significant differences in body mass index, serum cholesterol or triglycerides in subjects receiving chitosan for four weeks, compared to those receiving placebo (Pittler *et al*, 1999). In contrast, other authors reported that chitosan significantly lowered serum LDL cholesterol after 8 weeks of treatment (Wuolijoki *et al*, 1999). This study was per-protocol analysis intended to investigate whether oral chitosan can reduce total and LDL serum cholesterol in normal healthy females who maintain their usual diet.

Methods

Women with mild to moderate hypercholesterolemia were recruited via an advertisement. All subjects provided written informed consent. The study was approved by the Screening Committee for Alternative Medicine at the Shimane Institute of Health Science and conducted. An individual who was not involved in this study performed block randomization. The randomization code was sealed in an envelope, and broken just after the study of the trial. Subjects taking drugs that could affect lipid metabolism or cholesterol levels (eg HMG-CoA reductase inhibitors, cholestyramine, clofibrate, steroids or beta-blockers) were excluded. Liver or renal disease, poorly controlled hypertension, diabetes mellitus, heart failure, pregnancy or lactation were also grounds for exclusion. Subjects were asked to maintain their usual diet and document the type and gross amounts of food consumed. Food consumption was classified into 16 food groups (milk, eggs, meat, fish, beans, vegetables, seaweed, potatoes, fruit, grain, sweets, alcohol, soup, pickles, ham and deep-fried foods). For each food group, consumption was divided into three grades (much, average and little).

Chitosan capsules (Koyo Chemical Co. Ltd, Tokyo, Japan) contained 200 mg of chitosan. The actual amount of chitosan was 199.1 ± 6.7(mean ± s.d.) mg. Chitosan viscosity was 160 mPa s, and the extent deacetylation was 89.5%. Placebo capsules contained 300 mg of lactose. There was no difference between chitosan capsule and placebo capsule in the color, size, form, smell or taste. All subjects received three capsules of chitosan or placebo twice daily after a meal for 56 consecutive days.

Fasting blood samples were taken at baseline and after 28 days and 56 days of treatment: total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol and triglyceride levels were measured. Blood pressure, body weight and body mass index (BMI) were also assessed. Safety was evaluated with serum chemistry profiles, complete blood counts, and changes in physical findings and signs at each study visit.

Because risks for progression of atherosclerosis can now be tabulated based on age, subgroup analysis in subjects over 60 y of age was carried out.

Results were presented as means and standard deviation (s.d.). Changes in lipid variables, body mass index, and fat-weight ratio from baseline to 28 and 56 days were analyzed using paired *t*-tests; analysis of variance (ANOVA) for repeated measures was used to compare intervention and control values. The chi-square test was used to compare inter-group differences of subjects in each treatment group with adverse events. The statistical analyses were performed using Statview (SAS Institute Inc.).

Results

Ninety female volunteers entered this study and were randomly assigned to the placebo (46) or chitosan (44) group. Eighty-four subjects (41 chitosan, 43 placebo) were included in the data analysis: three withdrew from the study for personal reasons after the first visit, and three who had taken less than 80% of their allotted medication by their second visit were excluded (Figure 1). The compliance of both groups was generally good. The compliance of each subject was at least 80%.

The baseline characteristics of the subjects in the chitosan and placebo groups were not significantly different (Table 1). There were no significant changes in weight, BMI and blood pressure in either the chitosan or placebo groups after 4 or 8 weeks of treatment. No differences between the chitosan and placebo groups were found with respect to food consumption.

Compared with baseline, 8 weeks of chitosan therapy produced a statistically significant reduction in total cholesterol (241 ± 25 mg/dl to 233 ± 25 mg/dl, $P = 0.04$). Significant differences were observed between the curves of chitosan and placebo groups ($F = 3.19$, $P = 0.04$; Figure 2). In the chitosan group, LDL cholesterol also significantly decreased 152 ± 26 mg/dl at baseline to 143 ± 22 mg/dl at 8 weeks, ($P = 0.007$). However, no significant differences were observed

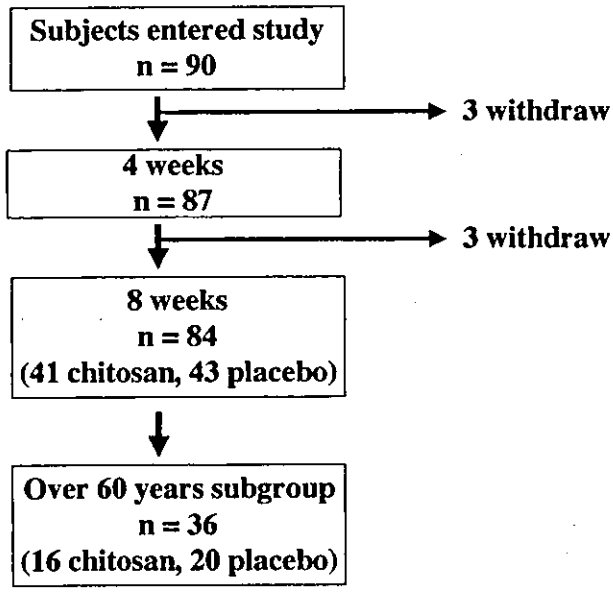

Figure 1 Flow chart for the number of the subjects.

Table 1 The characteristics and plasma lipids (mean ± s.d.)

	Baseline		Four week assessment		Eight week assessment	
Parameter	Chitosan	Placebo	Chitosan	Placebo	Chitosan	Placebo
Age (y)	56.7±8.0	56.4±9.0				
Weight (kg)	56.7±10.5	53.7±6.8	56.6±10.6	53.7±6.9	56.7±10.6	53.4±6.9
Body mass index (kg/m^2)	23.6±3.5	22.3±2.3	23.6±3.5	22.3±2.4	23.6±3.6	22.2±2.4
Blood pressure (mmHg)						
Systolic	135±19	134±16	135±21	125±18	132±21	129±16
Diastolic	75±10	73±8	74±11	69±9	77±11	75±11
Total cholesterol (mg/dl)	241±25	241±33	238±26	235±37	233±25*	241±33
HDL cholesterol (mg/dl)	68±15	71±15	66±15*	68±16**	65±12**	69±16*
LDL cholesterol (mg/dl)	152±26	153±32	149±25	146±33*	143±22**	150±29
Triglycerides (mg/dl)	122±95	96±35	110±48	98±42	114±77	90±31

Chitosan, $n=41$; placebo, $n=43$.
*$P<0.05$; **$P<0.01$ for the comparison with the baseline.

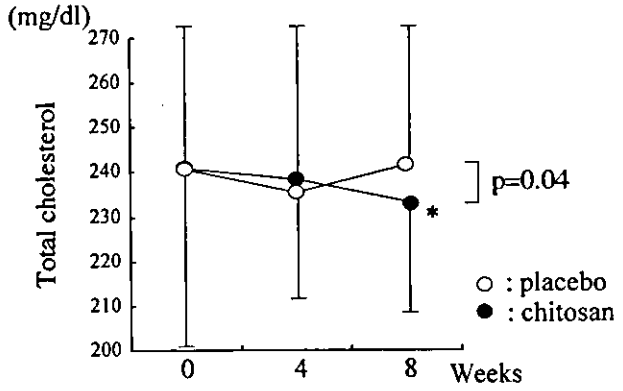

Figure 2 Changes of total cholesterol in all subjects treated with chitosan ($n=41$) and placebo ($n=43$). Closed and open circle are showed in chitosan group and placebo group, respectively. *$P<0.05$ for the comparison with the baseline. A significant differences were observed between the curves of chitosan and placebo groups ($P=0.04$).

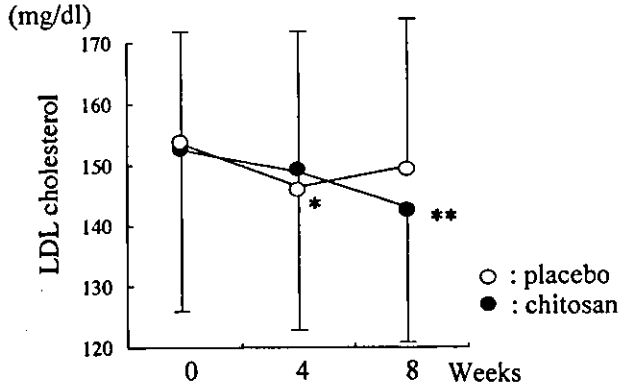

Figure 3 Changes of LDL-cholesterol in all subjects treated with chitosan ($n=41$) and placebo ($n=43$). Closed and open circle are showed in chitosan group and placebo group, respectively. *$P<0.05$, **$P<0.01$ for the comparison with the baseline.

between the curves of two groups ($F=2.73$, $P=0.07$; Figure 3). Although a significant decrease from baseline HDL cholesterol was also observed for both the placebo and chitosan study groups (at both the 4 and 8 week assessments), HDL cholesterol was maintained within normal limits. No significant differences were observed between two curves ($F=0.89$, $P=0.4$). Triglyceride levels did not change significantly in either the placebo or chitosan groups.

In a subgroup of subjects over 60 y of age (16 chitosan, 20 placebo), total cholesterol tended to decrease more in the chitosan group than in the placebo group (Table 2 and Figure 4). After 4 and 8 weeks of treatment, total cholesterol

Table 2 Values of serum lipids of subjects with more than 60 y of age

	Baseline		Four week assessment		Eight week assessment	
Parameter	Chitosan	Placebo	Chitosan	Placebo	Chitosan	Placebo
Total cholesterol (mg/dl)	241±30	237±26	232±27*	236±32	226±29*	242±27
HDL cholesterol (mg/dl)	66±18	67±12	65±19	65±11	64±15	66±12
LDL cholesterol (mg/dl)	153±28	152±27	144±25*	148±30	135±22**	151±24
Triglycerides (mg/dl)	130±62	95±38	108±41	94±47	110±55	94±35

Chitosan, $n=16$; placebo, $n=20$. *$P<0.05$; **$P<0.01$ for the comparison with the baseline.

