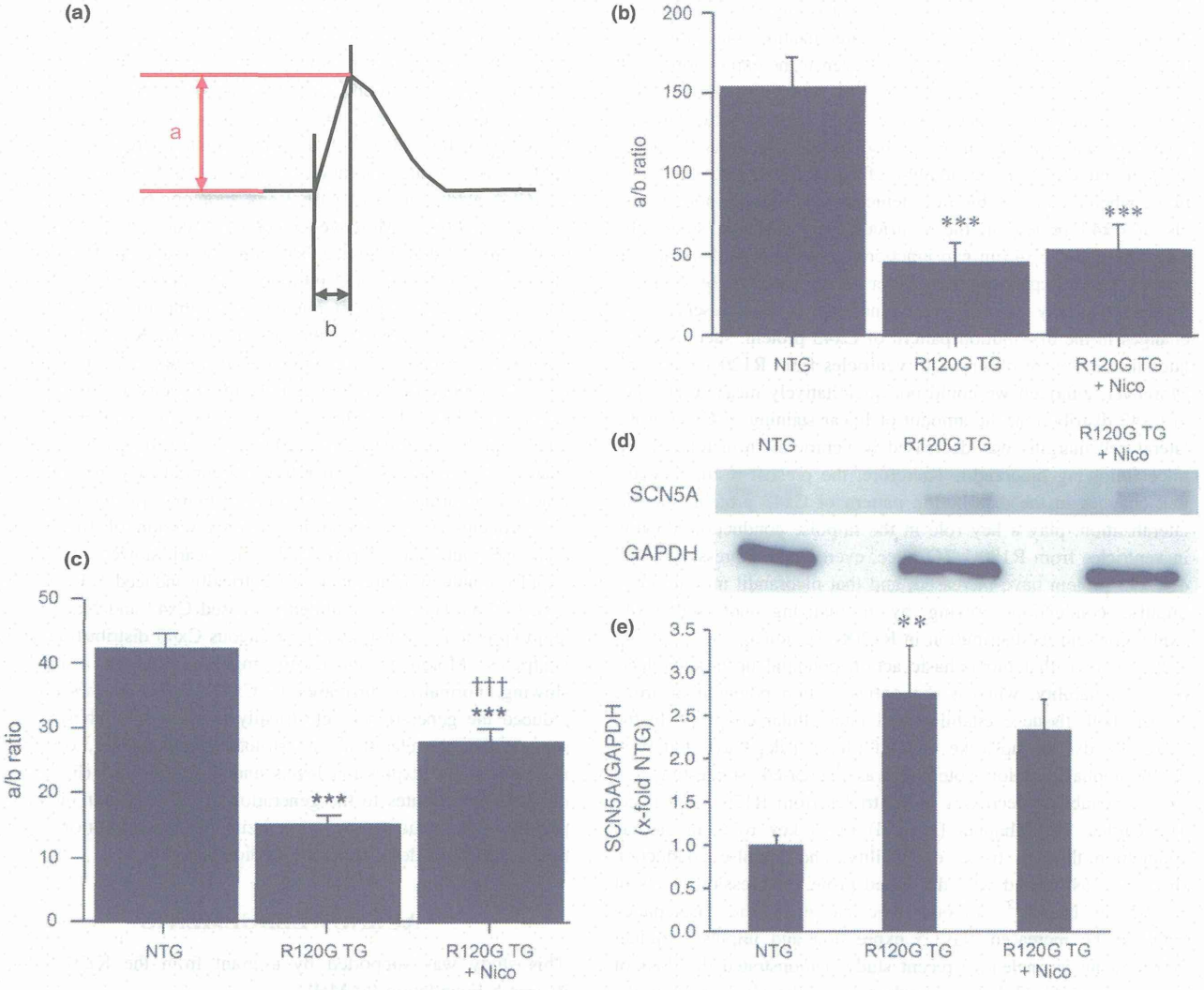

Fig. 6 (a) Method of determining the upstroke ratio of Phase 0 depolarization, calculated as the ratio of the width of *a* to the width of *b* from monophasic and optical action potentials. (b,c) The upstroke ratio of Phase 0 depolarization in the left ventricle calculated from the monophasic action potential (b) and the optical action potential (c) in non-transgenic (NTG) mice, mice overexpressing an arg120gly missense mutation in heat shock protein B5 (*HSPB5*; i.e. R120G TG mice) and nicorandil-treated (+ Nico) R120G TG mice. (d) Representative immunoblots of Nav1.5 (SCN5A) and GAPDH in ventricular tissue from NTG), R120G TG and R120G TG + Nico mice and summary of results of SCN5A expression (normalized against that of GAPDH, with that in the NTG group assigned a value of 1) in the three groups. Data are the mean±SEM (n = 6–8 mice in each group). $^{**}P < 0.01$, $^{***}P < 0.001$ compared with the NTG group; $^{\dagger\dagger\dagger}P < 0.001$ compared with the R120G TG group.

It is generally known that cardiac Cx43 plays key roles in cardiac impulse conduction and that impulse conduction slowing is associated with decreased protein expression levels of Cx43 in hearts.[16] The reasons for the discrepancy in Cx43 expression and impulse conduction in the present study are unknown, but such conflicting data could be explained, in part, by differences in the model of cardiac disease used. In fact, the mouse model of desmin-related cardiomyopathy differs from the model used in the present study, with Gard *et al.*[10] demonstrating that impulse conduction slowing was not associated with decreased Cx43 protein expression. Moreover, a recent study demonstrated that hindlimb unloading resulted in an increased predisposition to ventricular arrhythmia and that arrhythmia induction was associated with increases in left ventricular Cx43 expression.[17] In the present study, we also examined expression levels of p-Cx43 protein because decreases in it are known to cause electrical uncoupling.[18] Contrary to our expectations, p-Cx43 protein expression, like total Cx43 expression, was increased in TG mice. Moreover, the ratio of p-Cx43 : total Cx43 was similar between NTG and R120G TG mice, suggesting that the relative expression of p-Cx43 in total Cx43 is not changed between NTG and TG mice. Interestingly, a recent study suggested that ventricular impulse conduction slowing is associated with increased p-Cx43 expression.[19] These results suggest that factors other than Cx43 protein expression play crucial roles in impulse conduction slowing. Nevertheless, the improvement of conduction slowing was associated with normalized protein expression of total and p-Cx43 in R120G TG mouse hearts following nicorandil treatment, suggesting that increased Cx43 protein expression participates in ventricular impulse conduction slowing in this model.

