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ORIGINAL RESEARCH

Eccentric Stenosis of the Carotid Artery Associated with Ipsilateral Cerebrovascular Events

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BACKGROUND AND PURPOSE: Eccentric stenosis of the coronary artery is associated with plaque disruption and acute coronary syndrome. The purpose of the present study was to determine whether eccentric stenosis of the carotid artery contributes to cerebrovascular events.

MATERIALS AND METHODS: Of 6859 patients with vascular diseases who underwent duplex carotid ultrasonography, we studied 512 internal carotid arteries in 441 patients who had a maximum area stenosis at or more than 70%, which corresponds with approximately 50% or more by the NASCET method. The maximal (A) and minimal wall thicknesses (B) were measured on cross-sectional sonography images, and an eccentricity index was calculated using the following formula: (A − B)/A. Arteries in the lowest quartile of the eccentricity index (<0.69) were defined as having a concentric stenosis, whereas the others were defined as having eccentric stenosis. The underlying clinical characteristics and plaque morphologies, as well as the occurrence of ipsilateral ischemic stroke or transient ischemic attack in the preceding year, were compared between patients with eccentric and concentric stenosis.

RESULTS: Patient characteristics and plaque morphology were similar between the 2 groups. Cerebrovascular events occurred more frequently ipsilaterally to the artery with eccentric stenosis (13.5%) than to the artery with concentric stenosis (5.5%; $P = .013$); the difference was more evident when cerebrovascular events of presumed carotid arterial origin were assessed ($P = .005$). After adjusting for risk factors and plaque morphology, eccentric stenosis was independently related to the presence of recent cerebrovascular events (odds ratio = 2.76; 95% confidence interval = 1.19–6.40).

CONCLUSIONS: In patients with an area carotid stenosis of 70% or more, eccentric plaque was associated with a significantly increased incidence of ipsilateral cerebrovascular events compared with patients with concentric stenosis.

It is critical to identify patients with carotid stenosis that can lead to ischemic stroke. Several randomized prospective trials demonstrated that the degree of carotid stenosis is a common indicator that can be used to assess the risk of stroke.[1-4] However, because most patients with carotid stenosis without surgical revascularization are free from occurrence or recurrence of ischemic cerebrovascular events after many years, the use of stenosis severity as a measure of stroke risk has a relatively poor specificity.[5] Thus, additional indicators are needed to identify carotid artery lesions associated with a higher risk of stroke. The vulnerability of the carotid plaque morphology has been recognized as an important predictor for stroke.[6] Hypoechoic[7-10] or heterogenous[10-12] plaques on sonography, as well as the presence of intraplaque hemorrhage or a thin or ruptured fibrous cap on multicontrast weighted MR imaging,[13] have been reported to predict subsequent stroke. Plaque characteristics other than stenosis severity may be essential for assessing the risk of artery-to-artery embolism from a carotid plaque.

In the coronary artery, plaque distribution eccentricity is strongly associated with the acute coronary syndrome.[14-19] A prospective observational study using intravascular sonography found that, in all of the acute coronary syndrome patients, the pre-existing coronary lesions showed eccentric stenosis.[16] The reason for this association appears to be that the eccentric plaque is a vulnerable plaque and may indicate disruption and superimposed thrombus.[17]

In the carotid arteries, plaque eccentricity may also be an important marker of subsequent ischemic stroke. However, the correlation between the geometry of the carotid artery stenosis and cerebrovascular events has not been determined. The purpose of the present study was to identify differences in the clinical findings between eccentric and concentric carotid artery stenosis and to elucidate the relationship between the geometry of the stenosis and cerebrovascular events.

Methods

From January 2004 to February 2006, a total of 6859 consecutive patients were examined with duplex carotid ultrasonography in our hospital. Of them, 995 inpatients underwent ultrasonography for acute ischemic stroke, and the other patients were screened for carotid artery disease, because they had recent or chronic stroke, asymptomatic cerebrovascular disease, carotid bruit, vascular risk factors, cardiovascular disease, peripheral artery disease, or nonspecific symptoms, such as headache and dizziness. Of these, 370 patients had a plaque with maximum area stenosis at 70% or more in the unilateral internal carotid artery (ICA), and 71 patients had such plaques in both ICAs; these 512 arteries were studied. Area stenosis of the carotid artery at 70% or more on sonography roughly corresponds with stenosis of 50% or more by the North American Symptomatic Carotid Endarterectomy Trial Collaborators (NASCET) criteria.[1] Arteries

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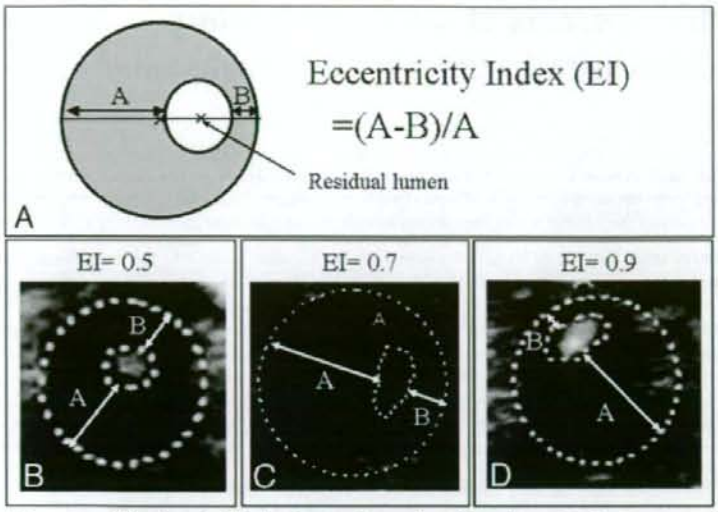

Fig 1. *A*, Eccentricity index = (A − B)/A. *B–D*, Examples of stenotic internal carotid arteries with eccentricity index values of 0.5, 0.7, and 0.9, respectively.

with severe calcification of which the degree of stenosis could not be measured due to the presence of an acoustic shadow on sonography were excluded from the study.

Sonography examination was performed using a duplex color-coded ultrasonographic device equipped with a linear array 7.5-MHz transducer (mainly Aplio XV; Toshiba, Tokyo, Japan). The most stenotic portion of the ICA was determined by using gray-scale and Doppler sonography, as described previously[20]; the cross-sectional image of the stenotic portion was stored on a computer hard disk together with the other sonography findings. Plaque morphology and the distribution of the stenotic portion were evaluated by investigators who were blinded to the patients' clinical information. The maximal (A) and minimal (B) thicknesses of the vessel wall were measured on the cross-sectional image by using Scion Image (Scion, Frederick, Md.), and the eccentricity index of the plaque was calculated by using the following formula: (A − B)/A (Fig 1).[16] The 512 ICAs studied were divided into quartiles based on the index; those in the lowest quartile were defined as having concentric stenosis, and those remaining were defined as having eccentric stenosis. The echogenicity of the carotid plaque was categorized as hypoechoic, isoechoic, or hyperechoic. A hypoechoic plaque was defined as having an echogenicity the same as that of the vessel lumen; an isoechoic plaque was defined as having an echogenicity of the soft tissues surrounding the carotid arteries; and a hyperechoic plaque was defined as having a brighter echogenicity than the surrounding soft tissues.[7] A heterogeneous plaque was defined as containing a mixture of hypoechoic, isoechoic, or hyperechoic lesions.[12]

The following clinical characteristics were evaluated: sex, age, hypertension (blood pressure of ≥140/90 mm Hg or use of antihypertensive medications), diabetes mellitus (fasting blood glucose ≥126 mg/dL, positive 75-g oral glucose tolerance test result, or use of insulin or oral hypoglycemic agents), hypercholesterolemia (serum total cholesterol ≥220 mg/dL or use of antihypercholesterolemic medications), ischemic heart disease, peripheral artery disease, aortic aneurysm, current smoking habit, and habitual alcohol consumption (≥2 drinks per day).

Recent cerebrovascular events, including ischemic stroke, transient ischemic attack (TIA), and transient monocular blindness (TMB) ipsilateral to the stenotic carotid artery within 1 year preceding the sonography study were reviewed from the medical records. For diagnosis of ischemic stroke, we required identification of culprit infarcts mainly on MR imaging, in addition to the episode of neurologic dysfunction. TIA was defined as a brief episode of neurologic dysfunction caused by focal brain or retinal ischemia, with clinical symptoms typically lasting less than 1 hour, and without evidence of acute infarction[21]; an episode caused by retinal ischemia was termed TMB here. As the cerebrovascular events of presumed carotid arterial origin, all of the cerebrovascular events other than those with the small infarct of less than 1.5 cm in diameter in the perforator arterial territory (lacune) and those with the high-risk sources of cardioembolism in Trial of Org 10172 in Acute Stroke Treatment classification[22] were also assessed.