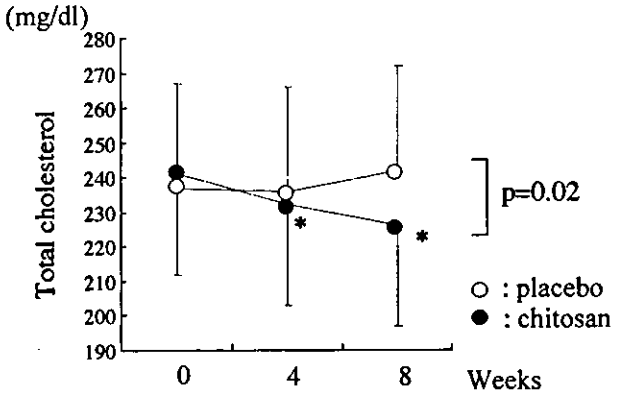

Figure 4 Changes of total cholesterol in over 60 y subjects treated with chitosan ($n=16$) and placebo ($n=20$). Closed and open circle are showed in chitosan group and placebo group, respectively. $^*P<0.05$ for the comparison with the baseline. A significant difference ($P=0.02$) was observed between two curves of groups.

decreased in the chitosan group from 241 ± 30 mg/dl to 232 ± 27 mg/dl and 226 ± 29 mg/dl, respectively, while in the placebo group total cholesterol changed from 237 ± 26 mg/dl to 235 ± 32 mg/dl and 242 ± 27 mg/dl, respectively. There was a significant difference between two curves of the chitosan and placebo groups ($F=4.21$, $P=0.02$). LDL cholesterol at baseline was 153 ± 28 mg/dl in the chitosan group and 152 ± 27 mg/dl in the placebo group, at 4 weeks was 144 ± 25 mg/dl in the chitosan group and 148 ± 30 mg/dl in the placebo group, and 135 ± 22 mg/dl and 151 ± 24 mg/dl, respectively at 8 weeks. A significant ($F=3.46$, $P=0.04$) difference was observed between the curves of two groups (Figure 5). HDL cholesterol and triglycerides did not change significantly in either group.

Adverse events are shown in Table 3. Nine adverse events were observed during the 8 week study period: five in the

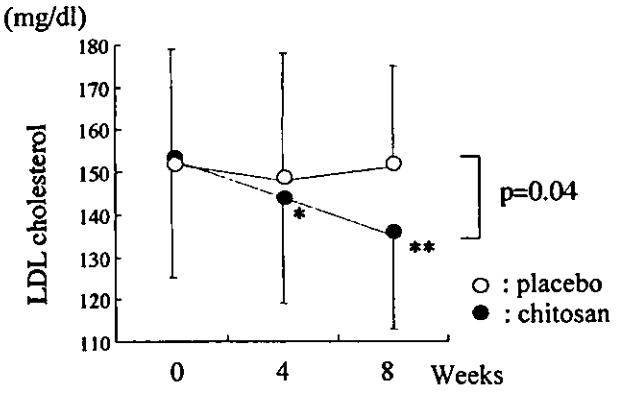

Figure 5 Changes of LDL-cholesterol in over 60 y subjects treated with chitosan ($n=16$) and placebo ($n=20$). Closed and open circle are showed in chitosan group and placebo group, respectively. $^*P<0.05$, $^{**}P<0.01$ for the comparison with the baseline. A Significant difference ($P=0.04$) was observed between two curves of groups.

Table 3 Adverse events during the 8 week of treatment

Symptoms	*Chitosan group*	*Placebo group*
Thirsty	3	0
Oral aphta	1	0
Abdominal fullness	0	1
Headache	0	1
Total	4	2

chitosan group and four in the placebo group. One subject in the placebo group complained of abdominal fullness and dropped out of the study. Three subjects in the chitosan group complained of thirst, the most frequent adverse event for chitosan. Changes in laboratory parameters were very small and transient; subjects with laboratory abnormalities did not show any associated symptoms.

Discussion

Although the recommended dose of chitosan is 2–3 g per day (Pittler *et al*, 1999), we found that, compared with placebo, 1.2 g per day of chitosan produced a statistically significant decrease in total cholesterol in healthy women volunteers after 8 weeks. These effects were more prominent in elderly individuals. Total and LDL cholesterol significantly decreased in the chitosan group in the over 60 y subgroup. Arai *et al* (2000) reported that LDL cholesterol levels in Japanese women increase significantly with menopause. High total and LDL cholesterol, or low HDL cholesterol have been reported to be significant coronary risk factors (Wilson & Kannel, 1993; Sheu *et al*, 2000). The rate of coronary heart disease among women increases with age. The data from large-scale population studies suggest that around the time of the menopause, LDL cholesterol levels increase by approximately 15–25% (Davidson *et al*, 2002). It is very important to control serum lipids in elderly women with hypercholesterolemia, for primary prevention of coronary heart disease (Rackley, 2002). For prevention of coronary disease, optimal LDL cholesterol is reported as < 100 mg/dl in both women and men (Sharrett *et al*, 2001). Our results show that chitosan may have value in the therapy of elderly women with hypercholesterolemia. Previous studies have shown that chitosan reduced serum LDL cholesterol (Yihua & Binglin, 1997; Wuolijoki *et al*, 1999), as also seen in our findings. Wuolijoki *et al* (1999) reported a lack of significant differences between chitosan and placebo with respect to the decrease in total cholesterol in their double-blind, placebo controlled trial. They assumed that they had few registered subjects who had normal serum cholesterol levels. For this reason they thought the decrease of total cholesterol was relatively small. Pittler *et al* (1999) pointed out that, after 4 weeks of treatment, total serum cholesterol was not significantly different in subjects receiving chitosan than in those receiving placebo. Our study also indicated that total serum cholesterol did not change

significantly with 4 weeks of chitosan treatment; a significant decrease was, however, observed after 8 weeks. These findings suggest that the beneficial effect of chitosan may require 8 weeks to appear, perhaps because chitosan does not reduce cholesterol synthesis but reduces lipid absorption from the gastrointestinal tract. However, many subjects were still hypercholestrolemic in terms of total and LDL cholesterol. The effect of chitosan for decreasing cholesterol is thought to be weaker than HMG-CoA reductase inhibitors.

HDL cholesterol levels were significantly decreased in both the chitosan and placebo groups. According to the food consumption records, this may be due to a relatively low-fat diet. In any case, chitosan would be expected to reduce absorption of cholesterol from the gastrointestinal tract. Nonetheless, serum HDL cholesterol remained normal in both groups after 8 weeks. A future direction of study should be to investigate the effect of chitosan on patients with low serum HDL cholesterol level.

As in previous reports, serum triglycerides did not change in the chitosan group. Previous studies also found that triglycerides were either slightly increased (Pittler *et al*, 1999) or not significantly different in subjects receiving chitosan compared with placebo (Wuolijoki *et al*, 1999).

Although previous studies demonstrated that oral chitosan was effective for body weight reduction (Colombo & Sciutto, 1996; Giustina & Ventura, 1995; Veneroni *et al*, 1996), this trial did not show an effect of chitosan on body weight or BMI. The preceding trials were, however, conducted with hypocaloric diets. Pitter *et al* (1999) reported in their randomized, double-blind trial that oral chitosan did not reduce body weight in the absence of dietary alteration. As we did not require the subjects to follow hypoenergetic diets, we consequently found no reduction in body weight.

In conclusion, our results suggest that chitosan is a safe and significantly reducing serum total cholesterol, especially in elderly women. However, the effect of chitosan on decreasing total and LDL cholesterol is mild.

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Meningeal involvement of chronic myelomonocytic leukemia

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Sirs: Chronic myelomonocytic leukemia (CMML) is classified as a clonal disorder of hematopoietic stem cells featuring myelodysplastic syndromes (MDS) and myeloproliferative disorders. CMML is present predominantly in elderly patients and unfortunately most medications prove disappointing. Therefore, the treatment is palliative and the prognosis is poor [4]. There are some reports of CMML involved in extramedullary tissues [2, 3, 11], but few in the central nervous system (CNS). The sole case reported [8] so far had a fatal outcome. Here we report two cases of CMML involving meningeal infiltration which were treated with intrathecal injections of cytosine arabinoside (Ara-C), methotrexate (MTX) and methylprednisolone or MTX alone resulting in favorable responses.

Patient 1. An 83-year-old man was admitted to our hospital for inguinal hernia. Postoperative peripheral blood examinations revealed sustained leukocytosis (the WBC count was 19.8–22.7 × 10^9/liter) with monocytosis (35–45 %) mild anemia (10.8–11.0 g/dl) and thrombocytopenia (91–100 × 10^9/liter). While the blast was not observed in the peripheral blood, 10 % blasts were counted in the bone marrow with hypercellularity. Bilineage dysplastic changes (micromegakaryocytes and hypersegmentation of neutrophils) were also noted. Chromosomal analysis revealed deletion of the Y chromosome. We considered this patient as CMML according to the FAB criteria [1] and did not perform any specific treatment because of the stable hematological condition. Six months later, he had double vision and right facial palsy. He showed left sixth, right fifth and seventh cranial nerve palsies and left Horner's syndrome. Lumbar puncture revealed an elevated mature monocyte count (13/μl, Fig. 1), but no neoplastic cells, with 52 mg/dl of glucose and 87 mg/dl of protein in the cerebrospinal fluid (CSF). Head magnetic resonance imaging (MRI) showed normal findings. Therefore, we presumed the diagnosis was CMML with meningeal infiltration, and performed 3 intrathecal injections of Ara-C (40 mg), MTX (15 mg) and methylprednisolone (4 mg) in one month. The patient recovered gradually and the symptoms disappeared on the 25th day after the treatment ended. The monocyte count in the CSF gradually decreased with improvement. His hematological condition is still stable without any treatment for CMML more than 2 years after discharge.