Recent studies have demonstrated that changes in the distribution pattern of Cx43, such as Cx43 lateralization, play important roles in impulse conduction slowing, even if the expression levels of Cx43 protein are not decreased.[20,21] Moreover, Gard *et al.*[10] indicated that increased amounts of Cx43 may be located within non-junctional pools in mice because confocal analysis showed a marked reduction in the amount of Cx43 present in gap junctions, whereas immunoblotting demonstrated roughly normal levels of Cx43 protein in the ventricular myocardium, suggesting that diminished Cx43 in gap junctions in the mice was not due to limited Cx43 expression but rather to an inability of Cx43 to localize normally at cell-to-cell junctions. In the present study, changes in the distribution pattern of Cx43 protein, such as Cx43 lateralization, were observed in ventricles from R120G TG mice. Moreover, although we could not quantitatively measure changes in Cx43 distribution, the amount of linear staining of Cx43 at the lateral cell margins was decreased in ventricles from R120G TG mice following nicorandil. Therefore, the present results suggest that changes in the distribution pattern of Cx43 protein, such as lateralization, play a key role in the impulse conduction slowing in ventricles from R120G TG mice, even when expression levels of Cx43 protein have increased, and that nicorandil may improve impulse conduction slowing by normalizing increased Cx43 expression and its distribution in R120G TG mice.

It is known that monophasic action potential upstroke reflects tissue excitability, whereas the optical action potential upstroke reflects both tissue excitability and intercellular coupling. In the present study, the upstroke ratio of Phase 0 depolarization from the monophasic action potential was decreased, suggesting that tissue excitability decreases in ventricles from R120G TG mice. The cardiac Na^+ channel (Nav1.5) plays key roles in cardiac conduction through tissue excitability, and impulse conduction slowing is associated with decreased protein expression levels of Nav1.5 in hearts.[22] Although the cause of the discrepancy between the increased Nav1.5 expression and impulse conduction slowing is unclear, a recent study demonstrated that loss of expression of Cx43 led to a reduction in the abundance of the Nav1.5 signal at the intercalated discs.[23] These results suggest that Cx43 modulates the expression of Nav1.5 protein. In the present study, both Cx43 and Nav1.5 protein expression was increased, and heterogeneous distribution of Cx43, such as lateralization, was observed. Therefore, although we did not examine the distribution pattern of Nav1.5, it is heterogeneous in R120G TG mouse hearts, which may induce impulse conduction slowing. However, in the present study chronic nicorandil treatment improved increased Cx43 protein expression levels, whereas it did not improve increased Nav1.5 expression, suggesting that Cx43 does not modulate the expression of Nav1.5 protein in the TG mice. Nevertheless, increased Nav1.5 may participate in ventricular conduction slowing because the action potential upstroke calculated from the monophasic action potential is reduced in ventricles from TG mice (i.e. decreases in tissue excitability). Moreover, nicorandil did not improve the decreased upstroke ratio of Phase 0 depolarization calculated from the monophasic action potential, whereas it improved the decreased upstroke ratio of Phase 0 depolarization calculated from the optical action potential, suggesting that nicorandil improves intercellular coupling, leading to improvements in impulse conduction slowing.

In the present study, nicorandil improved impulse conduction slowing and inhibited increases in total and p-Cx43 protein expression, leading to inhibition of electrically induced VT. In a previous study, we demonstrated that cell viability was dose-dependently recovered in myocytes overexpressing mutant *HSPB5* (i.e. R120G TG) following nicorandil treatment.[9] Nicorandil treatment also inhibited the increase in BAX, the decrease in BCL2, activation of caspase 3 and apoptotic cell death induced by mutant *HSPB5*. Moreover, nicorandil treatment prolonged the survival of R120G TG mice. Therefore, we concluded in the previous study that nicorandil prolonged the survival of R120G TG mice by protecting against mitochondrial impairment.[9] Recently, Zhang *et al.*[24] showed that the opening of mitoK_{ATP} channels in astrocytes can reverse rotenone-induced dysfunction of astrocytic Cx43 and therefore protect against the toxicity of rotenone in astrocytes. Several studies suggest that the cardioprotective effects of nicorandil are mediated by activation of mitoK_{ATP} channels in myocytes.[2,4] Therefore, nicorandil may improve dysfunction of cardiac Cx43 by opening of mitoK_{ATP} channels.

In conclusion, we found that overexpression of the R120G missense mutation in *HSPB5* in the heart slowed ventricular impulse conduction and caused electrically induced VT. In addition, R120G TG mice exhibited increased Cx43 and Nav1.5 protein expression, as well as heterogeneous Cx43 distribution in the ventricles. Moreover, nicorandil improved impulse conduction slowing, normalized increases in Cx43 protein expression and reduced the generation of electrically induced VT. These results suggest that the electrical and structural remodelling, especially in terms of the expression levels and heterogeneous distribution of Cx43, contributes to the generation of VT and that nicorandil prevents VT induction by normalizing Cx43 expression in this mouse model of desmin-related cardiomyopathy.

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DISCLOSURE

The authors declare no conflicts of interest.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Movie S1. Spiral re-entry.

Original Research Paper

Combination of blood flow asymmetry in the cerebral and cerebellar hemispheres on brain perfusion SPECT predicts 5-year outcome in patients with symptomatic unilateral major cerebral artery occlusion

Jun-ichi Nomura[1,2], Kuniaki Ogasawara[1,2], Hideo Saito[1,2], Kazunori Terasaki[2], Yoshiyasu Matsumoto[1,2], Yoshihiro Takahashi[1,2], Yasushi Ogasawara[1,2], Hiroaki Saura[1,2], Koji Yoshida[1,2], Yuiko Sato[1,2], Yoshitaka Kubo[1,2], Akira Ogawa[1,2]

[1]Department of Neurosurgery, School of Medicine, Iwate Medical University, Morioka, Japan, [2]Cyclotron Research Center, School of Medicine, Iwate Medical University, Morioka, Japan

Background and objective: Misery perfusion increases the risk of stroke recurrence in patients with symptomatic major cerebral artery occlusion. The ratio of brain perfusion contralateral-to-affected asymmetry in the cerebellar hemisphere to brain perfusion affected-to-contralateral asymmetry in the cerebral hemisphere (CblPR/CbrPR) indicates affected-to-contralateral asymmetry of oxygen extraction fraction (OEF) in the cerebral hemisphere. The purpose of the present study was to determine whether the CblPR/CbrPR on brain perfusion single-photon emission computed tomography (SPECT) predicts 5-year outcomes in patients with symptomatic unilateral occlusion of the middle cerebral artery (MCA) or internal carotid artery (ICA).