Statistical Analysis

Values are expressed as means ± SDs. The clinical variables of the concentric and eccentric plaque groups were compared by using Student *t* test for continuous variables and the χ^2 test for categoric variables. To determine the predictors for cerebrovascular events, multivariate logistic regression analysis was performed. To ascertain the reasonableness of our dividing arteries into 2 groups by using the first quartile vale of the eccentricity index, we constructed a receiver operating characteristic (ROC) curve and obtained the eccentricity index as the cutoff point for discriminating between patients with recent cerebrovascular events and those without. Statistical test results were considered significant with a P value < .05. SPSS software (SPSS, Cary, NC) was used for the analyses.

Results

The distribution of stenosis geometry of the 512 ICAs by the eccentricity index is shown in Fig 2. The index varied from 0.00 to 0.99; the first quartile, median, and third quartile values were 0.69, 0.87, and 0.95, respectively. Thus, the index

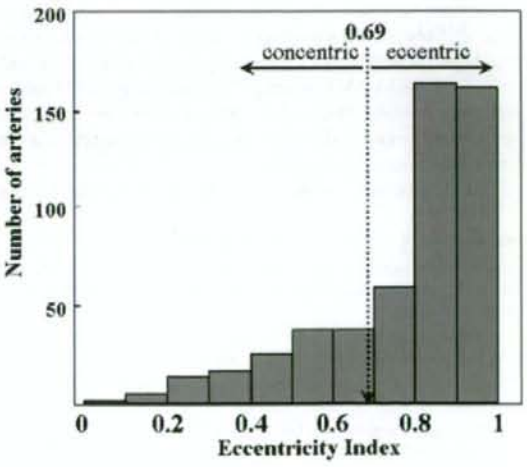

Fig 2. The eccentricity index histogram of 512 internal carotid arteries.

Table 1: Patient characteristics and plaque morphology

Variable	Concentric Stenosis (n = 128)	Eccentric Stenosis (n = 384)	P
Patient characteristics			
Age, mean ± SD, y	73.0 ± 7.5	72.4 ± 7.8	.476
Male, n (%)	107 (84)	339 (88)	.171
Hypertension, n (%)	116 (91)	335 (87)	.306
Diabetes mellitus, n (%)	56 (44)	159 (41)	.642
Hypercholesterolemia, n (%)	85 (66)	232 (60)	.227
Ischemic heart disease, n (%)	54 (42)	169 (44)	.719
Peripheral artery disease, n (%)	33 (26)	88 (23)	.509
Aortic aneurysm, n (%)	13 (10)	52 (14)	.319
Current smoking habit, n (%)	32 (25)	79 (21%)	.292
Alcohol consumption, n (%)	28 (22)	67 (17)	.264
Plaque morphology			
Degree of stenosis, mean ± SD, %	83.5 ± 9.0	83.6 ± 8.0	.909
70%–79%, n (%)	48 (37)	141 (37)	
80%–89%, n (%)	37 (29)	131 (34)	.489
90%–99%, n (%)	43 (34)	112 (29)	
Hypoechoic, n (%)	48 (38)	135 (35)	.538
Heterogeneous, n (%)	91 (72)	247 (64)	.097

Table 2: Comparison of cerebrovascular events between concentric stenosis and eccentric stenosis

Cerebrovascular Events	Concentric Stenosis (n = 128), n (%)	Eccentric Stenosis (n = 384), n (%)	P
Overall events	7 (5.5)	52 (13.5)	.013
Ischemic stroke	5 (3.9)	36 (9.4)	.048
Transient ischemic attack	0 (0)	9 (2.3)	.073
Transient monocular blindness	2 (1.6)	7 (1.8)	.601
Events of presumed carotid arterial origin	5 (3.9)	49 (12.8)	.005

cutoff value between the lowest quartile (concentric stenosis, n = 128) and the other quartiles (eccentric stenosis, n = 384) was 0.69. There were no significant differences in patient characteristics and plaque morphology between ICAs with concentric and eccentric stenoses (Table 1).

Ipsilateral to the concentric ICA stenosis, 5 ischemic stroke events and 2 TMBs occurred in the preceding year; of these 7 events, 1 was a lacunar stroke, 1 was an event with the high-risk sources of cardioembolism, and the remaining 5 were events of presumed carotid arterial origin (Table 2). Ipsilateral to the eccentric stenosis, 36 stroke events, 9 TIAs, and 7 TMB attacks occurred in the preceding year; of these 52 events, 1 was a lacunar stroke, 2 were events with the high-risk sources of cardioembolism, and the remaining 49 were events of presumed carotid arterial origin. Overall cerebrovascular events occurred more frequently ipsilaterally to the ICA with eccentric stenosis (13.5%) than with concentric stenosis (5.5%; P = .013); the difference was more evident when cerebrovascular events of presumed carotid arterial origin were assessed (eccentric, 12.8% versus concentric, 3.9%; P = .005).

After adjustment for age, sex, vascular risk factors, and plaque morphology, eccentric stenosis was independently related to overall cerebrovascular events (odds ratio = 2.76; 95% confidence interval [CI] = 1.19–6.40) and to cerebrovascular events of presumed carotid arterial origin (odds ratio = 3.69; 95% CI = 1.40–9.71; Table 3). In addition to eccentric stenosis, current smoking habit and severe stenosis at 90% or more were independently related to overall cerebrovascular events (current smoking habit, odds ratio = 2.29 and 95% CI = 1.23–4.29 and severe stenosis, odds ratio = 2.26 and 95% CI = 1.27–4.03) and to cerebrovascular events of presumed carotid arterial origin (current smoking habit, odds ratio = 2.25 and 95% CI = 1.17–4.32 and severe stenosis, odds ratio = 2.53 and 95% CI = 1.39–4.58). On the ROC curve for indicating recent cerebrovascular events, the optimal cutoff value of the eccentricity index was more than 0.71, with a sensitivity of 88% and specificity of 30%.

Table 3: Eccentric internal carotid artery stenosis as a predictor of recent cerebrovascular events

	Overall Cerebrovascular Events			Cerebrovascular Events of Presumed Carotid Arterial Origin		
Model	Odds Ratio	95% CI	P	Odds Ratio	95% CI	P
Model 1	2.60	1.15–5.90	0.022	3.46	1.34–8.90	0.010
Model 2	2.76	1.20–6.34	0.016	3.63	1.40–9.43	0.008
Model 3	2.76	1.19–6.40	0.018	3.69	1.40–9.71	0.008

Note:—Model 1 was adjusted for age and gender. Model 2 was adjusted for age, gender, and vascular risk factors (hypertension, diabetes mellitus, hypercholesterolemia, current smoking habit, and alcohol consumption). Model 3 was adjusted for age, gender, vascular risk factors, and plaque morphologies (degree of stenosis, hypoechoic plaque, and heterogeneous plaque). CI indicates confidence interval.

Discussion

This is the first study to investigate the association between the geometry of carotid artery stenosis and ipsilateral cerebrovascular events using ultrasonography. The major new finding was that, independent of underlying risk factors and other features of plaque morphology, eccentric ICA stenosis was associated with recent cerebrovascular events within the preceding year.

The carotid bifurcation often shows uneven development of atherosclerosis between the inner and outer walls because of different flow streamline patterns and shear stress.[23] The coronary artery may have a similar tendency. A previous study showed that 81% of the coronary arteries examined by intravascular sonography were eccentric, with an eccentricity index at 0.5 or more, and such eccentric plaques were strongly associated with the acute coronary syndrome.[16] Using the same 0.5 index cutoff value in the present study, eccentric carotid artery stenosis was present in 88% of all of the vessels. However, this percentage may be too high to use for appropriate comparisons of clinical features between eccentric and concentric stenosis. Instead, we used the first quartile value of the eccentricity index (0.69) to divide arteries into 2 groups. This value was close to the optimal cutoff value of the eccentricity index by the ROC curve analysis (0.71). Thus, eccentricity defined as an index at 0.69 or more appears to be appropriate.

The association of eccentric coronary artery stenosis with acute coronary syndrome is due to the presence of disruption of the eccentric plaque or superimposed thrombus.[14-19] To some extent, the same morphologic features may explain the association between eccentric carotid plaques and cerebrovascular events. In addition, hemodynamic changes induced by eccentric stenosis may be an important factor that leads to cerebrovascular events. Relatively low shear stress is reported to play a critical role in the development of atherosclerosis and vulnerable plaques.[6,23,24] A computational simulation study using carotid

bifurcation models demonstrated that there were differences between eccentric and concentric stenosis with respect to the size of the poststenotic recirculation zone, as well as the severity and distribution of wall shear stress.[5] Using the models, the deposition of platelet and monocyte-sized particles on the vessel wall was more distinct proximal to the eccentric stenosis than proximal to the concentric stenosis.[25] This suggests that eccentric stenosis is more prone to platelet activation and aggregation due to attenuated platelet deposition and plaque growth, as well as rupture due to attenuated monocyte deposition. Thus, eccentric stenosis may have a high potential for thrombus formation, which may lead to an increased risk of cerebrovascular events.

