of low-frequency rTMS applied over the nonlesional hemisphere on serum BDNF levels after stroke, although Yukimasa et al. have found that high-frequency (20 Hz) rTMS increases plasma BDNF levels in patients with depression who show significant symptomatic improvement with rTMS[22].

In the cascade of reorganization with rTMS application, long-term potentiation (LTP), which represents strengthening of synaptic functional connection, can be considered an important component[23,24]. Several researchers have demonstrated that BDNF immediately and robustly induces LTP in the mouse hippocampus[25,26]. Similarly, Escobal et al. have shown that intracortical infusion of BDNF induces LTP in anesthetized adult rats[27]. Therefore, we speculate that the beneficial effect on motor function of low-frequency rTMS applied over the nonlesional motor cortex is partly mediated by up-regulation of the serum BDNF level. With regard to intensive OT, there are no clinical data concerning its effect on the serum BDNF level. However, physical exercise in human subjects has been shown to increase peripheral BDNF concentrations[28]. In addition, Neeper et al. have reported that exercise training significantly up-regu-

lates BDNF messenger RNA within specific brain regions, such as the hippocampus, in adult rats[29]. Recently, Ke et al. have reported that voluntary exercise significantly up-regulates the hippocampal BDNF level and facilitates motor recovery in a rat brain model of ischemia[30].

Therefore, we believe that intensive OT featuring voluntary motor training, such as those applied in our protocol, can increase serum BDNF levels in patients after stroke. Considering these reports regarding rTMS application and intensive OT, we further believe that the increase in BDNF was induced by both interventions; i.e. low-frequency rTMS and intensive OT, although the extent of the contribution of each intervention to the BDNF increase should be clarified in further studies.

In the present study, the extent of motor functional improvement in the affected upper limb was significantly associated with the increase in the serum BDNF level, although the correlation between the extent of improvement and changes in BDNF levels was weak. In a rat model, MacLellan et al. investigated changes in motor function and BDNF levels in the brain after various forms of rehabilitation under different situations[31]. They showed that the extent of motor functional improvement was associated with the extent of BDNF increase with rehabilitation. In addition, BDNF crosses the blood-brain barrier in both directions[32]. As the peripheral levels of BDNF increased, the intracerebral levels of BDNF may also increase and facilitate beneficial plastic changes of specific brain resions. Indeed, Schabitz et al. have demonstrated that daily intravenous administration of BDNF improves sensorimotor function after stroke in rats[33,34].

On the other hand, Bocchio-Chiavetto et al have reported that BDNF levels were unchanged 1 week after electroconvulsive therapy but were higher 1 month after treatment than before treatment[35]. In the present study, the timing of this BDNF increase was unclear. Longer follow-up time may be needed to observe a significant correlation between the extent of motor functional improvement and changes in BDNF levels. In addition, the present results suggest that the time until beneficial plastic changes are generated by low-frequency rTMS/OT varies from patient to patient.

Our results suggest that the motor functional improvement in the affected upper limb after stroke is generated by many factors, i.e., low-frequency rTMS and intensive OT,

plastic changes in the brain associated with the increase in the BDNF level, an increase in the quantity of muscle, and an improvement in range of motion.

The study has several limitations. First, because no control groups (e.g., patients receiving only low-frequency rTMS, only OT, or no treatment) were included, it is difficult to draw strong conclusion regarding the changes in serum BDNF levels and in the measures of motor function. Further study with patients randomly assigned to treatment groups should be performed to confirm the effects of combined therapy and of each intervention on serum BDNF levels. Second, no data on serum BDNF levels and motor function after discharge from the hospital are available. Long-term follow-up beyond the end of treatment is needed to investigate this issue. Third, because of the small numbe of patients we were not able to identify "good-responders" in whom serum BDNF levels increase markedly after low-frequency rTMS/OT. Recently, BDNF val66met polymorphism has been shown in animal experiments to be associated with reduced activity-dependent BDNF secretion[36]. Therefore, BDNF genotyping might be used to predict how the serum BDNF level responds to low-frequency rTMS/OT.

Conclusions

In patients with upper-limb hemiparesis after stroke, daily application of low-frequency rTMS over the nonlesional motor cortex combined with intensive occupational therapy significantly increases serum BDNF levels. In addition, the increase in serum BDNF level is weakly correlated with the extent of motor functional recovery in the affected upper limb. Our results suggest that low-frequency rTMS/OT can improve motor functional recovery in paretic upper limb, at least in part through up-regulation of BDNF, although a randomized controlled trial including a control group should be performed to confirm the findings.

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