Methods: Brain perfusion was assessed using *N*-isopropyl-*p*-[^{123}I]-iodoamphetamine (^{123}I-IMP) SPECT in 70 patients. A region of interest (ROI) was manually placed in the bilateral MCA territories and in the bilateral cerebellar hemispheres, and the CblPR/CbrPR was calculated. All patients were prospectively followed for 5 years. The primary end points were stroke recurrence or death.

Results: A total of 17 patients exhibited the primary end points, 11 of whom experienced subsequent ipsilateral strokes. Multivariate analysis revealed that only high CblPR/CbrPR was significantly associated with the development of the primary end point or subsequent ipsilateral strokes (95% confidential limits [CIs], 1.130–3.145; $P = 0.0114$ or 95% CIs, 2.558–5.140; $P = 0.0045$, respectively). The CblPR/CbrPR provided 65% (11/17) or 91% (10/11) sensitivity and 88% (47/53) or 88% (52/59) specificity in predicting the primary end point or subsequent ipsilateral strokes, respectively.

Conclusions: The CblPR/CbrPR on brain perfusion SPECT predicts 5-year outcomes in patients with symptomatic unilateral occlusion of the MCA or ICA.

Keywords: Crossed cerebellar hypoperfusion, Brain perfusion SPECT, Subsequent stroke

Introduction

When cerebral perfusion pressure is reduced, cerebral blood flow (CBF) is initially maintained by dilation of precapillary resistance vessels in a process known as cerebrovascular autoregulation.[1,2] With more severe reductions in cerebral perfusion pressure, the capacity for compensatory vasodilation exceeds, autoregulation fails, and CBF begins to decline. In this context, a progressive increase in oxygen extraction fraction (OEF) can act to maintain cerebral oxygen metabolism and brain function.[1,3] This form of cerebral hemodynamic failure has been termed 'misery perfusion'.[4] In patients with symptomatic major cerebral arterial occlusive disease, misery perfusion increases the risk of stroke recurrence.[5–7] Therefore, identification and optimal treatment of patients with misery perfusion may help to prevent

Correspondence to: Kuniaki Ogasawara, Department of Neurosurgery, Iwate Medical University, 19-1 Uchimaru, Morioka 020-8505, Japan. Email: kuogasa@iwate-med.ac.jp

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stroke recurrence. However, direct detection OEF-related misery perfusion can only be accomplished using positron emission tomography (PET).

Brain perfusion single-photon emission computed tomography (SPECT) with an acetazolamide challenge is another reliable method for identifying patients with cerebral hemodynamic impairment[8,9] and for identifying those who are at high risk for stroke recurrence.[10,11] We previously demonstrated that reduced cerebrovascular reactivity to acetazolamide on brain perfusion SPECT is associated with a high 5-year risk of recurrence of ischemic stroke in patients with symptomatic occlusion of the middle cerebral artery (MCA) or internal carotid artery (ICA).[12] However, acetazolamide is associated with a variety of adverse side effects, including headache, nausea, dizziness, tinnitus, numbness of the extremities, and Stevens-Johnson syndrome.[13,14] In fact, 63% of patients who underwent SPECT study with acetazolamide challenge developed adverse effects after administration of acetazolamide, and these symptoms resulted in some impairment of the ability of patients to engage in their normal activities of daily living or work activities.[13] Thus, it would be beneficial to develop a SPECT method of detecting misery perfusion that does not require administration of acetazolamide.

Crossed cerebellar hypoperfusion (CCH) is defined as a reduction in blood flow in the cerebellar hemisphere contralateral to a supratentorial lesion.[15] This phenomenon can be demonstrated on brain perfusion images obtained by SPECT or by PET.[15–18] The mechanism underlying CCH reportedly consists of disruption of the corticopontocerebellar pathway that causes functional deafferentation and transneural metabolic depression of the contralateral cerebellar hemisphere.[17,18] A reduction in the blood flow in the cerebellar hemisphere corresponds with the degree of metabolic depression, resulting in CCH. The disruption of the corticopontocerebellar pathway also leads to metabolic depression of the ipsilateral cerebral hemisphere. Thus, the degree of CCH may reflect cerebral metabolism in the affected cerebral hemisphere relative to that in the contralateral cerebral hemisphere. A previous study demonstrated a correlation between the degree of CCH and affected-to-contralateral asymmetry of cerebral metabolic rate of oxygen ($CMRO_2$) in the cerebral hemispheres on PET in patients with unilateral carotid artery occlusive disease.[19] Oxygen extraction fraction is a function of the ratio of $CMRO_2$ to CBF, and we have demonstrated that the ratio of blood flow asymmetry in the cerebellar hemisphere (i.e. the degree of CCH) to blood flow asymmetry in the cerebral hemisphere on brain perfusion SPECT correlates with PET–OEF asymmetry in the cerebral hemisphere. Further, this blood flow ratio can detect misery perfusion in the affected cerebral hemisphere in patients with unilateral occlusion of the MCA or ICA.[20]

The purpose of the present study was to reanalyze the same patient cohort used in our previous studies[11,12] and to determine whether the ratio of blood flow asymmetry in the cerebellar hemisphere to blood flow asymmetry in the cerebral hemisphere on brain perfusion SPECT predicts 5-year outcomes in patients with symptomatic unilateral occlusion of the MCA or ICA.

Subjects and Methods

Patients

In all, 70 consecutive patients (53 men, 17 women) meeting the inclusion criteria and who were admitted from January 1993 to March 1996 prospectively entered into the study.[11,12] The mean age was 57 years (range, 38–69 years), and the population characteristics were described previously.[11] Inclusion criteria for the present study were as follows: (1) age younger than 70 years, (2) unilateral complete occlusion of the ICA or the horizontal portion of the MCA confirmed by angiography with arterial catheterization, (3) evidence of ischemic cerebrovascular events in the arterial distribution distal to the lesion within 3 months prior to study entry, (4) useful residual function (modified Rankin disability scale [RS] 0, 1, or 2), (5) no or border zone infarction on computed tomography (CT) or magnetic resonance (MR) imaging, and (6) informed consent obtained from the patient or relatives. Patients were excluded from the study if they had (1) cardioembolic infarction, based on the onset pattern, angiographic findings, and results of electrocardiography and echocardiography; (2) vascular lesions caused by other systemic diseases, such as aortitis syndrome, moyamoya disease, or fibromuscular dysplasia; (3) an occlusion, or moderate to severe stenosis (>50%) of major cerebral arteries in the contralateral carotid or vertebrobasilar system; or (4) systemic conditions such as cardiac failure, renal failure, hepatic failure, respiratory failure, severe diabetes mellitus (fasting blood sugar $\geq$ 300 mg/dl), and severe hypertension (diastolic blood pressure $\geq$ 110 mmHg).

All study protocols were reviewed and approved by the institutional ethics committee.