Patient 2. A 50-year-old man was admitted to our hospital because of fever, headache and disorientation. Lumber puncture revealed marked increase of cell numbers (296/μl) with dominant neutrophil population (96 %), 61 mg/dl of glucose and 49 mg/dl of protein. We could not detect any pathogen in CSF. The gadolinium-DTPA enhanced head MRI showed thickening of the meninges. He had double vision, right ptosis, in addition to hypesthesia and tingling of the bilateral lower extremities, after admission. MRI revealed a mass lesion which could not be enhanced by gadolinium-DTPA in the spinal cord at the L1 level (Fig. 2), this suggesting an intramedullary location. Unfortunately, we could not biopsy it. Then an infiltrative shadow appeared at the left lung. The persistent level of leukocytosis (the WBC count was 11.6–16.6 × 10^9/liter) with monocytosis (18–43 %), and the appearance of blastic cell (0.5–1 %) indicated the necessity for bone marrow examination. This showed hypercellular bone marrow occupied predominantly with myelomonocytic cells. The blast cell count was 25 % and dysplastic changes were observed in two lineages (myeloid and erythroid). These findings grossly accorded with CMML except for the percentage of blastic cells in the bone marrow. Cytogenetic abnormality with t[11, 19](q23;p13.1) was also recognized. Bronchoscopic biopsy of the pulmonary lesion revealed inflammatory change with monocytic infiltration. We diagnosed this patient as CMML with meningeal and pulmonary involve-

Fig. 1 Cytological examination of the CSF in patient 1. Only mature monocytes were detected

Fig. 2 Lumbar gadolinium-DTPA enhanced MRI in patient 2. A mass lesion was detected in the spinal cord at the L1 level (circle)

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ORIGINAL INVESTIGATION

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Event-related brain potential changes after Choto-san administration in stroke patients with mild cognitive impairments

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Abstract *Rationale:* Few drugs have been reported to be effective for the treatment of vascular dementia. Choto-san is a herbal medicine expected to be effectivein this condition, but it is unclear how this drug modulates brain activities and cognitive functions. P3 event-related brain potentials (ERP) provide reliable electrophysiological indices for some aspects of cognitive functions. *Objectives:* We measured P3 ERP to assess the effect of Choto-san administration on stroke patients with mild cognitive impairments. *Methods:* Choto-san was given for 12 weeks to ten chronic stroke patients. P3 ERP were recorded before and after drug administration in a modified auditory oddball paradigm including occasional novel sounds using a high-density array EEG recording system. The reproducibility of ERP was also assessed in other ten stroke patients with a 12-week interval. Cognitive functions were assessed with the Mini Mental State Examination (MMSE) and verbal fluency test. *Results:* Twelve-week administration of Choto-san significantly improved MMSE and verbal fluency test scores. The reproducibility of P3 latency and amplitude to target and novel sounds was excellent. P3 latency to target sounds was shortened in association with reduced reaction time to the sounds after drug administration. Furthermore, P3 amplitude to novel sounds was enlarged and its topography shifted from central to frontal sites. *Conclusions:* These results indicate that Choto-san improves electrophysiological indices related to attention and decision making, in addition to neuropsychological test scores in stroke patients with mild cognitive impairments.

Keywords Herbal medicine · ERP · Target P3 · Novelty P3 · Topography · Frontal lobe function

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Introduction

Treatment of dementia has been one of the biggest challenges in recent geriatric medicine. Cholinesterase inhibitors and estrogen replacement therapy have been proved to be effective for improvement of some cognitive functions in patients with Alzheimer's disease (AD) (Windisch 2000). There is also evidence for the efficacy of alternative herbal remedies such as Ginkgo biloba in the treatment of memory impairment (Kleijnen and Knipschild 1992). In contrast to AD, few effective treatments have been reported for vascular dementia (VD), although the incidence of VD is as frequent as that of AD in Japan (Yoshitake et al. 1995; Seno et al. 1999).

A herbal medicine termed Choto-san (Diao-Teng-San in Chinese) is a kampo (Japanese herbal) prescription, administered to older patients suffering from physical weakness and subjective symptoms such as headache, a heavy feeling of the head, vertigo, hot flashes, tinnitus, insomnia, and painful tension of the shoulder. Since these symptoms often occurred after stroke, Choto-san has also been expected to be effective to cognitive impairments after stroke. Recently, Terasawa et al. (1997) conducted a double-blind, placebo-controlled study of Choto-san in a fairly large number of patients with VD. The study demonstrated its effectiveness on several cognitive behavioral impairments after stroke. The dementia scale also tended to improve in the Choto-san administration group compared to the placebo group. However, it is largely unknown how brain neural activities are modulated by drug administration.

Event-related brain potentials (ERP) have been used to investigate electro- physiological indices of information processing in human. ERP latency and amplitude have been interpreted as the speed of perceptual or response-related processing and attentional resource allocation in memory updating, respectively (Kutas et al. 1977; Wickens et al. 1983; Friedman 1984). Cognitive impairments associated with aging and dementia can be reflected in ERP changes (Goodin et al. 1978; Polich 1997; Friedman et al. 1998). P300 is the most frequently

investigated ERP component in neurological and psychiatric populations, in addition to normal subjects (Polich and Kok 1995; Polich 1998). Correctly detected task-relevant stimuli generate a parietal maximal P300 (target P3), whereas non-target, deviant stimuli requiring no behavioral response generate an earlier latency, front-central P300 (novelty P3), which is considered to be an index of the orienting response (Courchesne et al. 1975; Squires et al. 1975; Knight 1984; Friedman and Simpson 1994; Katayama and Polich 1998). Target P3 is abolished by temporal-parietal lesion (Knight et al. 1989; Yamaguchi and Knight 1991a), suggesting a critical contribution of posterior association cortices on target P3 generation. On the other hand, novelty P3 is reduced in patients with lesions in prefrontal or medial temporal cortex (Knight 1984, 1996; Yamaguchi and Knight 1991a). Direct recording of neural activity using intracranial electrodes (Scabini and McCarthy 1993; Halgren et al. 1998), source analysis of scalp-recorded potentials (Friedman et al. 2001) and developmental study (Cycowicz and Friedman 1997) suggest its association with frontal lobe function. Our recent study demonstrated that VD patients manifested distinctive patterns of ERP changes from AD patients and that novelty P3 was affected more severely in VD patients (Yamaguchi et al. 2000). A pharmacological study also suggested that ethanol affects the amplitude of novelty P3 differentially compared to target P3 (Grillon et al. 1995).

In this study, we assessed alterations of brain neural activity by measuring target and novelty P3 ERP before and after the administration of Choto-san in patients with stroke-related cognitive impairments. When ERP are used for assessing drug effects on cognitive functions, the reproducibility of ERP within repeated measurements is of great concern. Several reports demonstrated good reproducibility of target P3 measures over the interval of months or years (Segalowitz and Barnes 1993; Walhovd and Fjell 2002). However, there are no reports on long-term reproducibility of novelty P3, whereas novelty P3 amplitude shows rapid habituation within a session (Knight 1984; Yamaguchi and Knight 1991b; Friedman and Simpson 1994). Thus, in order to assess drug effects on both P3 components reliably, we also studied the reproducibility of the two P3 components in patients with stroke who showed clinical features similar to those included in the drug study.

Materials and methods

Subjects

Ten stroke patients were selected from the neurology section of the outpatient clinic in the Shimane Medical University Hospital. They consisted of two females and eight males, and ranged from 52 to 85 years of age [mean=71.3±9.8 (SD) years]. The criterion for patient selection was that they had at least one episode of cerebrovascular accident and showed mild cognitive impairments after stroke. Their brain lesions were confirmed by MRI scan. The lesion locations were right frontal lobe for one subject, left frontal lobe for three subjects, right caudate nucleus for one subject, right parietal lobe for one subject, bilateral basal ganglia for three subjects, and bilateral thalamus for one subject. No patients showed marked brain atrophy. The duration after the latest stroke onset was from 6 months to 3 years, and their clinical symptoms were stable when they were enrolled in this study. The cognitive abilities were evaluated using Mini Mental State Examination (MMSE) and verbal fluency test. The emotional state was assessed using the Zung Self-rating Depression Scale (SDS) (Zung 1965).

In order to confirm reproducibility and reliability of ERP, we performed repeated measurements of ERP with a 12-week interval in ten other stroke patients without medication (control group; one female and nine males, 68.0±8.6 years old). They also had at least one stroke episode before the measurement. We did not apply cognitive criteria for selection of this control group. Their lesion locations were largely comparable to those of patients with drug administration: The lesions were located in the right frontal lobe for two subjects, left frontal lobe for four subjects, bilateral basal ganglia for three subjects, and left thalamus for one subject. The study was approved by the review board of the Shimane Medical University, and informed consent was obtained from the patients and/or their family members prior to enrollment in this study.

Drug administration

All patients in the medication group were administered Choto-san extract (TJ-47, Tsumura, 7.5 g/day) between meals three times a day for 12 weeks. During the drug administration, no new medications were given to the patients. A Choto-san granular extract of 7.5 g contains 4.5 g of the extract of 11 kinds of dried medical herbs mixed in the following ratio: Uncariae Uncis Cum Ramulus (3.0 g, hooks and branch of *Uncaria sinensis* Oliver), Aurantii Nobilis pericarpium (3.0 g, peel of *Citrus unshiu* Markovich), Pinelliae tuber (3.0 g, tuber of *Pinellia ternata* Breitenbach), Ophiopgonis tuber (3.0 g, root of *Ophiopogon japonicus* Ker-Gawler), Hoelen (3.0 g, fungus of *Poria cocos* Wolf), Ginseng radix (2.0 g, root of *Panax ginseng* C.A. Meyer), Chrysanthemi flow (2.0 g, flower of *Chrysanthemum morifolium* Ramatulle), Saphoshnikoviae radix (2.0 g, root and rhizome of *Saposhnikovia divaricata* Schischkin), Glycyrrhizae radix (1.0 g. root of *Glycyrrhiza uralensis* Fisher), Gypsum Fibrosum (5.0 g, $CaSO_4$ $2H_2O$) and Zingiberis rhizoma (1.0 g, rhizome of *Zingiber officinale* Roscoe).

ERP measurements

ERP were recorded before and after the 12-week administration of the drug in the medication group. The recordings were also repeated with the same interval in the control group. Subjects were tested while sitting on a chair in a sound-attenuated room with dim lighting. Before the recording binaural audiometric thresholds were determined at 1000 Hz for each subject. All stimuli were presented binaurally at 60 dB above each individual's threshold through headphones in the experimental sessions. The auditory stimuli consisted of 1000 Hz pure tone bursts as standard stimuli and 2000 Hz pure tone bursts as target stimuli. In addition, environmental and computer-generated sounds were delivered as novel stimuli. To keep the stimuli novel during the experiment, 39 different novel sounds were prepared for one session; each novel sound was used only once during each session. Novel sounds in the second session were also unique and different from those used in the first session. The stimulus duration was 100 ms for pure tones and 200 ms for the novel sounds. The interstimulus interval was 1.0–1.3 s. The probability of each sound category was 0.65 for standard, 0.20 for target, and 0.15 for novel stimuli. The three types of sounds were presented randomly in a stimulus sequence. The subjects were requested to press a button using the right index finger when they heard the target sound. No patients had severe hemiparesis. They were not informed beforehand that novel sounds would be presented. The experiment consisted of 260 trials. Before the experimental session, the subjects had a practice session that

consisted of 20 stimuli without novel stimuli to be familiarized with the task.