Similar plaque morphology can be easily seen by using multidetector row CT (MDCT) angiography and some types of MR techniques.[13,26-30] In a recent study on MDCT, the relationship between stroke symptoms and plaque morphology was assessed.[30] In the study, expansive carotid remodeling was greater in patients with cerebral ischemic symptoms than in asymptomatic patients, though there was no significant difference in the plaque eccentricity between symptomatic and asymptomatic patients. Thus, MDCT and MR techniques, as well as ultrasonography, seem to be available for evaluation of the geometry of carotid artery stenosis.

In the present study, hypoechoic plaque and heterogenous plaque were not indicative of recent cerebrovascular events, though they were often reported to be risk factors in the events.[7-12] This might be because of the small patient number for appropriate statistical analysis or because the echogenicity of the carotid plaque was not evaluated objectively and quantitatively by using the gray-scale median. Other limitations of the present study include the following reasons. First, carotid arteries with advanced calcification were excluded from the study, because the severe acoustic shadow did not allow the eccentricity index to be measured on sonography. In our study population, such arteries might account for 10% of the arteries with maximum area stenosis at 70% or more. Although the contribution of carotid calcification to cerebrovascular events is uncertain,[27,28,31,32] the present results may have been affected by the exclusion of these calcified arteries from the analysis. Second, although the present retrospective analysis demonstrated an association between stenosis geometry and pre-existent cerebrovascular events, a prospective trial is required to assess the contribution of eccentric stenosis to future cerebrovascular events.

In patients with a cross-sectional area carotid stenosis of 70% or more on sonography, eccentric plaque with an eccentricity index at 0.69 or more was associated with a significantly increased incidence of recent ipsilateral cerebrovascular events compared with a cohort of patients with concentric stenosis. The presence of eccentric stenosis, as well as the severity of the stenosis, may be an important indicator for use in the selection of patients for surgical revascularization of the carotid artery.

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BRAIN RESEARCH

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Repeated application of an electric field increases BDNF in the brain, enhances spatial learning, and induces infarct tolerance

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Neurotrophic factor

ABSTRACT

Development of a safe method to increase brain-derived neurotrophic factor (BDNF) in the brain is expected to have utility in enhancing learning and memory, in protecting the brain, and in suppressing appetite. We investigated the effects of whole-body exposure to high voltage electric potential (HELP), which generates an electric field and current density in the body, on BDNF levels in the brain, spatial learning, or resistance to cerebral infarction development after focal ischemia. Adult mice (C57BL/6J) were exposed to 3.5 kV, or 5.8 kV for 5 h a day, making indirect contact with the ground via room air, over 1, 3, 6 or 12 consecutive weeks. After treatment, BDNF levels, performances in the Morris water maze task (MWM), or development of infarct lesion after focal ischemia was analyzed. Treatment with 3.5 kV for 1, 3, 6 or 12 weeks, or with 5.8 kV for 1, 3 or 12 weeks increased BDNF levels in the cortex ($P<0.05$, one-way ANOVA). Every HELP treatment differentially improved escape latency in the MWM, compared with the corresponding untreated controls ($P<0.05$, one-way ANOVA). Treatment with 3.5 kV for 6 or 12 weeks, but not with 5.8 kV protected the brain suppressing cerebral infarction development ($P<0.05$). The HELP treatment with 3.5 kV for 6 or 12 weeks improves spatial learning, gently suppressing body weight gain, and protects the brain against cerebral infarction.

1. Introduction

Repetitive exposure of the body to an electric field by direct contact with high voltage electric potential (HELP), ≦30 kVrms (V) at the HELP-generator, 50 or 60 Hz, under the condition that the HELP-exposed body is insulated from the surroundings making indirect contact with the ground (=0 V) via room air, has been shown to improve headache, shoulder muscle stiffness, insomnia, and chronic constipation (Hara, 1961; Harakawa et al., 2002). For the treatment of these symptoms, the Ministry of Health,

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Labour and Welfare (HLW) in Japan approved the HELP-application for 1 h to several hours a day as a safe alternative therapy more than 40 years ago. However, while it is known that HELP therapy suppresses a restraint stress-induced increase in plasma lactate levels in rats (7.0 kV, 50 Hz) (Harakawa et al., 2004b), the underlying biological mechanisms by which HELP acts on the body as well as the optimum protocols for the improvement of these symptoms remain unknown.

Brain-derived neurotrophic factor (BDNF) (Barde et al., 1982), the most abundant neurotrophin in the brain, is known to promote neuronal differentiation/maturation in the developing central nervous system (CNS) (Binder and Scharfman, 2004). After the maturation of the CNS, BDNF participates in multiple forms of learning and memory formation by promoting dendritic outgrowth and synaptic development (Kuipers and Bramham, 2006; Rex et al., 2007). Upregulation of BDNF has been observed in the inferior temporal cortex during declarative memory formation in monkeys (Tokuyama et al., 2000). Genetic downregulation of BDNF expression has been found to impair long-term potentiation (LTP) (Korte et al., 1995), learning for a pattern discrimination task (Gorski et al., 2003), learning of a spatial task (Gorski et al., 2003), or complex and/or stressful learning (Minichiello et al., 1999). Such impairment in LTP caused by BDNF-deficiency was completely resolved by administration of exogenous BDNF (Patterson et al., 1996).

In addition to its role in learning and memory, BDNF promotes neuronal survival (Hofer and Barde, 1988), protecting neurons from lethal ischemic stresses (Beck et al., 1994; Kobayashi et al., 1995). Increased BDNF levels after intracerebral infusion of exogenous BDNF has protected the brain from focal ischemia (Yanamoto et al., 2000a,b). Furthermore, BDNF has been known to regulate appetite. Heterozygous BDNF-deficient mice have been found to develop hyperphagia and aggressiveness (Lyons et al., 1999). Direct intracerebral infusion of exogenous BDNF has been found to suppress appetite, decrease blood glucose levels by increasing insulin sensitivity, and cause body weight loss in a dose-dependent manner without any metabolic toxicity in normal rats (Pelleymounter et al., 1995). Therefore, development of a safe method to increase BDNF levels in the brain is expected to have utility in

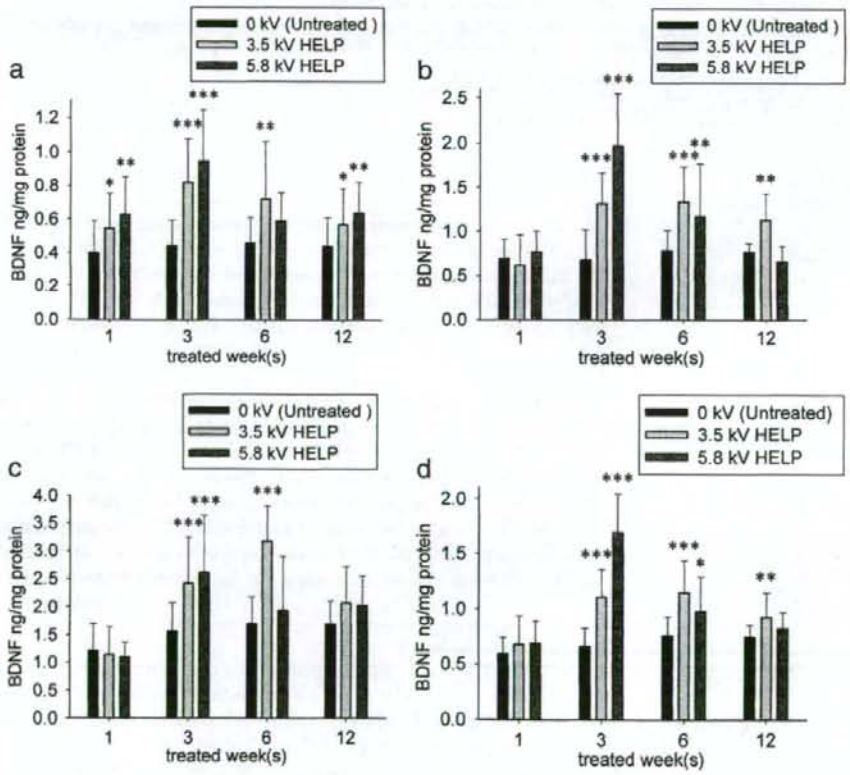

Fig. 1 – BDNF levels in the cortex (a), the thalamostriatum (b), the hippocampus (c), or the total forebrain (d), after HELP treatment, in comparisons with untreated controls. In the cortex (a), BDNF levels increased significantly after HELP treatment, excepting treatment with 5.8 kV for 6 weeks. In the thalamostriatum (b) or the total forebrain (d), BDNF levels increased significantly after either HELP treatment for 3 or 6 weeks, or after treatment with 3.5 kV for 12 weeks. In the hippocampus (c), BDNF levels increased after either HELP treatment for 3 weeks, or treatment with 3.5 kV for 6 weeks. HELP: high voltage electric potential. Asterisk, $P<0.05$; double asterisk, $P<0.01$; triple asterisk, $P<0.001$; compared with the corresponding untreated controls. $n=20$; error bars indicate S.D.

enhancing learning and memory, in protecting the brain from ischemic stroke, and in suppressing excessive appetite and body weight.