Patient Management and Outcome Measures

All patients were treated with antiplatelet therapy (81 mg/day aspirin or 200 mg/day ticlopidine HCl), and treatment of other risk factors and medical therapy were based on the individual case. Although the attending physicians were unaware of the findings of the SPECT studies, treatment did not differ among the patients. No patients underwent bypass surgery. Patients were examined at 1-month intervals and followed for 60 months after study entry. An interim

history was obtained, and a neurologic examination was performed at each visit.

The study terminated on 31 March 2001.[12] The primary end point was stroke recurrence or death, and observation was terminated if stroke recurred or if death occurred. Magnetic resonance imaging was obtained and was compared with initial studies to confirm recurrent stroke. Stroke in the previously symptomatic arterial territory without evidence of primary intracranial hemorrhage was classified as an ipsilateral ischemic stroke.

Single-Photon Emission Computed Tomography Methodology

Brain perfusion was assessed using *N*-isopropyl-*p*-[^{123}I]-iodoamphetamine (IMP) and SPECT. All patients underwent SPECT study 1 month or more after the last ischemic event and within 2 weeks before study entry. Single-photon emission computed tomography images were obtained using a multidetector ring-type scanner (Headtome-SET031, Shimadzu Corp., Kyoto, Japan), consisting of 64 NaI crystals in a 38-cm diameter circle. After tomographic reconstruction, the spatial resolution and slice thickness in the center of the plane were 9 and 16 mm full width at half maximum (FWHM), respectively. The device can obtain six tomographic slices in a single scanning process. The energy window in this study was 140 keV ($\pm$15%). Projection data were processed with Ramachandran's filtered backprojection after introduction of a Butterworth prefilter. A 64×64 image matrix was used.

An intravenous injection of 111 MBq, ^{123}I-IMP was administered. After 15 minutes, SPECT imaging with a high resolution collimator was performed, and tomographic data were continuously obtained over a 30-minute period.

Two tomographic planes, located 50–66 mm above (plane of the cerebrum) and 2–18 mm below (plane of the cerebellum) and parallel to the orbitomeatal line, were analyzed for each patient, and the region of interest (ROI) was placed directly on each selected SPECT image. Magnetic resonance-single-photon emission computed tomography imaging coregistration was not used. Following the atlas developed by Kretschmann and Weinrich,[21] one investigator, who was blinded to patient data, manually drew a large irregular ROI in the cerebral cortex perfused by the ipsilateral MCA on the plane of the cerebrum. On the same plane, a mirror-image ROI was also placed in the corresponding region on the contralateral side. Further, an irregular ROI was symmetrically placed in the bilateral cerebellar hemispheres on the plane of the cerebellum. Next, the mean count was determined in each ROI. The determination was accomplished at the time of study entry in all patients. Before the initiation of patient entry, the same SPECT studies were performed in 10 normal subjects (8 men and 2 women; age range, 35–65 years; mean age, 52.3 years) for whom informed consent was obtained.

Based on these SPECT data obtained at the time of patient entry, several analyses were retrospectively performed. First, the ratio of the mean count in the MCA territory on the occluded side to that on the contralateral side was calculated and was defined as the cerebral perfusion ratio (CbrPR). In the 10 normal subjects, the CbrPR was calculated when the left and right cerebral hemispheres were defined as the occluded side and the contralateral side, respectively. Second, the ratio of the mean count in the cerebellar hemisphere on the contralateral side to that on the occluded side was calculated and was defined as the cerebellar perfusion ratio (CblPR). In the 10 normal subjects,[12] the CblPR was calculated when the left and right cerebellar hemispheres were defined as the contralateral side and the occluded side, respectively. Finally, the value of CblPR/CbrPR was calculated for each patient and for each normal subject.

Statistical Analysis

Descriptive data are expressed as mean$\pm$standard deviation (SD). The relationship between CblPR/CbrPR in patients and those in normal subjects was evaluated using the Mann–Whitney's *U*-test. The relationship between each variable and the development of the primary end point or subsequent ipsilateral strokes was evaluated by univariate analysis using the Mann–Whitney's *U*-test or chi^2-test. A multivariate statistical analysis of factors related to development of the primary end point or subsequent ipsilateral strokes was also performed using a logistic regression model. Variables with $P < 0.2$ in the univariate analyses were selected for analysis in the final model. Regardless of the *P* value, modified RS and border zone infarction were adopted as confounders of the baseline clinical status in the model. Differences were deemed statistically significant if $P < 0.05$. The accuracy of CblPR/CbrPR at entry to predict the development of the primary end point or subsequent ipsilateral strokes was determined by a receiver operating characteristic curve when the relationship between the two variables was significant. The curve was calculated in increments or decrements of 0.5 SD from the mean value of CblPR/CbrPR obtained in normal subjects.

Results

All 70 patients were followed for 5 years or until stroke recurrence or death.[12] A total of 13 strokes were identified, 11 of which were ipsilateral to the ICA or MCA occlusion; the other two patients suffered stroke in the contralateral cerebral hemisphere or in the contralateral brain stem, respectively.[12] One death

each was attributed to lung cancer, gastric cancer, myocardial infarction, and a motor vehicle accident.[12] Thus, 17 patients were defined as having the primary end point.

Cerebellar perfusion ratio (CblPR)/cerebral perfusion ratio (CbrPR) was higher in the 70 patients [0.801–1.468 (mean, 1.072; SD, 0.122)] than in the 10 normal subjects [0.942–1.066 (mean, 1.002; SD, 0.050)] ($P = 0.0432$).

Results of univariate analysis of factors related to the development of the primary end point or subsequent ipsilateral strokes are summarized in Table 1. The CblPR/CbrPR was significantly higher in patients with the primary end point or subsequent ipsilateral strokes than in those without (Fig. 1). Other variables were not significantly associated with the development of the primary end point or subsequent ipsilateral strokes. After eliminating closely related variables in the univariate analyses, the following confounders with $P < 0.2$ in addition to modified RS and border zone infarction were adopted in the logistic regression model for the multivariate analysis: hyperlipidemia and CblPR/CbrPR. The multivariate analysis revealed that only high CblPR/CbrPR was significantly associated with the development of the primary end point or subsequent ipsilateral strokes (95% confidential limits [CIs], 1.130–3.145; $P = 0.0114$ or 95% CIs, 2.558–5.140; $P = 0.0045$, respectively).