EEG was recorded using a dense array 128 channels EEG system [Electrical Geodesics Inc. (EGI)]. All electrodes were referenced to the vertex channel during recording. The impedance of electrodes was kept below 40 kOhm. This level of electrode impedance was achieved by applying saline onto the spongy placed between the skull and electrode, and this relatively high electrode impedance was not a problem in EEG recording with a modern high input-impedance amplifier (265 MOhm at 60 Hz) (Ferree et al. 2001). To avoid a problem of cross talk between adjacent electrodes (Tenke and Kayser 2001), we followed EGI procedure by (1) insuring that no sensors were resting on a mat of hair, which can result in bridging, and that each sensor was seated under the hair on the scalp, which avoids bridging, and (2) examining the recordings to determine that there was sufficient variance between adjacent channels. The EEG samples were amplified with a 0.1–50 Hz bandpass filter, digitized at a rate of 250 samples per second, and stored on a hard disk for off-line analysis. The EEGs were averaged over 1200 ms, time-locked to stimuli, including 200 ms of a prestimulus baseline. The EEGs to target stimuli were averaged only for the trials accompanied with correct response occurring within the time range of 200–1000 ms after stimulus onset. The ERP to novel stimuli were obtained only from the trials without response execution (i.e. false alarm). Individual trials with excessive muscle activity (greater than 100 μV peak-to-peak) or eye movement (greater than 100 μV peak-to-peak amplitude) were excluded. The averaged data were re-referenced algebraically to an averaged value across all electrodes and the vertex channel was used as the 129th channel for the analysis.

Statistical analysis

We analyzed two types of P3 components, i.e. target and novelty P3. Target P3 was generated by detection of target sounds and was defined as the largest positive peak over the parietal site in the latency range of 300–600 ms post-stimulus. Novelty P3 was a response to task-irrelevant novel sounds, and was the largest positive peak over the fronto-central site within the latency range of 300–600 ms. These time windows were determined by inspection of grand average waveforms. The peak amplitudes of target and novelty P3 were determined in all channels within the time windows for each subject at first. Then a single channel that showed the maximal peak amplitude among all channels was identified in each subject. The amplitude and latency measures from that channel were used for the group analysis. They were subjected to repeated measures analysis of variance (ANOVA), to evaluate drug effects and reproducibility of P3 components. In addition to changes in amplitude and latency, drug effect on scalp topography was also analyzed for each P3. First, the amplitude was normalized within each subject for each P3 component (McCarthy and Wood 1985). Then the statistical analysis for the topography was performed on the data from midline electrodes, because both P3 components distributed maximally over midline scalp sites. Finally, the lateral expansion of the activity was analyzed over the parietal site for target P3 (Ch 58, 59, 60, and 61 for the left hemisphere and Ch 79, 86, 92, and 97 for the right hemisphere) and the frontal site for novelty P3 (Ch 20, 25, 28, and 34 for the left hemisphere and Ch 4, 122, 123, and 124 for the right hemisphere). The data were analyzed using repeated measures ANOVA with adjustment by the Greenhouse-Geisser method to correct for deviations from sphericity and to account for auto-correlation of the data.

Behavioral data were also compared between before and after the 12-week drug administration using a paired t-test. A level of $P<0.05$ was accepted as statistically significant. The statistical analyses were performed using Statview (SAS Institute, Inc.) and SPSS (SPSS, Inc.).

Results

Behavioral data

The score of MMSE was significantly increased from 23.8±3.6 (mean±SD) to 25.2±4.0 after the administration of Choto-san for 12 weeks (t=2.33, $P<0.05$). The number of words in the verbal fluency test was also increased from 8.3±3.8 to 10.0±3.7 (t=2.76, $P<0.05$). The SDS score did not change (36.5±6.8 versus 35.0±6.1). The control group showed no significant changes in MMSE (26.8±2.8 versus 27.0±2.5), verbal fluency (12.3±2.5 versus 12.8±2.8), and SDS (29.3±6.4 versus 31.6±6.2) between the first and second measurement.

We measured reaction time and correct response rate to target stimuli during the ERP experiment. The reaction time was significantly decreased from 684.1±134.3 ms to 624.6±95.6 ms (t=3.03, $P<0.02$), and the correct response rate was also significantly increased from 82.5±12.2% to 87.8±9.8% (t=2.59, $P<0.05$) after the drug administration. There was no significant change in either reaction time or correct response between the first and second measurement in the control group (442.7±58.6 ms versus 435.5±57.5 ms and 87.7±9.5% versus 91.0±10.1%).

ERP data

The reproducibility of ERP between the first and second measurement were demonstrated in Fig. 1 for target P3 and Fig. 2 for novelty P3 in the control group. The maximal amplitude of target P3 was located around the parietal sites. The peak amplitude of target P3 showed no significant difference between the first and second measurement (4.7±1.6 μV versus 4.5±1.5 μV, $P>0.7$). In contrast to target P3, novelty P3 amplitude was maximal over the frontal-central sites. There was no difference between the first and second measures of novelty P3 peak amplitude (5.6±1.9 μV versus 5.3±1.6 μV, $P>0.6$). The peak latency also showed good reproducibility between the first and second measurement for both target and novelty P3 (442.6±69.0 ms versus 430.2±70.3 ms for target P3, 384.3±44.6 ms versus 399.0±59.3 ms for novelty P3). Correlation was calculated between the first and second measures for each P3. Novelty P3 amplitude showed good reliability within repeated measures (r=0.86, $P<0.005$), while the correlation was slightly lower for target P3 amplitude (r=0.71, $P<0.05$). Latency measures showed high correlations for both target and novelty P3 (r=0.97, $P<0.001$ for target P3, r=0.86, $P<0.005$ for novelty P3).

The drug effect on target P3 is shown in Fig. 3. The peak amplitude of target P3 was 2.4±1.1 μV before drug administration. The amplitude was marginally increased to 3.3±1.8 μV (t=1.98, $P<0.1$) following drug administration. The pre-drug peak latency of target P3 was 494.6±58.8 ms and was significantly shortened to 465.4±46.7 ms after drug administration (t=2.93, $P<0.02$). The change of novelty P3 is shown in Fig. 4. The peak

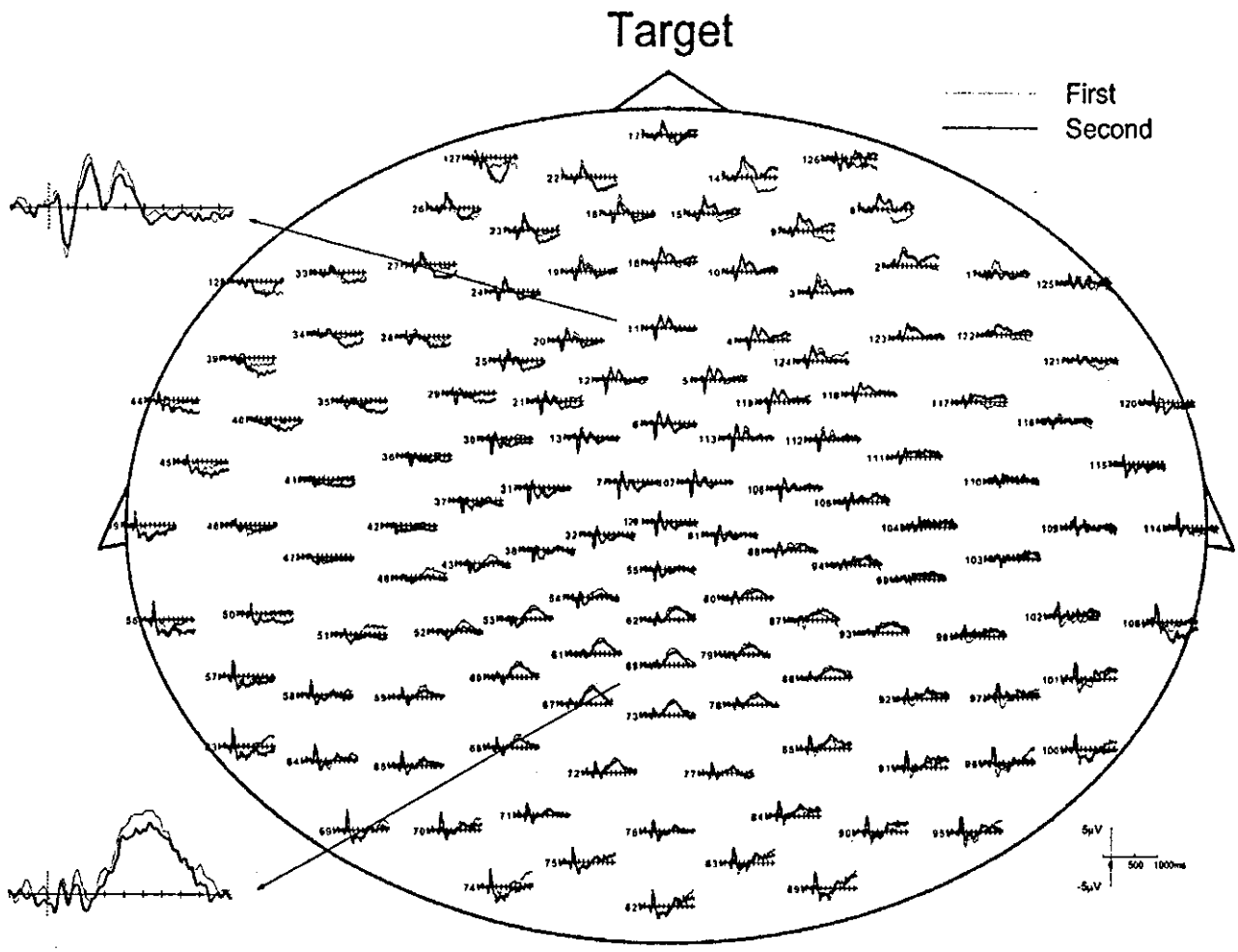

Fig. 1 Grand averaged ERP to target sounds at 129 electrode sites over the scalp surface. *Thin* and *thick* lines represent ERP for the first and second measurement, respectively. All data are referenced using an averaged value across all electrodes. *Left column* shows representative ERP with larger scale at the frontal and parietal sites

amplitude of novelty P3 was significantly increased from 3.3±1.2 μV to 4.9±1.4 μV after drug administration (t=2.95, P<0.02). The peak latency of novelty P3 was not different between pre- and post-administration of the drug (418.4±63.2 ms versus 399.1±65.1 ms).