Specific types of electric stimulation that have been shown to be associated with elevated levels of BDNF in the brain include the following: a brief period of direct current electrification (3V-DC, 100 μs, 20 Hz for 1 h) (Al-Majed et al., 2000), an epileptiform discharge (Takahashi et al., 1999), and chronic electroconvulsive therapy (ECT) (Nibuya et al., 1995). In this study, we investigated the effect of HELP therapy; 3.5 kV or 5.8 kV at the HELP-applying surface, for 5 h a day over consecutive 1, 3, 6, or 12 weeks, on BDNF levels in the brain, on spatial learning and memory formation, on infarct lesion development after the induction of transient focal ischemia, and on body weight gain in normal adult mice.

2. Results

2.1. *BDNF protein levels after HELP treatment*

After HELP treatment, the BDNF levels in the cortex (Fig. 1a), the thalamostriatum (Fig. 1b), the hippocampus (Fig. 1c), or the total forebrain (Fig. 1d) significantly increased, compared with the corresponding untreated (0 V-treated) controls. After treatment with 5.8 kV for 3 weeks, the increase in BDNF levels in the thalamostriatum or the total forebrain were significantly larger than that after 3.5 kV for 3 weeks ($P<0.001$) (Figs. 1b, d).

2.2. *Alteration in body weight after HELP*

Body weight gain was significantly reduced after treatment with 3.5 kV for 6 ($P=0.018$) or 12 weeks ($P=0.024$), or 5.8 kV for 6 ($P<0.001$) or 12 weeks ($P<0.001$), compared with the corresponding untreated controls (Fig. 2). There were no deaths or weaknesses in HELP-treated or untreated groups during the observation period.

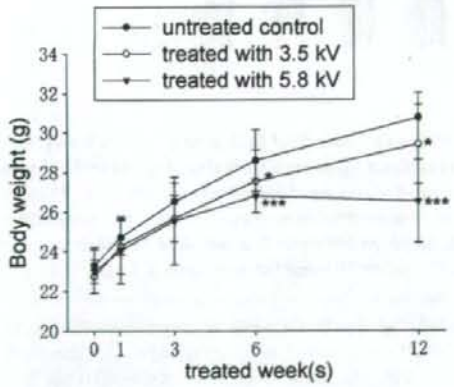

Fig. 2 – Alterations in body weight after treatment with HELP. The treatment with either HELP for 12 weeks significantly reduced body weight compared with the corresponding untreated controls. Asterisk, $P<0.05$; triple asterisk, $P<0.001$, compared with the corresponding untreated controls. $n=20$; error bars indicate S.D.

2.3. *Water maze task after HELP treatment*

Every mouse underwent 5 MWM sessions and the subsequent probe trial successfully, with the exception of one mouse treated with 5.8 kV for 12 weeks, in which the MWM was interrupted because of a near-drowning appearance in the 1st trial. The escape latencies after 3.5 or 5.8 kV-treatment for 1, 3, 6, or 12 weeks showed differential improvement, compared with the corresponding untreated controls (Figs. 3a–d) (escape latencies are expressed as the mean ± S.E.M.). After treatment with 5.8 kV for 3 weeks, mean escape latencies of 2–5 sessions were decreased to a minimum of 35% compared with the corresponding control (Fig. 3e). After 5.8 kV for 3 weeks, the total path lengths were also decreased; down to a minimum of 31% in the 4th session, compared with the corresponding controls (Table 1). V_{max} did not differ among the groups with HELP for various periods and the corresponding untreated controls (Table 1).

In the probe trial, the HELP-treated groups, at either 3.5 kV for 1, 3, 6, or 12 weeks, or 5.8 kV for 3, or 12 weeks, were found to cross over the location where the platform had been placed previously more frequently than the corresponding untreated controls (Table 1).

In the untreated control groups, significant improvements in escape latency (Fig. 3f), total path length ($P<0.001$ in the 2nd–4th sessions), the probe trial ($P<0.001$), and maximum velocity ($P<0.001$) (Table 1), were observed in 20–21-week-old mice, compared with 8–9-week-old mice.

2.4. *Sizes of cortical infarct lesion*

Infarct lesion sizes corrected by the edema ratio were significantly decreased following treatment with 3.5 kV for 6 (54.2%, $P=0.021$) or 12 weeks (47.9%, $P=0.004$), compared with the corresponding controls (=100%) (Fig. 4). The reduction (61.6%) after treatment with 5.8 kV for 3 weeks did not achieve a significant difference ($P=0.073$), compared with the control.

2.5. *Regional CBF (rCBF) measurement*

After the HELP (3.5 kV)-or 0 V-treatment for 6 weeks, the rCBF in the penumbra area before, during, and after ischemia was monitored by LDF (Fig. 5). Results of blood pressure (BP), and heart rate (HR) monitored before, during, and after ischemia are shown in Table 2. There were no significant differences in the rCBF values during or post-ischemia, or in the physiological parameters between the groups.

3. Discussion

HELP treatment improved the scores in escape latency or the probe trial, and shortened the total path length to the hidden platform in the MWM. Because HELP treatment was not associated with an increase in the V_{max}, the significant decrease in these parameters cannot be due to improvements in sensorimotor function or locomotor activity, or in direct visual search,

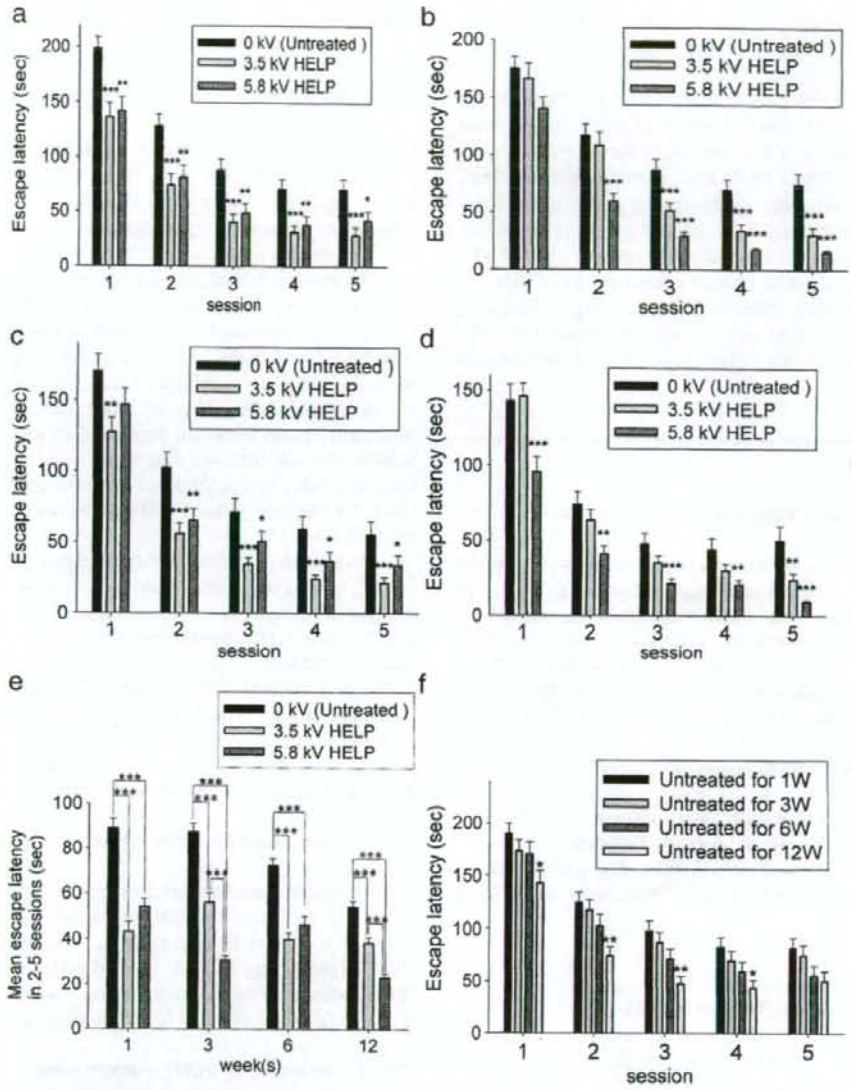

Fig. 3 – Escape latencies in each session of the MWM after treatment with an electric field (0, 3.5, or 5.8 kV, 5 h/day) for 1 (a), 3 (b), 6 (c), or 12 weeks (d). Escape latency in each treatment, mean of 2–5 sessions (e). Escape latencies of the MWM in untreated groups (f). HELP treatment significantly improved escape latencies compared to the corresponding controls (a–e). There were significant differences between the 3.5 kV- and 5.8 kV-treatments for 3, or 12 weeks (e). In untreated groups (f), escape latencies gradually improved in the observation period. There were significant differences between 1-week- and 12-week-untreated groups. Asterisk, $P<0.05$; double asterisk, $P<0.01$; triple asterisk, $P<0.001$. $n=24–25$; error bars indicate S.E.M.

but rather to enhanced spatial learning. Even if V_{max} values were not affected by HELP treatment, a significant enhancement of physical activities, if any, may improve escape latencies by moving faster to the platform. However, enhanced physical activities without any improvement in spatial learning cannot shorten the total path length (Table 1).