Sensitivity and specificity for the CblPR/CbrPR in the cut-off point lying closest to the left upper corner of the receiver operating characteristic curve were 65% (11/17) and 88% (47/53) (cut-off point = 1.102: the mean+2 SD of the control value obtained from normal subjects), respectively, for prediction of development of the primary end point and were 91% (10/11) and 88% (52/59) (cut-off point = 1.102: the mean+2 SD of the control value obtained from normal subjects), respectively, for prediction of the development of subsequent ipsilateral strokes (Figs. 1 and 2). With the same cut-off point, the positive- and negative-predictive values were 65% (11/17) and 88% (47/53), respectively, for prediction of the development of the primary end point or were 59% (10/17) and 98% (52/53), respectively, for prediction of the development of subsequent ipsilateral strokes.

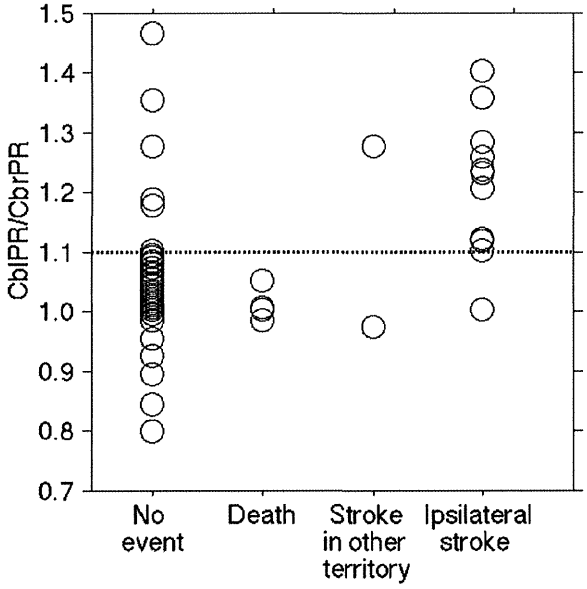

Figure 1 Relationship between cerebral perfusion ratio (CblPR)/cerebellar perfusion ratio (CbrPR) and the primary end point (death, stroke in the other vascular territory, and subsequent ipsilateral stroke). Dashed horizontal line denotes mean+2 SD (1.102) of CblP R/CbrPR obtained in healthy volunteers.

Single-photon emission computed tomography images at entry for a patient who developed a subsequent ipsilateral stroke are illustrated in Fig. 3.

Discussion

The present study demonstrated that the ratio of blood flow asymmetry in the cerebellar hemisphere to

Table 1 Univariate analysis of factors related to the development of the primary end point or subsequent ipsilateral stroke

	Primary end point			Subsequent ipsilateral stroke		
Variables	Yes (n = 17)	No (n = 53)	P value	Yes (n = 11)	No (n = 59)	P value
Age (years, mean±SD)	57.7±7.3	56.5±8.7	0.8477	58.6±6.1	56.4±8.7	0.6336
Male gender	12 (71%)	41 (77%)	0.7458	8 (73%)	45 (76%)	0.7215
Modified RS of zero	10 (59%)	22 (42%)	0.2678	7 (64%)	25 (42%)	0.3231
Border zone infarction	11 (65%)	29 (55%)	0.5778	8 (73%)	32 (54%)	0.3305
Site of lesion						
ICA	10 (59%)	31 (58%)	> 0.9999	7 (64%)	34 (58%)	0.7532
MCA	7 (41%)	22 (42%)		4 (36%)	25 (42%)	
Hypertension	10 (59%)	27 (51%)	0.5917	7 (64%)	30 (51%)	0.5219
Diabetes mellitus	4 (24%)	17 (32%)	0.5610	2 (18%)	19 (32%)	0.4852
Hyperlipidemia	3 (18%)	1 (2%)	0.0519	2 (18%)	2 (3%)	0.1136
Prior MI	1 (6%)	2 (4%)	0.5720	1 (9%)	2 (3%)	0.4061
Smoking	9 (53%)	38 (72%)	0.2344	64 (53%)	68 (72%)	> 0.9999
CblPR/CbrPR	1.155±0.138	1.045±0.104	0.0018	1.212±0.118	1.046±0.104	0.0001

SD: standard deviation; RS: Rankin disability scale; ICA: internal carotid artery; MCA: middle cerebral artery; MI: myocardial infarction; CblPR: cerebellar perfusion ratio; CbrPR: cerebral perfusion ratio.

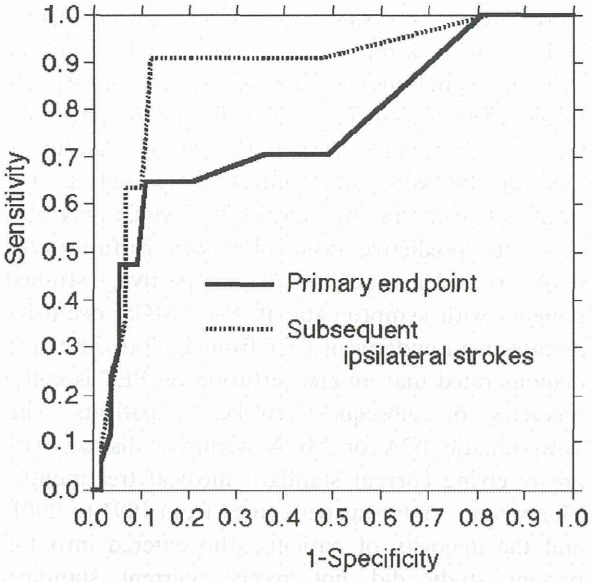

Figure 2 Receiver operating characteristics curves used to determine the accuracy of cerebellar perfusion ratio (CblPR)/cerebral perfusion ratio (CbrPR) at entry to predict the development of the primary end point or subsequent ipsilateral strokes.

blood flow asymmetry in the cerebral hemisphere on brain perfusion SPECT predicts 5-year outcomes in patients with symptomatic unilateral occlusion of the MCA or ICA.

We recently demonstrated that the CblPR/CbrPR on brain perfusion SPECT reflects OEF asymmetry in the MCA territory and that this blood flow ratio detects misery perfusion in the affected cerebral hemisphere with a sensitivity and negative-predictive value of 100% in patients with unilateral stenosis or occlusion of the MCA or ICA.[20] In that previous study, the misery perfusion in the affected MCA territory was defined as an affected-to-contralateral PET–OEF ratio greater than the mean+2 SDs (1.089) of the normal values. Grubb *et al.* categorized patients with the affected-to-contralateral PET–OEF ratio greater than 1.082 due to unilateral ICA steno-occlusive disease as having misery perfusion and reported that such patients are at high risk for subsequent stroke when treated medically.[6] The cut-off point for the affected-to-contralateral PET–OEF ratio in our recent study was similar to that defined by Grubb *et al.* Further, in the same study, the receiver operating characteristic analysis showed that the optimal cut-off point of the CblPR/CbrPR on brain perfusion SPECT for detection of the misery on OEF PET was the mean+2 SDs (1.101) of the control value obtained from normal subjects,[20] which is similar to the optimal cut-off point (the mean+2 SDs of the control value obtained from normal subjects, 1.102) of the CblPR/CbrPR in predicting development of the primary end point or subsequent ipsilateral strokes in the present study.