We examined the effect of drug administration on scalp topographies of target and novelty P3. Target P3 distributed maximally over the parietal site and this pattern did not change after drug administration. On the other hand, the peak of novelty P3 shifted anteriorly after the drug administration (Fig. 5). To confirm the anterior shift of novelty P3, we performed repeated measures ANOVA for novelty P3 amplitude along midline 11 electrode sites after amplitude normalization within each subject. A significant interaction was observed between drug administration and electrode site [F(4.0,36.4)=4.55, P<0.005]. As shown in Fig. 6, this interaction was due to increased amplitudes at the frontal site for post-drug novelty P3. We also examined lateral expansion of the activity due to drug administration by comparing normalized target and novelty P3 between pre- and post-drug administrations over the lateral scalp sites. These analyses showed no evidence of changes in amplitude distribution over the lateral sites for both P3 components.

Discussion

The present study demonstrated that Choto-san improved MMSE scores in stroke patients with mild cognitive impairments. In addition to the improvement of general intelligence level, the score in the verbal fluency test, which has been used as a test of frontal lobe functions, also increased due to Choto-san administration. These results are consistent with the outcome of the double-blind, placebo-controlled study in VD patients (Terasawa et al. 1997).

The main purpose of the present study was to investigate electrophysiological correlates of improvement in cognitive functions by Choto-san administration. To our knowledge, this is the first report that demonstrated changes in brain electrical activity associated with cognitive improvement by herbal remedies. Several modulations were observed in P3 components by Choto-

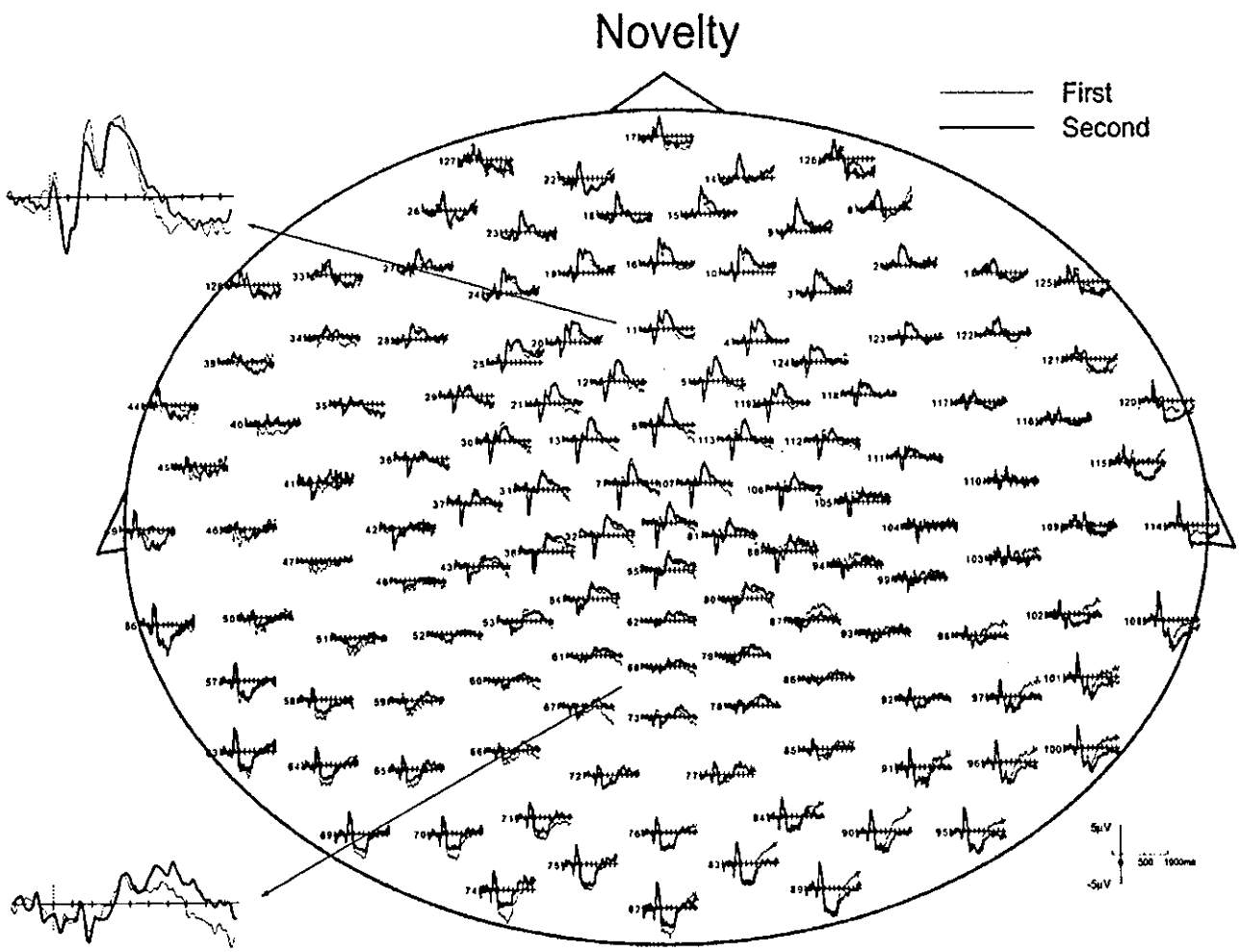

Fig. 2 Grand averaged ERP to novel sounds. Graphical format is the same as Fig. 1

san administration. First, target P3 latency was significantly shortened and amplitude marginally increased, indicating improvement of the efficacy in the neural system involved in voluntary detection of environmental changes and task-relevant information processing. The task requires several processing operations: focusing attention and detection of the relevant frequency of the sounds, comparison with the memorized template, and selection of the response that matches the sample. Target P3 probably reflects the final step of stimulus evaluation and decision making (Hillyard and Picton 1987). The ERP data were also consistent with the behavioral measures, i.e. shortened reaction time and increased correct response to target stimuli.

Second, the response to novel stimuli was enhanced by Choto-san. This was represented by the enlarged amplitude of novelty P3. Novelty P3 is thought to reflect orienting response in the central nervous system (Friedman et al. 2001). Although target P3 contains the aspect of the orienting response as reflecting the mismatch between standard and target stimuli, the larger part of target P3 is contributed by the later stage of task-related information processing (i.e. P3b). The network for novelty detection is known to involve the prefrontal cortex, temporal-parietal association cortex, and medial part of the temporal lobe, which have been demonstrated by lesion studies (Knight 1984, 1996; Yamaguchi and Knight 1991a). Additional converging evidence from intracranial recording (Halgren et al. 1998), pharmacological (Grillon et al. 1995) and imaging studies (Clark et al. 2000; Kiehl et al. 2001) has suggested that different neural networks contribute to generations of target and novelty P3. According to these network models, it is apparent that Choto-san does not modulate only a single brain region or neural system. The finding that Choto-san affected two P3 differentially supports this notion. The neural systems affected by Choto-san seem to include the frontal lobe, because topographical analysis of novelty P3 demonstrated anterior shift of peak amplitude after the 12-week drug administration. One characteristic feature of VD is frontal lobe dysfunction (Fukuda et al. 1990; Yamaguchi et al. 2000), which plays a central role in numerous executive functions such as working memory, inhibitory control, planning, and emotional control (Stuss and Knight 2002). Further study is desirable, to clarify

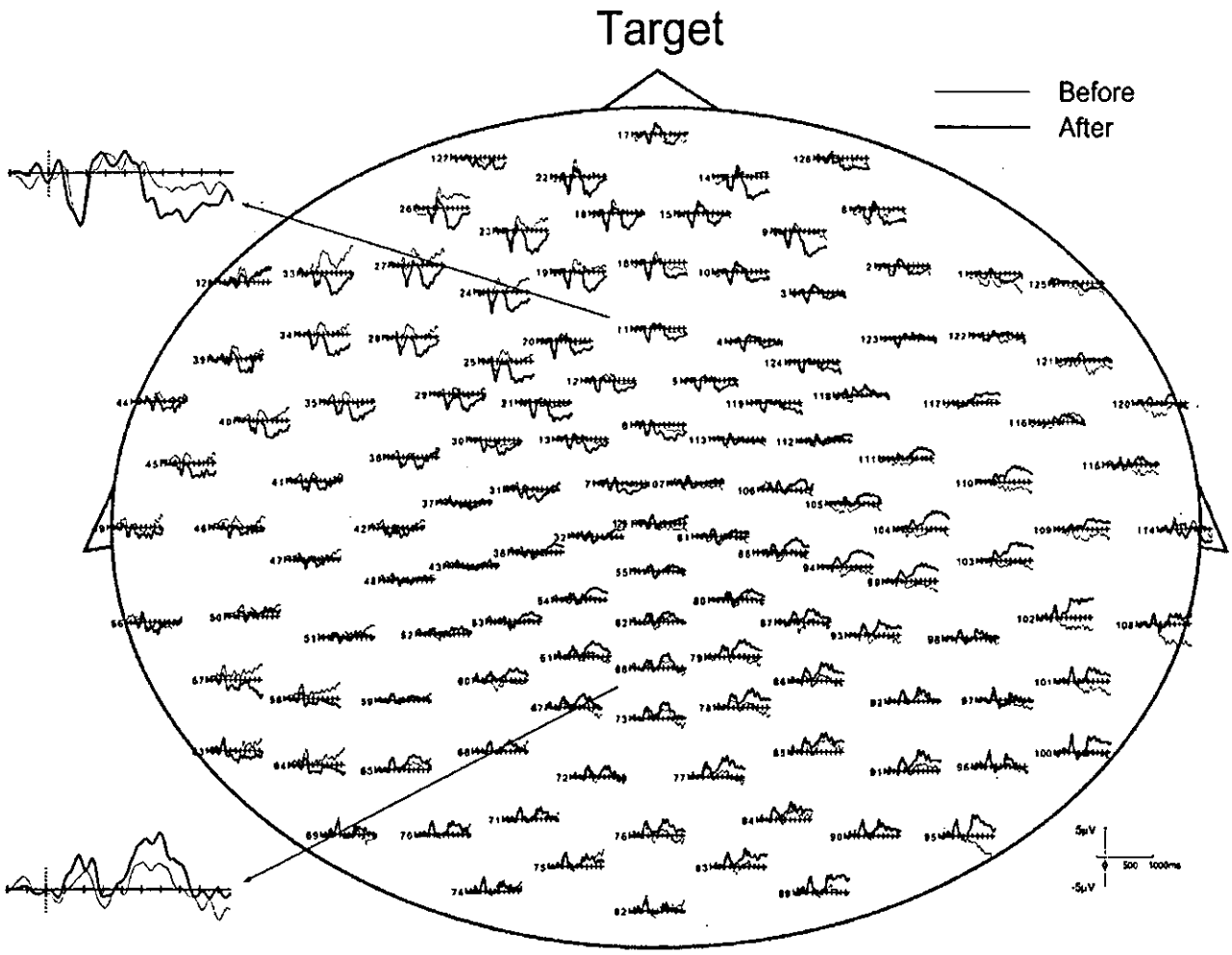

Fig. 3 Grand averaged ERP to target sounds. *Thin* and *thick lines* represent ERP before and after Choto-san administration, respectively. Other format is the same as Fig. 1

how Choto-san modulates these distinct frontal lobe functions.