A statistical difference was detected in a single session (3.5 kV for 12 weeks or 5.8 kV for 6 weeks), 3 sessions (3.5 kV for 3 weeks), 4 sessions (3.5 kV for 6 weeks or 5.8 kV for 3 weeks), or in all 5 sessions (3.5 or 5.8 kV for 1 week or 3.5 kV for 12 weeks) (Figs. 3a–d) in the MWM. Given these results, as well as the results expressed as mean escape latencies (from 2–5 sessions) (Fig. 3e), HELP treatment causes various levels of improvements in spatial learning depending on the voltage level and the treatment period.

Mice engineered to overexpress the BDNF gene show improved performance in a spatial learning task compared with wildtype littermates (Yanamoto et al., 2005). In the present study, significant improvement in spatial learning was observed after either HELP treatment for 1 week, at the time

Table 1 – The maximum swimming velocity (V_{max}), the total swimming path length, and the number of mice entering the location of "removed platform" (probe test), in the MWM

Water maze test		1 W	3 W	6 W	12 W
The velocity (V_{max}, cm/s)	Untreated	40.4±1.9	37.3±3.1	36.7±2.0	36.8±2.4
	3.5 kV	40.0±3.1	37.3±3.4	36.3±2.1	36.5±3.0
	5.8 kV	40.1±3.1	37.7±2.5	36.5±2.5	36.3±1.8
The path length in the 1st session (cm)	Untreated	1684±791	1550±473	1466±574	1448±628
	3.5 kV	***1340±638	1537±773	***1207±571	1463±637
	5.8 kV	*1438±689	1298±379	**1218±455	**1209±592
The path length in the 2nd session (cm)	Untreated	1028±602	963±520	877±617	662±401
	3.5 kV	***659±637	969±562	***522±447	628±520
	5.8 kV	***712±175	***619±558	**665±299	**480±249
The path length in the 3rd session (cm)	Untreated	892±569	680±434	637±537	438±319
	3.5 kV	***449±338	***512±277	***398±259	378±267
	5.8 kV	***524±473	***239±151	579±390	***292±216
The path length in the 4th session (cm)	Untreated	652±451	516±391	411±360	355±398
	3.5 kV	***327±261	***349±390	***242±158	295±204
	5.8 kV	***323±260	***159±146	***264±196	**243±194
The path length in the 5th session (cm)	Untreated	584±369	526±400	327±321	310±444
	3.5 kV	***241±275	***367±228	**224±175	**187±162
	5.8 kV	***306±183	***189±113	**232±212	***169±115
The probe test after the 5th session	Untreated	2.5±2.9	2.8±3.1	3.4±2.5	5.7±2.8
	3.5 kV	***6.0±3.6	***5.9±3.1	*5.2±2.4	**8.1±2.9
	5.8 kV	3.4±2.2	***6.1±1.9	4.0±2.7	*7.8±3.4

Asterisk, $P<0.05$; double asterisk, $P<0.01$; triple asterisk, $P<0.001$, compared with the corresponding untreated controls. $n=24–25$; data are expressed as the mean±SD.

point when the BDNF levels in the cortex increased; at this time, BDNF levels were not elevated in the other areas. In the cortical BDNF levels after HELP for various periods (Fig. 1a), fluctuations similar to the various levels of improvements in spatial learning (Fig. 3e) were observed. The hippocampus has been thought to participate in declarative memory, but is not thought to play a role in spatial memory (Squire, 1992). The role of the hippocampal formation in memory storage has been considered to be temporary (Squire, 1992), with spatial working memory subserved by a network of other areas, especially the frontal cortex of the brain (Courtney et al., 1998).

Restoration of decreased BDNF levels in the brain is a proposed aim of antidepressant treatment (Khundakar and Zetterstrom, 2006). Genetic downregulation of BDNF expression shows vulnerability to the deleterious effects of stress (Advani et al., 2007). BDNF-knock-in mice, in which activity- and calcium-dependent expression of BDNF is abolished in the medial frontal cortex, shows difficulty in reversal learning or fear memory extinction, suggesting that BDNF expression are required for behavioral flexibility and fear memory extinction (Sakata and Lu, 2007). Interestingly, the four symptoms targeted by HELP therapy are related to masked depression. Increased BDNF levels after HELP therapy may improve fear and depressive mood disorders, resulting in improvements of the four symptoms.

Activation of voltage-dependent calcium channels increases intracellular calcium levels, increases BDNF levels, and enhances activity-dependent neuronal survival in embryonic cortical neurons (Ghosh et al., 1994). Repetitive spreading depressions (SD), which reversibly depolarize cellular membrane potential, increase intracellular calcium levels, and increase BDNF levels in the brain (Yanamoto et al., 2000a,b). Thus, transient increases in intracellular calcium levels can be triggers leading to increased BDNF levels in the brain. An electric field (1 or 10 Hz, 10 V/cm) has been found to increase intracellular calcium ion concentrations via Ca^{2+} influx across the plasma membrane in cultured hepatoma cells (Cho et al., 1999). Stimulations with an electric field (60 Hz, 6 $\mu A/cm^2$ for 12 min) upregulated lectin-induced cytosolic Ca^{2+}-elevation, via enhancing Ca^{2+}-influx, in cultured splenocytes (Harakawa et al., 2004a). Thus, HELP treatment has the potential to facilitate intracellular Ca^{2+}-elevation that upregulates BDNF expression. The estimated current density in the mouse body induced by the HELP-application ranges from 0.0019 $\mu A/cm^2$ to 2.2 $\mu A/cm^2$ (calculated from data in rats) (Kaune and Phillips, 1980).

In our previous study, repetitive SD-stimulations that increase BDNF levels in the brain (Yanamoto et al., 2000a,b) induced potent tolerance to infarcting after lethal ischemia (infarct tolerance) (Yanamoto et al., 1998). Infarct tolerance has been observed in genetically normal mice but not in BDNF-deficient mice, indicating that increase in the BDNF levels is required for the induction of infarct tolerance (Yanamoto et al., 2004). The development of infarct tolerance has been observed 2 weeks after repetitive SD in rats, or after 1 week in mice (Yanamoto et al., 1998, 2004). Intracerebral infusion of recombinant BDNF (8 μg total) continuing for 1 or 2 weeks (gradual and prolonged increase in the BDNF levels) was associated with the development of infarct tolerance, but there was no such protective effect seen with infusion for only 3 days (rapid and transient increase) in rats (Yanamoto et al., 2000a,b). Thus, the development of infarct tolerance appears to require an appropriate elevation in the intracerebral BDNF levels and a maturation period. In line with these previous findings, in the present study, significant reduction in infarct lesion size did not occur until after a specific interval period following the "gradual and prolonged" elevation in the cortical BDNF levels. Because the

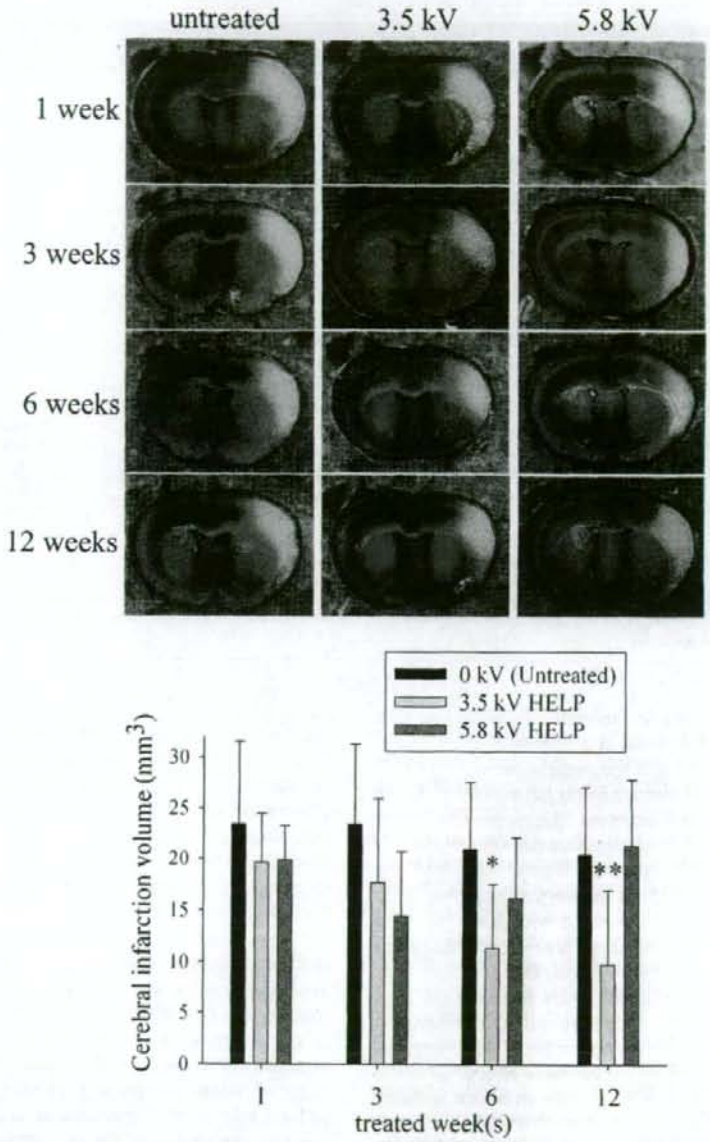

Fig. 4 – Cerebral infarct lesion 24 h after focal cerebral ischemia. Representative images of cerebral infarction in the group treated with 0 (untreated), 3.5 or 5.8 kV, for 1, 3, 6, or 12 weeks (photos). Treatment with 3.5 kV for 6, or 12 weeks significantly reduced the size of cerebral infarction, compared with the corresponding controls (graph). Asterisk, $P<0.05$; compared with the corresponding untreated controls. $n=7$; error bars indicate S.D.