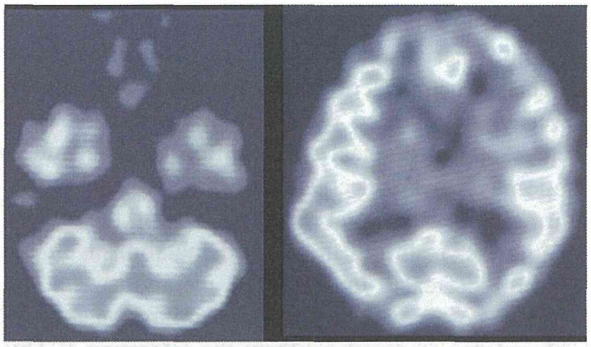

Figure 3 Single-photon emission computed tomography (SPECT) images of a 58-year-old woman with symptomatic occlusion of the left middle cerebral artery (MCA). While blood flow asymmetry in the cerebellar hemisphere is not observed, blood flow in the left MCA territory is reduced when compared with blood flow in the right MCA territory. This patient developed stroke recurrence in the left MCA territory 5 months after entry into the study.

Although contralateral-to-affected side blood flow asymmetry in the cerebellar hemisphere essentially reflects $CMRO_2$ in the affected cerebral hemisphere relative to that in the contralateral cerebral hemisphere,[19,20] the degree of the blood flow asymmetry in the cerebellar hemisphere underestimates the degree of $CMRO_2$ asymmetry in the cerebral hemisphere when assessed at later than 3 months after the last ischemic event.[20] These findings are supported by previous reports in which CCH was not always seen in patients with decreased $CMRO_2$ in the cerebral hemisphere ipsilateral to a major cerebral artery occlusion.[22] In fact, CCH often disappeared 2–3 months after onset in patients with stroke.[23] As a result, while the CblPR/CbrPR overestimates the OEF asymmetry in patients who are assessed at later than 3 months after the last ischemic event, the specificity (83%) and positive-predictive value (72%) of the CblPR/CbrPR for detection of the misery perfusion are elevated when assessed in patients within 3 months after the last ischemic event.[20] The present study had included only the latter type of patients, and this inclusion criterion might explain why CblPR/CbrPR predicted subsequent stroke in this study.

The simplicity of requiring only the evaluation of asymmetry on blood flow images makes the present SPECT method practical. The present SPECT method also has the advantage of not requiring acetazolamide, which is otherwise associated with a significant risk of various adverse side effects.[13,14] In particular, this SPECT method is useful as a screening test to determine whether a PET study is needed to predict the development of subsequent strokes in patients within 3 months after the last ischemic event due to unilateral occlusion of the MCA or ICA.

Blood flow in the cerebral hemisphere ipsilateral to the arterial lesion and in the contralateral cerebellar hemisphere could be affected by stroke severity, as reflected by the infarct volume or the degree of neurological deficits. Border zone infarction may cause selective neuronal damage in the normal-appearing cerebral cortex beyond the regions of infarcts, resulting in reduced metabolism in the cerebral cortex.[24] In addition, metabolism in the cerebral cortex with border zone infarction may be reduced due to diaschisis from the infarction.[24] This reduction in metabolism in the affected cerebral hemisphere leads to CCH as well as hypoperfusion in the ipsilateral cerebral hemisphere.[17] Under such conditions, CblPR/CbrPR is not elevated, suggesting 'matched hypometabolism'[20] and a low risk of stroke recurrence. On the other hand, blood flow in the affected cerebral hemisphere is often reduced to a greater degree than its metabolism, even in patients with border zone infarction. In this condition, CblPR/CbrPR is elevated, suggesting 'misery perfusion'[20] and a high risk of stroke recurrence. These factors might account for why neither modified RS nor border zone infarction was associated with the development of the primary end point or subsequent ipsilateral strokes in the present study. These findings also suggest that when a patient with border zone infarction due to symptomatic unilateral chronic ICA or MCA occlusive disease exhibits a decrease in perfusion in the ipsilateral cerebral hemisphere on brain perfusion imaging, CblPR/CbrPR can distinguish 'misery perfusion' from 'matched hypometabolism'.[20]

Superficial temporal artery-MCA bypass for patients with recent symptomatic carotid artery occlusion and misery perfusion (as in the patients in the present study) was not effective in reducing recurrent, ipsilateral ischemic events in the Carotid Occlusion Surgery Study.[25] One of the factors that led to the negative outcome of that study was the high rate of ipsilateral strokes during the first 2 days after surgery.[26] The mechanism of these perioperative ischemic strokes was attributable to hemodynamic factors and the inability of patients with a symptomatic ICA occlusion and impaired hemodynamics in the cerebral hemisphere distal to the ICA occlusion to tolerate surgery in the majority of cases.[26] On the other hand, 2-year rates for the primary end point were 23% for patients who received current standard medical treatments in the Carotid Occlusion Surgery Study.[25] Thus, the optimal treatment strategy for patients with misery perfusion due to symptomatic ICA or MCA occlusive disease remains undetermined.

The present study possesses several limitations that require discussion. First, although misery perfusion is a predictor of subsequent stroke in medically treated patients with symptomatic major cerebral artery disease, this finding has been demonstrated in studies in the 1990s.[5,6] Advances in medical therapies over the last few decades, such as the use of angiotensin receptor blockers and statins, may reduce the recurrent stroke rate in general,[27–30] which may also affect the predictive value of misery perfusion for stroke risk. Yamauchi *et al.* prospectively studied patients with symptomatic ICA or MCA occlusive diseases who underwent PET from 1999 to 2008 and demonstrated that misery perfusion on PET is still a predictor of subsequent stroke in patients with symptomatic ICA or MCA occlusive disease who are receiving current standard medical treatments.[7] The present study was performed from 1993 to 2001, and the majority of patients who entered into the present study did not receive current standard medical treatments. Thus, even when considering the findings demonstrated by Yamauchi *et al.*, further investigation regarding the relationship between the blood flow ratio on brain perfusion SPECT used in the present study and the risk of stroke recurrence is needed in patients with symptomatic major cerebral artery occlusive disease who are receiving current standard medical treatments. Second, the study population included only patients with unilateral ICA or MCA occlusive disease and used blood flow asymmetry on SPECT images to detect misery perfusion in the affected cerebral hemisphere. However, impairments in cerebral hemodynamics are more severe in patients with bilateral major cerebral artery occlusive disease than in those with unilateral major cerebral artery occlusive disease,[31] and impairments in bilateral cerebral hemodynamics in patients with bilateral major cerebral arterial occlusive disease may not be detected by the present SPECT method. Third, irregular ROIs were manually drawn in the cerebral cortexes perfused by the bilateral MCAs and in the bilateral cerebellar hemispheres on SPECT images using the atlas developed by Kretschmann and Weinrich.[21] Because of the age of the scanner, a program for coregistration or anatomic standardization of SPECT data was not used. Furthermore, whether only one selected tomographic plane reliably reflects the hemodynamics on the entire territory perfused by the MCA is an important issue. The SPECT machine used in the present study can obtain only six tomographic slices for 123I-IMP SPECT in a single scanning process. In addition, the obtained SPECT tomographic slices are not sequential, and data are missing in every other slice. Therefore, we could not perform volumetric analysis of the SPECT data in the present study. Lastly, cerebral hemodynamic impairment can be identified using other less-invasive and lower cost methods, such as determination