Anterior distribution of novelty P3 may be interpreted as a contribution of no-go P3 potential, which is also reported to distribute over frontal scalp site (Falkenstein et al. 1995; Bokura et al. 2001). In the paradigm employed in this study, subjects had to withhold their response to novelty stimuli. However, the latency of nogo P3 is typically longer than that of target P3, while novelty P3 latency is shorter than target P3 latency (Katayama and Polich 1998). This distinction suggests that novelty P3 is a different component from no-go P3.

In this study, we conducted a test of reproducibility of P3 components in repeated measurements to draw stronger conclusions for drug effects. Target P3 has been proved to be reliable and reproducible between repeated measurements (Segalowitz and Barnes 1993; Walhovd and Fjell 2002). On the other hand, because novelty P3 is reported to show rapid habituation to repeated exposure to stimuli in the early part of session (Knight 1984; Yamaguchi and Knight 1991b; Friedman and Simpson 1994), its long-term reproducibility must be evaluated before this component is adopted to drug studies. The present study demonstrated good reproducibility of novelty P3, as seen for target P3, in repeated measurements with a 12-week interval. Although contextual novelty is supposed to be stronger in the early part of the first measurement, stimulus novelty may be kept unchanged across two measurements, because all novel stimuli are unique in each presentation throughout the experiment. This finding suggests that the present ERP paradigm provide useful tools for assessing and electrophysiological modulation by drug administration.

The improvement in cognitive improvement and electrophysiological indices could be ascribed to several pharmacological actions of Choto-san and its extracted components, i.e. Uncariae Uncis Cum Ramulus. One of the relevant mechanisms may be improvement of cerebral circulation via changing endothelial function or activation of nitric oxide synthase (Sugimoto et al. 2000). It is reported that Choto-san improved ischemia-induced amnesia for passive avoidance in mice (Yuzurihara et al. 1999). The modulatory effects of Choto-san on serotonin and dopamine transmitter systems were also reported

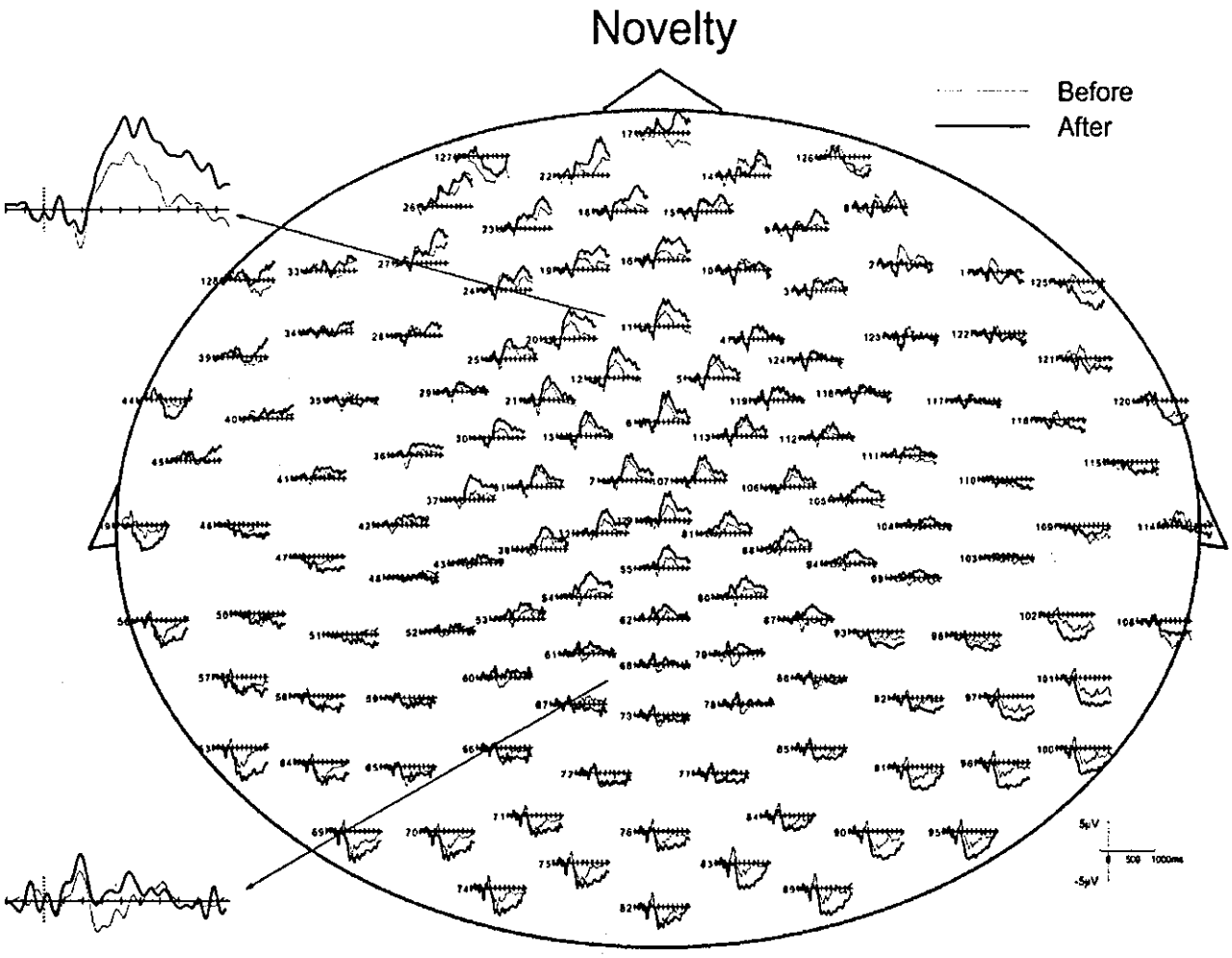

Fig. 4 Grand averaged ERP to novel sounds. Graphical format is the same as Fig. 3

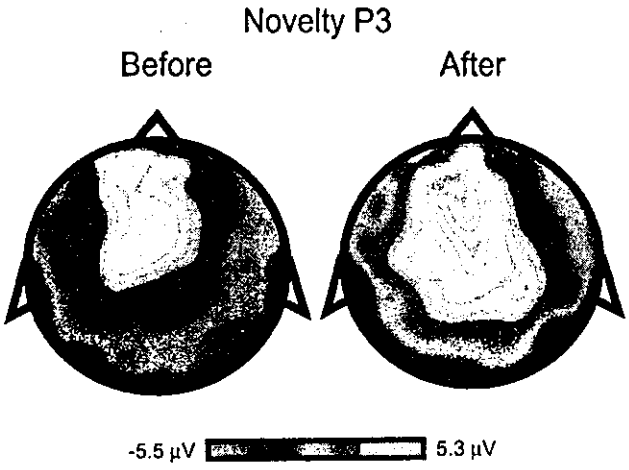

Fig. 5 Topographic maps of the ERP to novel sounds over the scalp before and after Choto-san administration. The ERP latency adopted for these maps is 416 ms and 392 ms for before and after drug administration, respectively. In this figure the data are not normalized because amplitude difference can be also shown. The maps are calculated using spherical spline functions

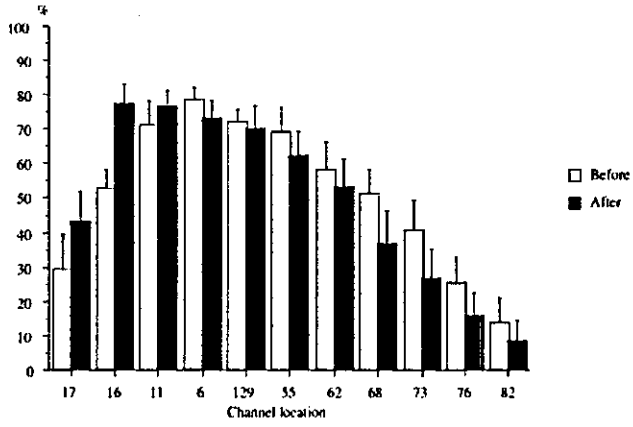

Fig. 6 Topographical changes of novelty P3 along the midline electrodes. Values are mean±SE. Note that the amplitude distribution shifted anteriorly after drug administration

(Kanatani et al. 1985). It is plausible that these modulatory effects contribute to the electrophysiological changes by Choto-san, although the relationship between neurotransmitter systems and ERP components has not been fully uncovered (Hill et al. 1998; Kahkonen et al. 2001).

Since the impairments of cognitive functions were mild in the present patients and any subjects were not diagnosed as VD according to the criteria of DSM-IV, the effect of Choto-san on brain electrical activity in VD patients should be further clarified in future studies. A patient group with selective frontal lobe dysfunction might be a good candidate for the treatment with Choto-san, but studies with a larger, more homogenous, sample of frontal patients may be needed to obtain stronger conclusions. Finally, the present study provides a novel approach to evaluate drug effects on human cognitive functions, and shows a potent effect of Choto-san on modulating brain functions in stroke patients.