suppression of body weight gain was severe (the mean value declined than the previous level) and one mouse showed a strange behavior in the MWM, after the treatment with 5.8 kV for 12 weeks, the voltage level in the HELP treatment should be selected carefully aiming to induce an appropriate increase in intracerebral BDNF levels over a prolonged period.

In addition to the improvements seen in HELP-treated mice, there were significant improvements in learning/memory and sensorimotor function in the untreated groups (Fig. 3f, Table 1). While the maximum velocity representing sensory–motor function was improved to some extent (10%) during the period, there were greater improvements in escape latency (51%), total path length (51%), and in the probe trial (128%), suggesting that spatial learning and memory retention improve in young adulthood under normal conditions.

Physical exercise (Neeper et al., 1996), and chronic dietary restriction (Duan et al., 2001) have been shown to elevate BDNF mRNA, BDNF protein, or trkB-signaling in the brain. The

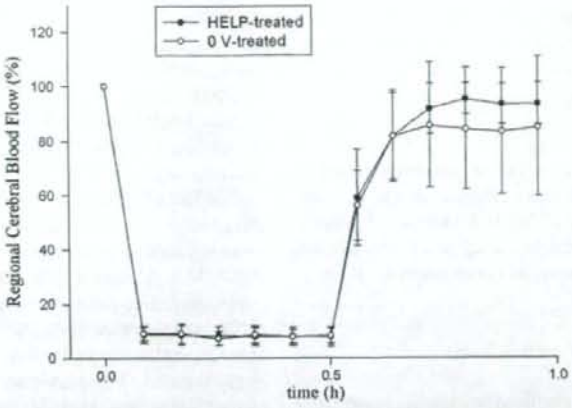

Fig. 5 – Regional cerebral blood flow (rCBF) in the ischemic penumbra during and after the temporary focal ischemia compared to the pre-ischemic control value (=100%). During ischemia, rCBF was reduced to 8.3±2.3% on average in the 0 V-treated group, or 8.1±3.4% in the 3.5 kV-treated group. Significant reperfusion was observed in both groups after ischemia, however, the rCBF values were relatively variable and lower in the 0 V-treated group. Which might indicate that cerebral damage is relatively less after the ischemic stress in the HELP-treated group. n=7; error bars indicate S.D.

present study demonstrates that appropriate usages of HELP therapy have utility in increasing BDNF levels in the brain, in enhancing learning and memory, and in protecting the brain. Additional studies focusing on impaired cognitive/learning function, depressive mood disorders, blood glucose levels (Krabbe et al., 2007), or obesity (Lebrun et al., 2006; Pelleymounter et al., 1995) are required to better understand the therapeutic potential of HELP in dementia, ischemic stroke, depression, other neurological disorders associated with reduced BDNF levels in the brain, diabetes type II, or metabolic syndrome.

4. Experimental procedures

The experimental protocols were approved by the NCVC Animal Research Committee. All efforts were made to minimize suffering and the number of animals used.

4.1. *Experimental animals and treatment with HELP*

Male adult C57BL/6J mice (8–9 weeks old, 22–24 g, a total of 638, Clear Japan Inc., Osaka, Japan) were used. Mice were placed in a temperature-controlled room under a regular light/dark cycle and had free access to food and water *ad libitum* throughout the experiment.

Each mouse cage, 17*23*12 cm-size, composed with 2 mm-thick acryl boards, housed 5–6 mice. A mattress type HELP-supplier, 30*50 cm in size, connected to the HELP-generator (an AC-transformer; 60 Hz), designed to generate 5.5 kV or 9.0 kV (=4.7 kV or 7.8 kV at the working electrode, respectively), made for humans originally, was placed under the cage. The counter electrode, 50*150 cm in size, grounded (=0 V), was located 20 cm below the HELP-supplier. The HELP-area, consisting of the HELP-supplier, the cage and the mice, was electrically insulated from its surroundings by means of an insulating table, so that the HELP-exposed mice made indirect contact with 0 V (ground) via room air. The HELP-generator elevated the electric potential at the HELP-applying surface: the floor of the cage, to 0 V (untreated control), 3.5 kV, or 5.8 kV (measured by Statiron-DZ3, SSD Electrostatic Ltd., Tokyo, Japan), for 5 h a day over consecutive 1, 3, 6, or 12 weeks. In this setting, the electric field that is generated above the HELP-supplier (vertical direction to the floor), in the absence of mice, was 0 V/m, 14–15 kV/m, or 24–25 kV/m, respectively (FOVM-03

Table 2 – Physiological parameters pre, during, and after transient focal ischemia, which was induced after the HELP (3.5 kV)- or 0 V-treatment over 6 weeks

	0 V-treated group			HELP-treated group		
	Pre-ischemia	Intra-ischemia	Post-ischemia	Pre-ischemia	Intra-ischemia	Post-ischemia
Systolic BP (mm Hg)	86±32	104±23	95±23	89±21	80±18	80±27
Mean BP (mm Hg)	51±21	68±19	66±20	53±11	54±19	49±19
Diastolic BP (mm Hg)	47±29	55±23	51±19	43±22	42±21	35±18
Heart rate (beat/min)	393±49	396±43	385±54	396±65	436±50	438±51
Temperature (°C)	36.8±0.1	37.1±0.2	37.1±0.2	36.8±0.2	37.0±0.2	37.1±0.1

There was no significant difference in these parameters between the groups. Although not significant, BP during and after ischemia in the HELP-treated group were relatively lower and HR were relatively higher, compared to the 0 V-treated control group.
Data are expressed as the mean±SD. n=7 in each experimental group.

optical fiber voltmeter, Sumitomo Electric Co. Ltd., Tokyo). The relative positions of the HELP-applier, the body, and the counter electrode exactly mimicked those of human HELP therapy.

4.2. Experimental groups

The experimental animals were randomly assigned to one of the treatment groups described above. The number of animals for each analysis was designed to detect deviations of 30% for the analysis on BDNF or spatial learning, or of 40% for the analysis on ischemic injury, from the mean normal values in our previous studies.

4.3. Measurement of BDNF protein levels

In the first group, animals were perfused intracardially with ice-cold PBS (pH 7.5) with an overdose of sodium pentobarbital within 24 h after the end of 0, 3.5, or 5.8 kV-treatment over 1, 3, 6, or 12 weeks. The cortex, thalamostriatum, or the hippocampus was excised, and stored at −80 °C until use. BDNF levels in the brain were measured quantitatively using two-site enzyme-linked immunosorbent assay (ELISA) (BDNF Emax™ immunoassay system, Promega, Madison, WI) (Radka et al., 1996). The protein concentration in each sample was measured using a BCA protein assay kit (Pierce, Rockfold, IL) to standardize the BDNF levels. Body weight was measured before and at the end of each HELP treatment.

4.4. Analysis of spatial learning

In the second group, spatial learning, comprising cognition/orientation, working memory/consolidation, and recall/navigation, were examined after 0, 3.5, or 5.8 kV-treatment over 1, 3, 6, or 12 weeks, using the Morris water maze task (MWM) with our original modifications. In a 64*91 cm-sized pool, a 10*10 cm-square-shaped platform, made opaque with a non-toxic white agent, was hidden at a fixed position under the water. The temperature of the water was kept at 24–25 °C during the procedure. After HELP treatment, each animal performed 4 trials per day (=1 session), over 5 consecutive days without any prior or subsequent training. The 1st session was started within 24 h after the last HELP (or 0 V for control)-application. The cut-off time in a trial was set at 300 s; animals that failed to reach the platform in 300 s were removed from the water. In each trial, the time needed to escape to the platform (escape latency), the total path length needed to navigate to the platform, and the fastest swimming speed (maximum velocity, V_{max}), were analyzed, using an automated video tracking system (Smart, Panlab s.l., Spain). To analyze memory retention, a probe trial was performed for 60 s in the absence of the platform, starting at 30 min after the final session of the MWM. The incidence of successful navigation, that is, when the mouse crossed over the "exact previous location of the platform" was analyzed (Smart).