of cerebrovascular reactivity to hypercapnia, as measured by transcranial Doppler ultrasonography and the breath-holding index values in the MCA flow velocity.[32] This method also identifies patients who are at high risk for stroke onset due to ICA occlusive disease.[33] However, transcranial Doppler ultrasonography with breath-holding often cannot be accomplished due to technical problems caused by poor insonation of the temporal bone window or subject problems caused by intolerance to breath-holding.[32,33] Although brain perfusion SPECT is more expensive than transcranial Doppler ultrasonography, it always displays left-to-right asymmetry of blood flow in the cerebral and cerebellar hemispheres, resulting in the prediction of patient outcomes.

In conclusion, the ratio of blood flow asymmetry in the cerebellar hemisphere to blood flow asymmetry in the cerebral hemisphere on brain perfusion SPECT predicts 5-year outcomes in patients with symptomatic unilateral occlusion of the MCA or ICA.

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Postoperative Changes in Cerebral Metabolites Associated with Cognitive Improvement and Impairment after Carotid Endarterectomy: A 3T Proton MR Spectroscopy Study

H. Saito, K. Ogasawara, H. Nishimoto, Y. Yoshioka, T. Murakami, S. Fujiwara, M. Sasaki, M. Kobayashi, K. Yoshida, Y. Kubo, T. Beppu, and A. Ogawa

ABSTRACT

BACKGROUND AND PURPOSE: Cognitive function can improve or decline after carotid endarterectomy. Proton MR spectroscopy can be used evaluate cerebral metabolites, such as *N*-acetylaspartate, choline, and creatine, in vivo. The purpose of the present study was to determine whether postoperative changes in cerebral metabolites measured by using 3T proton MR spectroscopy were associated with changes in cognitive function after CEA.

MATERIALS AND METHODS: In 100 patients undergoing CEA for ipsilateral cervical internal carotid artery stenosis (≥70%), brain proton MR spectroscopy was performed before and after surgery. NAA/Cr and Cho/Cr ratios were measured in regions of interest placed in the centrum semiovale of both cerebral hemispheres. Neuropsychological testing was also performed preoperatively and 1 month postoperatively. Multivariate statistical analysis of factors related to postoperatively changed cognition was performed, and odds ratios with 95% confidence intervals were calculated.

RESULTS: On the basis of the neuropsychological assessments, 10 (10%), 80 (80%), and 10 (10%) patients were defined as having postoperatively improved, unchanged, and impaired cognition, respectively. A positive and high ΔNAA/Cr ratio (postoperative value–preoperative value) in the cerebral hemisphere ipsilateral to the operative site was significantly associated with postoperatively improved cognition (95% CI, 13.3–21.3; $P = .0016$). Negative and high absolute values of the ΔNAA/Cr ratio (95% CI, 0.018–0.101; $P = .0039$) and ΔCho/Cr ratio (95% CI, 0.042–0.135; $P = .0046$) in the ipsilateral cerebral hemisphere were significantly associated with postoperatively impaired cognition.

CONCLUSIONS: Postoperative changes in cerebral metabolites measured by using proton MR spectroscopy were associated with changes in cognitive function after CEA.

ABBREVIATIONS: CEA = carotid endarterectomy; CI = confidence interval; Rey test = Rey-Osterreith Complex Figure Test; WAIS-R = Wechsler Adult Intelligence Scale Revised

Carotid endarterectomy can reduce the risk of stroke in appropriately selected patients.[1,2] While CEA may also improve cognitive function,[3,4] cognitive impairment occurs in 10%–30% of patients following CEA.[5-8] A recent study reported that 11% of patients undergoing CEA experienced improvement in cognitive function after surgery, while another 11% experienced a decline in cognitive function after surgery.[9] Cerebral metabolism may also change along with these postoperative changes in cognitive function, but the relationship between these 2 factors remains unclear.

Proton MR spectroscopy enables noninvasive chemical analysis in vivo, because the proton is the most sensitive stable nucleus for MR spectroscopy and nearly all metabolites contain protons.[10] Proton MR spectroscopy can also measure relative changes in metabolites, including *N*-acetylaspartate, choline-containing compounds, and total creatine.[11] Investigators have suggested that the level of NAA in the brain is an index of neuronal viability[12] and that the level of choline in the brain is associated with membrane synthesis or degeneration in neural tissues.[13] On the basis of these hypotheses, proton MR spectroscopy has been applied for the study of various pathologic conditions of the cen-

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From the Department of Neurosurgery (H.S., K.O., M.K., K.Y., Y.K., T.B., A.O.) and Advanced Research Center (H.N., T.M., S.F., M.S.), School of Medicine, Iwate Medical University, Morioka, Japan; and Biofunctional Imaging (Y.Y.), Immunology Frontier Research Center, Osaka University, Osaka, Japan

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Please address correspondence to Kuniaki Ogasawara, MD, Department of Neurosurgery, Iwate Medical University, Uchimaru, 19-1, Morioka 020-8505, Japan; e-mail: kuogasa@iwate-med.ac.jp

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tral nervous system.[11,12,14-22] Several studies have also investigated relative changes in NAA and/or choline following CEA by using proton MR spectroscopy.[23-26] However, the clinical significance of such postoperative changes remains unknown.

Correlations between MR spectroscopic measures and neuropsychological function have been reported in patients with Alzheimer disease, mild cognitive impairment, idiopathic normal pressure hydrocephalus, and cerebral infarction.[14,17-22] Thus, the purpose of the present prospective study was to determine whether a postoperative increase or decrease in cerebral metabolites measured by using proton MR spectroscopy was associated with improvement or impairment in cognitive function after CEA.