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□ CASE REPORT □

Successful Treatment with Succinate in a Patient with MELAS

Hiroaki OGURO, Kenichi IIJIMA, Kazuo TAKAHASHI, Atsushi NAGAI, Hirokazu BOKURA, Shuhei YAMAGUCHI and Shotai KOBAYASHI

Abstract

We present a case report of a 27-year-old man with MELAS, who presented with general convulsions and left flaccid hemiparesis. Anticonvulsant drugs failed to achieve complete control of his convulsions. A good response to oral administration of succinate has been maintained for more than 30 months, with no recurrence of any stroke-like episode. Succinate therapy may have potential for treatment of uncontrolled convulsive MELAS patients.
(Internal Medicine 43: 427–431, 2004)

Key words: MELAS, succinate, therapy

Introduction

MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes) is characterized by mitochondrial dysfunctions in multiple organs, resulting in generalized convulsions and recurrent stroke-like manifestations (1). These symptoms are often intractable. Several effective drug therapies have been reported for the treatment of mitochondrial encephalopathy. They include coenzyme Q, cytochrome c, nicotinamide, dichloroacetate (2) and succinate (3, 4). These therapeutic drugs, which are mitochondrial respiratory chain enzymes or their substrates, are thought to compensate for defects in the corresponding pathways (5). Recently, trials of several new therapeutic drugs have been reported. Among them, idebenone is a synthetic analogue of coenzyme Q (6). L-arginine is a potent donor of nitric oxide, reducing ischemic damage in brain ischemia (7). Creatine enhances adenosine triphosphatase synthesis and serves as an activator of glycolysis (8). Taurine, which is absent in mutant mt tRNA of MELAS, can be imported into mitochondria through a taurine transporter (9). However, the long-term prognosis with these treatments remains unknown. Here, we report a patient with MELAS, in whom two-year administration of succinate at 6 g/day effectively prevented relapse of the cardinal neurological symptoms.

Case Report

A 27-year-old man was diagnosed with secondary generalized seizure and diabetes mellitus at the age of 18. Insulin therapy was started two years later. At the age of 24, an A3243G mutation in mitochondrial (mt) tRNA-Leu (UUR) 3243 allele 1 and wild-type in mt tRNA-Leu (UUR) 3243 allele 2 or 3271 allele 1 were identified from peripheral whole blood cells (by Athena Diagnostics Co. Mass.), which indicated the diagnosis of MELAS. At the age of 26, he was hospitalized with diabetic ketoacidosis and stroke-like episodes (convulsion of the right side of the body, loss of consciousness and aphasia). He recovered after administration of ATP, cytochrome c and coenzyme Q, and was continuously treated with sodium valproate. Sensorineural hearing loss was also found by audiometry in May 2000. Because of the detrimental effects of valproate on oxidative phosphorylation, sodium valproate was changed to phenytoin in August 2000 (10).

At the age of 27, he was admitted to our hospital because of stroke-like episodes with myoclonus and drop attack of the left upper limb in November 2000. These episodes progressed to general convulsions followed by left flaccid hemiparesis in December 2000. Electro-encephalography (EEG) showed a 2 Hz multiple spike and wave complex during general convulsions (Fig. 1A). Furthermore, his consciousness state was lethargic due to diabetic ketoacidosis. The concentration of lactate in CSF was elevated to 46.2 mg/dl, and pyruvate concentration was also increased to 1.66 mg/dl with a high lactate/pyruvate ratio (range 19 to 25) (11). Diffusion-weighted head MRI demonstrated a high sig-

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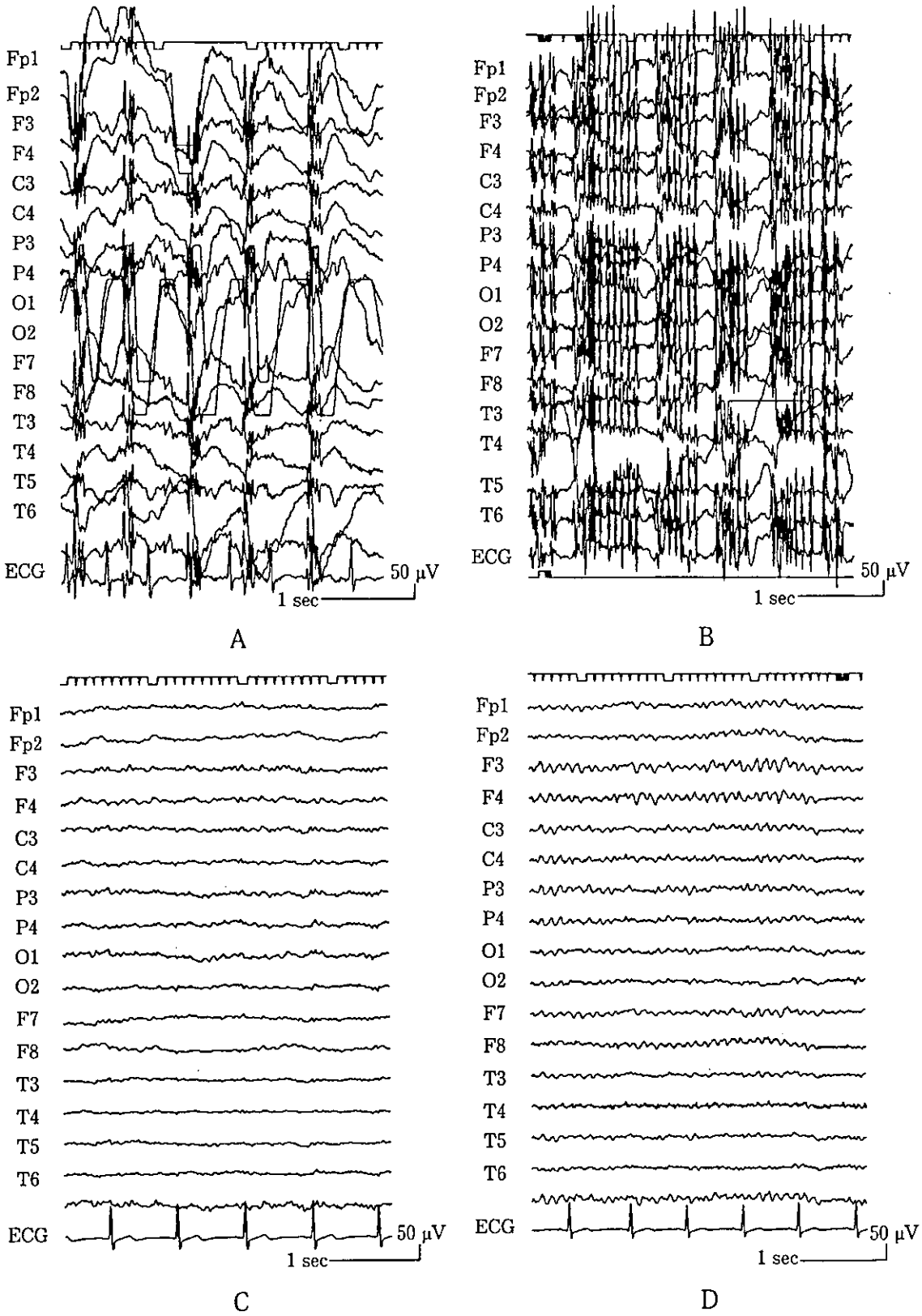
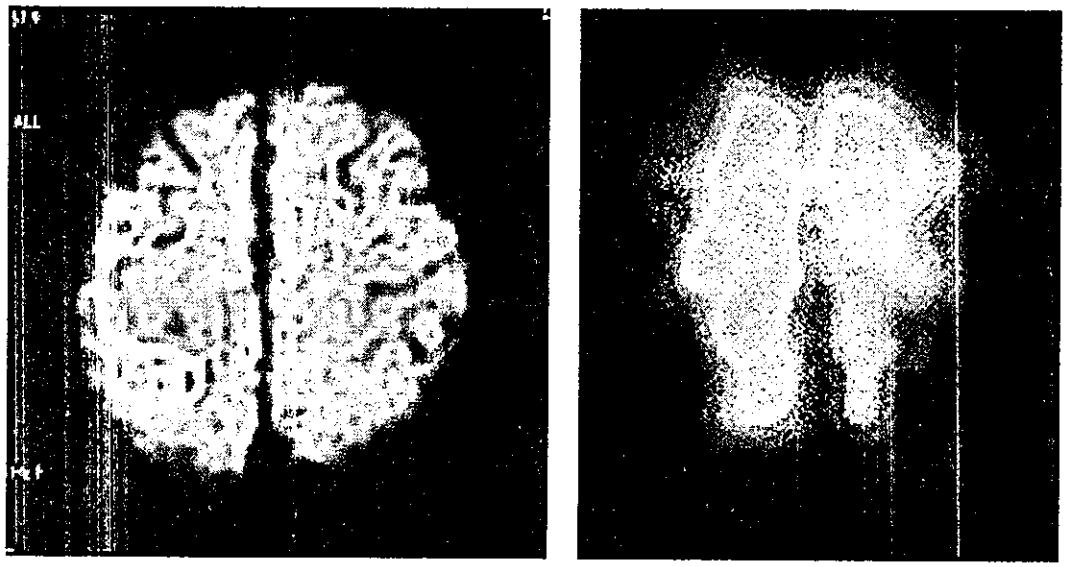


Figure 1. A) 2 Hz multiple spike and wave complex seen on EEG during general convulsions in December 2000. B) Poly-spikes seen on EEG during intermittent myoclonic seizures in February 2001. C) A normal pattern without any spike or sharp wave seen on EEG in February 2002. D) A normal pattern seen on EEG in June 2003.



A B

Figure 2. A) MR diffusion-weighted image showing an epileptogenic focus at the post central gyrus on admission in November 2000. B) Tc-99m HMPAO brain SPECT in January 2001.

nal intensity region at the post central gyrus (Fig. 2A) and fluid attenuated inversion recovery (FLAIR) images showed multifocal cortical hyperintensities located bilaterally in the temporo-occipital lobes (Fig. 3A). Tc-99m HMPAO brain SPECT showed increased blood flow in the right front-parietal lesion (Fig. 2B). Muscle biopsy of the quadriceps femoris revealed a deficiency of cytochrome c oxidase in muscle fibers (Fig. 4A) and strongly SDH-reactive blood vessels (SSV) (Fig. 4B), but ragged-red fibers (RRF) were not detected (Fig. 4C). Aggregates of enlarged mitochondria or paracrystalline inclusion bodies characteristically detected in RRF were not found by electron microscopy. The point mutation of A3243G in mt DNA was also recognized in the muscle sample.

Anticonvulsant drugs (clonazepam and zonisamide) were partially effective, but failed to give complete control of convulsions. Poly-spikes were seen on EEG during intermittent myoclonic seizures in February 2001 (Fig. 1B). Then, we started oral administration of succinate at 6 g per day. After one week his consciousness level improved and abnormal neurological signs, including myoclonus and hemiparesis, disappeared. The same amount of succinate and the anticonvulsants were continued for 30 months, during which he showed no neurological abnormalities.