4.5. Induction of focal ischemia

In the third group, temporary focal ischemia was induced using the 3 vessel-occlusion technique as described elsewhere (Yanamoto et al., 2003), after 0, 3.5, or 5.8 kV-treatment over 1, 3, 6, or 12 weeks. It has been established that this technique can produce a consistent reduction in regional cerebral blood flow, generating constant infarct lesions in mice (Yanamoto et al., 2004). Briefly, the left middle artery (MCA) was cauterized at the lateral border of the olfactory tract under a surgical microscope (OME-5000, Olympus, Tokyo), and bilateral common carotid arteries were clip-occluded for 30 min. The induction of ischemia was started within 24 h after the last HELP (or 0 V for control) treatment. Rectal (core) temperature was regulated so that it stayed within the physiological range (36.5–37.5 °C) during the operation (NS-TC10 temperature controller, Neuroscience, Tokyo). The surgical wound for the MCA was closed within 3 min after the opening the skull and subsequent cauterization of the MCA, to prevent regional hypothermia. The regulation of core temperature was continued into the reperfusion period for 30 min to avoid hypothermic neuroprotection against reperfusion injury (Yanamoto et al., 1996). Before and during ischemia, blood pressure (BP) and heart rate (HR) were monitored via the tail artery (BP-98AW BP monitor, Softron Co. Ltd., Tokyo).

4.6. Regional CBF (rCBF) measurement

In a separate set of animals after HELP (3.5 kV)- or 0 V-treatment over 6 weeks, which was found to reduce cerebral infarction sizes in this study, rCBF was monitored before, during and after ischemia using a laser-Doppler blood flowmetry (LDF) system (LDF meter, TBF-LN1, Unique Medical, Tokyo, Japan). The region of measurement was set at 2 mm caudal and 1 mm dorsal to the crossover point of the left MCA and the rhinal fissure, in the ischemic penumbra of the ischemic lesion. During placement of the probe, all visible small vessels were avoided with the aid of a surgical microscope. The rCBF measurements were taken just before (as in the 100% control), during, and after the release of bilateral common carotid arteries. BP, HR, and rectal temperature were monitored before, during, and after ischemia.

4.7. Analysis of cortical infarct lesion volume

Animals were perfused intracardially with PBS at 70–90 mm^3 Hg with an overdose of sodium pentobarbital 24 h after ischemia. It has been established that infarcted lesion become liquefied/destroyed, and subsequently separate from intact brain tissue 48 h after temporary focal ischemia (Yanamoto et al., 2003). The brain was removed, cut from the frontal tip into 1 mm-thick slices and immersed in a 2% solution of 2,3,5-triphenyltetrazolium chloride (Bederson et al., 1986). The slices were then fixed by immersion in 4% paraformaldehyde/PBS. The infarct area or the total hemispheric areas of each section were measured by tracing the borders in a computer-assisted image-analysis system (Win Roof, Mitani Co., Osaka). An edema index was calculated by dividing the total volume of the hemisphere ipsilateral to the MCA occlusion by the total volume of the contralateral hemisphere. The infarct lesion size was calculated as the infarct size divided by the edema index (Yanamoto et al., 1996, 2003).

4.8. Statistical analysis

Escape latencies, total path lengths and V_{max} in each session, regional BDNF levels in the brain, and infarct volumes were all

analyzed using one-way ANOVA with the post-hoc Holm–Sidak method at each time point. The results are presented as the mean±S.D., with the exception of the values for escape latencies; these range from 4 to 300 (s), and are reported as mean±s.e.m. A value of $P<0.05$ was considered significant.

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Neurological and MRI Findings as Predictors of Progressive-Type Lacunar Infarction

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Key Words
Progressing stroke · Lacunar infarction · Magnetic resonance imaging

Abstract
Aims: To find neurological or neuroimaging signs to predict neurological deterioration in acute lacunar infarctions. ***Methods:*** Sixty-one consecutive patients with a supratentorial lacunar infarct, who were admitted within 48 h, were studied retrospectively. Progressive-type stroke (PS) was defined as progressive motor deficits that arose within 7 days after onset, by using the motor ratings of the National Institutes of Health Stroke Scale. ***Results:*** Sixteen patients (26%) were classified into the PS group. In the PS group, fluctuating or progressing onset (81 vs. 42%, p = 0.009), leg-predominant motor deficits on admission (63 vs. 16%, p = 0.001) and corona radiata lesion on diffusion-weighted MRI (100 vs. 69%, p = 0.013) were all more frequent than in the non-PS group. ***Conclusion:*** Bedside neurological assessment and MRI findings may allow us to predict PS and start early intensive treatment for preventing further neurological deterioration.

Introduction

Although the majority of patients with lacunar infarct have good clinical outcome, approximately one fourth of such patients show a progressive time course during the first several days and thus have a poor functional outcome [1–3]. It seems that, once neurological symptoms start deteriorating, any treatment can do little to stop the progression. Because a predictive diagnosis, prior to the neurological deterioration, may allow us to start early intensive treatment – which may be the only way to prevent further deterioration – identification of various predictors has been attempted [4]. However, such markers are often unfeasible in practice. We therefore attempted to clarify neurological and neuroimaging findings which may have predictive value for the diagnosis of neurological deterioration in acute lacunar infarction.

Subjects and Methods

We retrospectively reviewed the clinical records of 66 consecutive patients with acute lacunar infarct, who were admitted within 48 h after stroke onset, between January 1999 and January 2004. Patients with a supratentorial perforating artery territory infarct, which was identified on diffusion-weighted MRI (DWI) performed within 72 h after onset (mean, 26.3 h; range 3.7–

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Table 1. Demographic and clinical characteristics on admission

	Non-PS (n = 45)	PS (n = 16)	p value
Age, years	67 ± 10	71 ± 10	0.151
Male	32 (71)	9 (56)	0.355
Arterial hypertension (sBP >160 mm Hg or dBP >90 mm Hg)	37 (82)	10 (63)	0.164
Hyperthermia (body temperature >37.0° C)	5 (11)	1 (6)	>0.999
Hyperglycemia (serum glucose >110 mg/dl)	29 (64)	7 (44)	0.236
Fluctuating or progressing onset	19 (42)	13 (81)	0.009
Mean duration from onset to admission, h	12.8	13.6	0.718
NIHSS			0.291
Range	1–12	2–15	
Median value	4	5	

sBP = Systolic blood pressure; dBP = diastolic blood pressure. Figures in parentheses are percentages.

Table 2. Predominance of motor deficits

Motor deficits	Non-PS (n = 45)	PS (n = 16)			
		on admission	p	after progression	p
Arm dominant	5 (11)	0 (0)	0.313	1 (6)	>0.999
Arm = leg	33 (73)	6 (38)	0.016	12 (75)	>0.999
Leg dominant	7 (16)	10 (63)	0.001	3 (19)	0.713

Figures in parentheses are percentages.

69.2 h), were included in this study. Patients with an infratentorial or a cortical infarct were not included in this series. CT and blood analysis were performed immediately after admission in all cases. Stroke neurologists obtained the onset information from the patients or others who had witnessed the patients' stroke episodes, and then evaluated the neurological findings on admission. The types of onset were classified as follows: sudden onset, or fluctuating or progressing onset. Each case was treated with the appropriate medication, including antithrombotic therapy.

A quantitative assessment of motor deficits was conducted by using the modified motor ratings of the National Institutes of Health Stroke Scale (NIHSS) for both the affected arm and leg. The deficits were categorized into three degrees: mild (grade 0 or 1 NIHSS motor rating), moderate (grade 2), or severe (grade 3 or 4). The predominance of motor deficits in arms and legs was assessed by using these three degrees. The progressive-type stroke (PS) was defined as a case that showed deterioration in motor deficits within 7 days after stroke onset rated from mild to either moderate or severe, or from moderate to severe. The patients were then divided into two groups: the PS group and the non-PS group. In order to avoid recording transient neurological fluctuations, any deterioration resolving within 24 h was not regarded as PS.

MRI was performed with a 1.5-tesla Siemens Vision MR system, equipped with echo planar imaging data acquisition capability designed to obtain rapid diffusion images. Diffusion imaging was performed with a slice thickness of 4 mm, with a 2-mm interslice gap, and 2 levels of diffusion sensitization (b = 0, 1,000 s/mm^2). The diffusion gradients were applied sequentially in three orthogonal directions. To minimize the effects of diffusion anisotropy, an average of the three diffusion directions was calculated to give a trace of the diffusion tensor. Based on DWI findings, the location of the lesion was classified into three regions: the basal ganglia including the internal capsule, thalamus, or corona radiata (the paraventricular region including the body of the caudate nucleus).

Stenotic lesions in the internal carotid artery and the middle cerebral artery, which were less than 50% in all cases, were diagnosed by carotid sonography and MR angiography.