Although the concentrations of lactate and pyruvate in CSF were still high (61.4 mg/dl and 2.12 mg/dl, respectively), EEG showed a normal pattern without any spike or sharp wave in February 2002 (Fig. 1C) and June 2003 (Fig. 1D). Follow-up MRI demonstrated a mixture of remissive and recurrent foci, and similar findings were seen in February 2002 (Fig. 3B) and June 2003 (Fig. 3C). Diabetes mellitus was also well controlled. After succinate therapy the Hasegawa Dementia Rating Scale was improved from 21 points to 26 points. The mean hearing thresholds measured by audiometry slightly worsened from 65.0 dB (rt) and 63.8 dB (lt) in May 2000, to 72.5 dB (rt) and 80.0 dB (lt) in May 2002. He showed a progression to deafness and needed an audiphone in spite of succinate treatment. We advised him to avoid the stresses of strong sunlight, sea bathing and alcohol intake for two years (12). We used pure succinate free of sodium, because sodium succinate can cause hypernatremia, and his serum sodium levels remained in the normal range with no adverse effect for two years.

Discussion

To obtain good efficacy in the treatment of mitochondrial encephalopathy, drugs need to be delivered to the mitochondrial matrix through cell membranes. Succinate has a low molecular weight of 162.06 and is small enough to be transported into the mitochondrial matrix. There are distinct electron transport systems in the mitochondria, i.e., complexes I and II. Complex I receives electrons from NADH originating from α-ketoglutarate, pyruvate and fatty acids, whereas complex II receives electrons directly from succinate in the TCA cycle. These two systems cooperatively create an electrochemical gradient that is used for the production of ATP. MELAS is a mitochodrial disorder due to a deficiency of the subunits of complex I (3). Thus, despite the defect in complex I, the respiratory capacity in a MELAS patient can be restored by succinate administration, through activation of the complex II system. It has been reported that

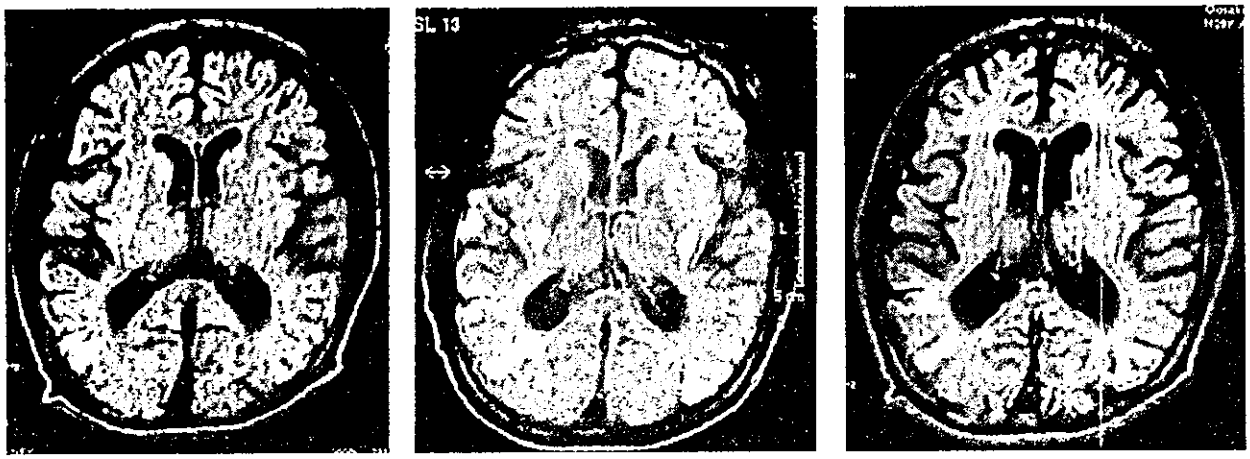


Figure 3. MR FLAIR image shows a mixture of remissive and recurrent foci. A) January 2001, B) February 2002, C) June 2003.

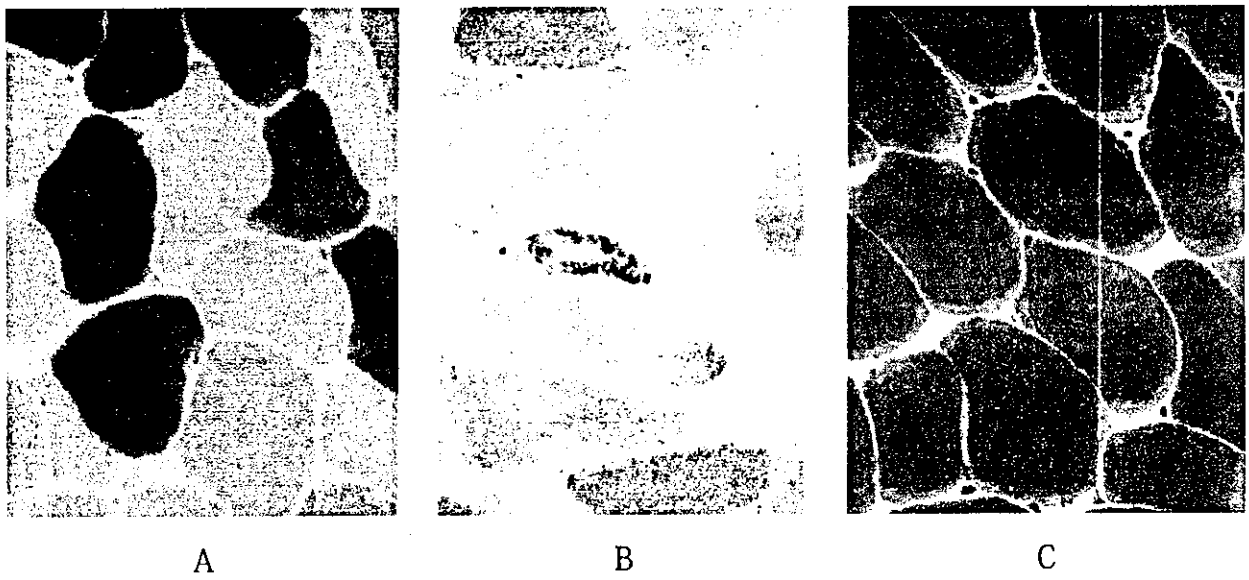


Figure 4. Muscle biopsy specimens (M. quadriceps femoris) ×400. A) Cytochrome c oxidase (CCO) stain: Several fibers show loss of activity. B) SDH stain: Strongly SDH-reactive blood vessel (SSV) can be seen. C) Gomori trichrome stain: No ragged-red fibers are seen.

succinate increases electron flow from complex II to complex III (CoQ-cytochrome c reductase) and complex IV (cytochrome c oxidase), enabling these two energy coupling sites to operate normally (3).

A MELAS patient treated with L-arginine showed significant improvements in lactate and pyruvate levels, and symptoms of headache, nausea and clinical disability were dramatically improved (7). In contrast, lactate and pyruvate concentrations in CSF remained high even following the succinate therapy in the present case. One out of two reported cases successfully treated with succinate also showed sustained elevation of pyruvate and lactate concentrations in CSF (3, 4). The concentrations of these compounds were not correlated with neurological symptoms, and may not be useful indexes of the effectiveness of succinate therapy.

The present patient showed positive SSV but no RRF in a muscle biopsy specimen. The intensity of histochemical staining with SDH more specifically reflects the number and activity of mitochondria, as compared with other stains (13). Two MELAS cases with positive SSV and without RRF were described in previous reports (13, 14), and they showed no apparent muscle symptoms. The present case also showed

no muscle symptom. Absence of RRF in muscle biopsy does not exclude mitochondrial disease (13–15). The diagnosis of MELAS in this case was confirmed by the SDH staining of muscle.

In the present patient also, the neuroimaging findings were not characteristic. FLAIR images showed increased signal intensities bilaterally in the temporo-occipital lobes (Fig. 2B, C). The major pattern of neuroimaging changes in MELAS is reported to be migrating infarct lesions and progressive focal and generalized atrophy in follow-up MRI study, and during the course of the illness, new lesions progressively appear in other cerebral regions regardless of improving clinical signs (16). Our patient showed migrating lesions, but no atrophic changes. Consequently the correlation between symptoms and imaging findings is unclear.

More than 60 percent of MELAS patients have bilateral progressive sensorineural hearing loss (17), as seen in our patient. Longitudinal audiometry tests did not show any improvement due to succinate therapy, and this is consistent with a previous report showing no correlation between degrees of hearing loss and severity of neurological symptoms in MELAS patients (18). Succinate therapy might not completely prevent the progressive symptoms of MELAS.

This is the first report of successful long-term treatment of intractable neurological symptoms in a MELAS patient by administration of succinate. A large-scale clinical trial of succinate treatment for long-term control of MELAS syndrome would seem to be warranted.

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ments, and administered 7 intrathecal injections of MTX (15 mg) during a 12-week period, and daily subcutaneous injections of Ara-C (15 mg × 2/day) simultaneously for 3 weeks followed by combination therapy with Ara-C (15 mg × 2/day, 14 days), M-CSF (Macrophage – colony stimulating factor) (8×10^6 U/day, 14 days) and aclarubicin (20 mg/day, 4 days). Neurological symptoms improved gradually with this treatment. Monocytes in the CSF and the mass lesion in the spinal cord both disappeared at the 12th weeks after the treatment ended. However, hematological response was not enough and he died of acute transformation thereafter.

The prognosis of patients with CMML is generally poor and the median survival length is approximately 8 to 30 months [4, 5, 7]. Although some treatments have been reported to have a small benefit, there is no definite treatment [4, 6, 9]. The incidence of CNS involvement in chronic leukemia is low while it is frequently observed in acute leukemia [10]. In this report, we showed two cases of CMML with meningeal infiltration. It should be noted that mature monocytes rather than leukemic monoblasts were observed in the CSF of these patients. Specifically, in patient 2, monoblasts did not appear in CSF despite the impending acute transformation during the initial course. These cases imply that neurological symptoms might be caused by the infiltration of mature monocytes. Indeed, this phenomenon was reported in patients with chronic myelogenous leukemia as myeloproliferative disorder [12]. The rare CMML case involving CNS reported so far [8] did not receive any specific treatment. Ara-C and MTX can exert an antiproliferating effect, and intrathecal administrations have been reported in patients with CNS involvement in other acute leukemias and malignant lymphoma [10]. Patient 1 was solely treated with intrathecal chemotherapy whereas patient 2 was treated with intrathecal and systemic chemotherapy because of the pulmonary involvement and hematological progression. We cannot conclude yet which combination of intrathecal chemotherapy will be the most favorable unless we consider the long-term prognosis. Nevertheless, these cases indicate the effectiveness of these treatments even for the meningeal involvement of CMML caused by infiltrations of mature monocytic cells.

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