Baseline clinical variables (blood pressure, body temperature, serum glucose levels and NIHSS), the types of onset (sudden onset, or either fluctuating or progressing onset), the duration from onset to admission, the initial CT and DWI findings, and the functional outcome upon discharge (modified Rankin Scale and Barthel Index) were all compared between the two groups.

Proportions between the PS group and the non-PS group were compared by Fisher's exact test. The Mann-Whitney U test was used to compare ordinal variables. A value of $p < 0.05$ was considered significant.

Results

Of the 66 patients studied, 5 who already had a dense hemiplegia on admission were excluded from the following analysis. Of the remaining 61 patients, 16 (26%) showed progression of motor deficits (PS group). The demographic and clinical characteristics of these two groups are compared in table 1. No significant differences between these two groups were observed in regard to age, gender, risk factors, frequency of hyperthermia, duration from the onset to admission, or their initial NIHSS scores. However, a fluctuating or progressing onset was more frequent in the PS group than in the non-PS group (81 vs. 42%, p = 0.009). That parameter predicted PS with 0.81 sensitivity, 0.58 specificity and 0.41 positive predictive values.

The prevalence of internal carotid and middle cerebral artery stenosis on the lesion side was not significantly different between these two groups: 2 (13%) and 3 patients (19%) in the PS group, and 6 (13%) and 7 (16%) in the non-PS group (p = 1.000, p = 0.713, respectively).

Table 2 shows the predominance of motor deficits. On admission, 17 patients (28%) displayed leg-predominant

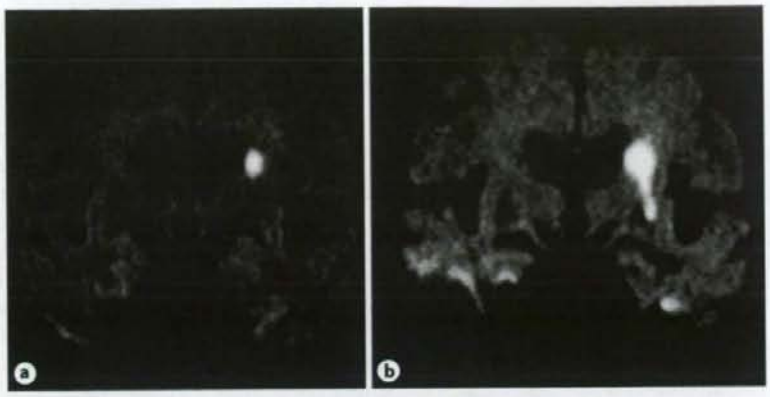

Fig. 1. Coronal sections of DWI in a typical PS patient. **a** On admission, the ischemic lesion was located in the paraventricular corona radiata. **b** After progression, the lesion extended downward, involving the basal ganglia besides the corona radiata.

motor deficits, which were more common in the PS group than the non-PS group (63 vs. 16%, p = 0.001), whereas patients with equally affected arms and legs were more common in the non-PS group than the PS group (73 vs. 38%, p = 0.016). All 5 patients with arm-predominant deficits belonged to the non-PS group. Leg-predominant deficits predicted PS with 0.63 sensitivity, 0.84 specificity and 0.59 positive predictive values. After progression, 13 patients (81%) had arm-predominant deficits or were equally affected in arms and legs. All but 1 patient had a progression of motor deficits in their arms.

Initial CT findings, as well as the location of lesions detected by DWI, are shown in table 3. Frequencies of positive CT findings between the two groups were not different statistically. Regarding DWI findings, the corona radiata was affected in all PS group patients, while in only 69% of non-PS group patients. The frequency of corona radiata involvement was significantly higher in the PS group than the non-PS group (p = 0.013). Corona radiata involvement predicted PS with 1.00 sensitivity, 0.31 specificity and 0.34 positive predictive values. DWI was performed repetitively before and after progression in 5 of 16 patients in the PS group. In all of them, ischemic lesions involved the paraventricular corona radiata before progression, and those lesions extended downward after progression (fig. 1). The final size of the infarctions was large, exceeding 15 mm in diameter, in all of the patients.

Eight patients had a combination of fluctuating or progressing onset, leg-predominant motor deficits on admission and corona radiata lesion on DWI. All but 1 showed subsequent neurological deterioration.

Table 3. Initial CT and DWI findings

	Non-PS (n = 45)	PS (n = 16)	p value
Positive CT finding	14 (31)	5 (31)	>0.999
Location of lesions on DWI			
BG	23 (51)	9 (56)	0.777
TH	9 (20)	0 (0)	0.096
CR	31 (69)	16 (100)	0.013

BG = Basal ganglia including internal capsule; CR = corona radiata; TH = thalamus. Figures in parentheses are percentages.

Patients in the PS group had poorer outcomes than those in the non-PS group. Both the modified Rankin Scale (range, 1–4 vs. 0–3; median, 4 vs. 1) and the Barthel Index (range, 20–100 vs. 45–100; median, 60 vs. 100) at discharge were significantly different between the two groups ($p < 0.001$, $p < 0.001$, respectively).

Discussion

PS was found in 26% of cases, and clinical outcome was worse in the PS group than in the non-PS group. These findings agree with those reported in previous studies [1–3]. Various clinical, biochemical and radiological markers were identified as predictors of PS [4]. However, biochemical markers often require special measurement techniques requiring excessive time. It is more desirable to predict PS on the basis of clinical history, neurological findings or neuro-imaging findings. The

present study indicates that a fluctuating or progressing onset, leg-predominant motor deficits and involvement of the corona radiata on acute DWI were all important for predicting neurological deterioration in a single lacunar infarction.

Regarding the mode of stroke onset, few studies have been performed on this subject since the early study by Britton and Rödén [5], which reported no difference between patients with and without PS. In their study, all types of stroke patients were included, and 20% of them were classified into the unknown type of onset, whereas, in the present study, only patients with a lacunar infarction were included and none of them were classified into the unknown type – and then a fluctuating or progressing onset was found to be a predictor of PS. Fluctuating or progressing onset is, however, rather common for lacunar stroke [6] and the specificity was not sufficient to predict PS.

Some investigators reported higher frequencies of neurological deterioration in subcortical infarction [1–3]. In the study by Nakamura et al. [1], 27% of 92 patients with supratentorial lacunar infarction had progressive motor deficits. In the study by Steinke and Ley [3], subcortical infarction was found more frequently in patients with progressive motor deficits (59.1%) than in those without progression (32.9%). In these studies, neurological worsening was restricted to progressive motor deficits, for the reason that persistent severe motor dysfunction is a predominant cause of long-term disability and that the deterioration of consciousness could be influenced by extracerebral factors, such as fever, infections, electrolyte imbalances, dehydration and myocardial infarction [7]. For the same reasons as above, we also evaluated only motor deficits of arms and legs to investigate progressive-type lacunar infarction. In the present study, by comparing arm motor deficits with those of the legs, leg-predominant deficits predicted PS with high specificity.

In the PS group, 10 patients (63%) showed leg-predominant motor deficits on admission, but 13 patients (81%) did not exhibit leg-predominant deficits after symptomatic progression. In other words, the progression occurred mainly in the arms. The corticospinal fibers descend through the corona radiata and converge in the posterior limb of the internal capsule, in keeping with the somatotopic representation of body parts [8]. We speculated that ischemia might affect leg fibers first in the distal areas of the penetrating arteries and then arm fibers in the proximal areas of the same arteries. This view is supported by the serial DWI findings that were obtained before and after progression in 5 patients (fig. 1). In all these patients, ischemic lesions extended from the distal area to the proximal area of the penetrating arteries. The mechanism causing such an extension could be a thrombus propagation in an end artery; that is, initial occlusive changes at the distal portion of the penetrating arteries extending proximally toward the orifice. Alternatively, by applying the concept of branch atheromatous disease proposed by Caplan [9], small atheromatous changes located at the orifice of the penetrating arteries play the major role in the mechanism of progression. Obstructive change at the orifice of the penetrating arteries may initially reduce blood flow in the distal areas and later decrease blood flow in the proximal areas, thus evolving into ischemia from the distal to the proximal portion of the penetrating arteries. The final pathological observations are the same, however, irrespective of whether the occlusive changes start from the orifice or from the distal portion of the penetrating arteries. The accumulation of pathological data in patients with progressive-type lacunar infarction may clarify the question of whether obstructive changes start at the distal or proximal portion of the penetrating arteries.

This study has several limitations. First, our study was retrospective with a small population; therefore, the result of this study should be confirmed by large-scale prospective studies. Second, we estimated only the location of DWI lesions, instead of the lesion volumes. Previous studies have also shown a correlation of DWI lesion volumes with clinical outcome [10]. However, DWI lesion volumes can vary exceedingly in the acute phase of stroke, especially under treatment [11, 12]. Castillo and Leira [4] regarded DWI findings as a possibly modifiable predictor of PS. Third, we had no MR-pathological correlation. A direct comparison between MRI and histological analysis is the next step to consolidate our knowledge. Fourth, each factor of itself was not an exclusive predictor of PS. However, these three findings still have value as predictors of PS to start intensive therapy for preventing further progression and expecting a better outcome.

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