MATERIALS AND METHODS

Inclusion and Exclusion Criteria of Patients

We prospectively selected patients scheduled to undergo CEA and satisfying the following inclusion criteria: 75 years of age or younger; having ipsilateral cervical internal carotid artery stenosis (≥70%) on angiography with MR imaging, CT, or arterial catheterization according to the method of the North American Symptomatic Carotid Endarterectomy Trial[1]; having preoperative useful residual function (modified Rankin Scale, 0 or 1); the presence of episodes of ipsilateral carotid territory ischemic symptoms that had occurred between 2 weeks and 6 months before presentation to our department (defined as symptomatic carotid stenosis) or the absence of episodes of ipsilateral carotid territory ischemic symptoms or the presence of episodes of ipsilateral carotid territory ischemic symptoms that had occurred >6 months before presentation to our department (defined as asymptomatic carotid stenosis)[2]; having no infarction in the cerebral cortical area perfused by ≥1 branch of the middle cerebral artery confirmed by MR imaging, including T2-weighted and fluid-attenuated inversion recovery sequences, that was performed 2 weeks before surgery; and obtaining written informed consent. Patients who satisfied the following criteria after surgery were excluded from the present study: new neurologic deficits lasting for 2 weeks after surgery; and additional ischemic lesions on MR imaging, including T2-weighted and FLAIR sequences, performed 2 weeks after surgery compared with preoperative MR imaging. A 1.5T whole-body imaging system (Signa MR/i; GE Healthcare, Milwaukee, Wisconsin) was used for evaluation of ischemic lesions before and after surgery.

All study protocols were reviewed and approved by the local institutional ethics committee.

MR Spectroscopy

A 3T scanner (Signa Excite HD; GE Healthcare) with a "birdcage" quadrature head coil was used for this study. First, all subjects underwent axial T2-weighted imaging. In the T2-weighted images for each subject, 1 section through the upper gray matter above the centrum semiovale was selected, and a single-voxel region of interest was manually and symmetrically placed in the bilateral cerebral hemispheres so that the proportion of the cortical gray matter and CSF occupying the region of interest was as high and as low as possible, respectively (Fig 1). Voxel size was $17 \times 50 \times 15\ mm^3$. Next, acquisition of proton MR spectroscopy

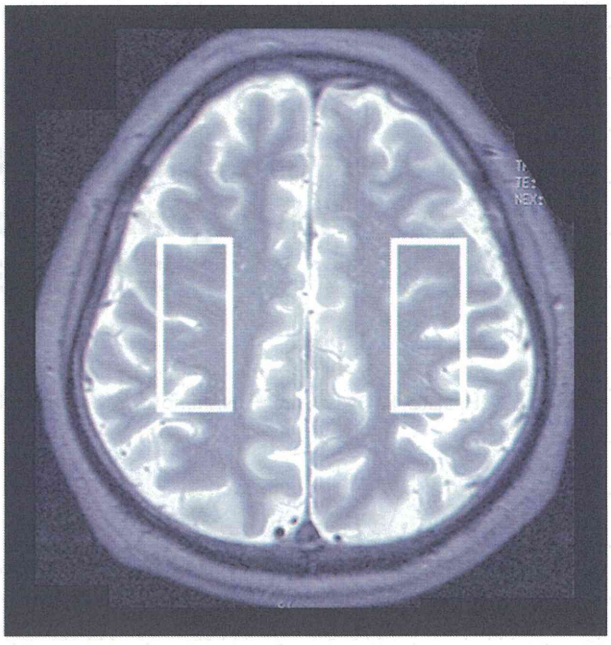

FIG 1. Regions of interest placed on 1 section through the upper gray matter above the centrum semiovale in an axial T2-weighted MR image to obtain MR spectroscopy.

was performed by using point-resolved spectroscopy with the following parameters: TR, 2000 ms; TE, 144 ms; data size, 4 K points; spectral width, 5000 Hz; 96 acquisitions (3.9 minutes). An area under each peak of 3 main metabolites was automatically obtained on the MR imaging console: choline-containing compounds at 3.2 ppm, total creatine at 3.0 ppm, and NAA at 2.0 ppm. Area ratios for NAA and Cho peaks were expressed as the relative ratio to Cr in each spectrum.

Patients underwent MR spectroscopy within 7 days before surgery and between 2 and 4 weeks after surgery. On each occasion, care was taken to place the region of interest in the same position on the T2-weighted image. NAA/Cr and Cho/Cr ratios in each cerebral hemisphere were calculated before and after surgery, and the difference between these 2 values (postoperative values minus preoperative values) was calculated and defined as ΔNAA/Cr and ΔCho/Cr ratios, respectively.

Neuropsychological Evaluation

For each patient, a battery of neuropsychological tests was administered, consisting of the Japanese translation of the Wechsler Adult Intelligence Scale Revised,[27] the Japanese translation of the Wechsler Memory Scale,[28] and the Rey test.[29] WAIS-R generates a verbal and performance intelligence quotient. The Rey test evaluates copy and recall of a complex figure. Thus, 5 scores (WAIS-R verbal IQ, WAIS-R performance IQ, Wechsler Memory Scale, Rey copy, and Rey recall) were used to evaluate cognitive function.

The neuropsychological tests were performed within 7 days before surgery and were repeated 1 month after surgery. All examinations were administered by a trained neuropsychologist who was blinded to the patient clinical information.

Postoperative cognition was categorized as improved, unchanged, or impaired for each patient on the basis of the definition described previously.[9] Briefly, 40 healthy volunteers served as

controls and underwent the same neuropsychological tests on 2 separate occasions (intertest interval, 1–2 months).[9] Differences in each neuropsychological test score between the 2 tests (the second test score–the first test score) were calculated. For the neuropsychological test scores of patients undergoing CEA, a significant increment was defined as a postoperative test score greater than the preoperative score plus the value of the mean minus 2SDs of the difference between the 2 test scores in the controls; a significant decrement was defined as a postoperative test score less than the preoperative score minus the absolute value of the mean: 2SDs of the difference between the 2 test scores in the controls. A patient was defined as having postoperative cognitive improvement or impairment when there was a significant increment or decrement in ≥1 postoperative neuropsychological score, respectively; a patient was defined as having an unchanged cognition after surgery when there was no significant increment or decrement in any postoperative neuropsychological scores.[9]

Intraoperative Management

All patients underwent surgery under general anesthesia. An intraluminal shunt during ICA clamping was not used in any of the patients. The mean duration of ICA clamping was 37 minutes (range, 26–49 minutes).

Statistical Analysis

Data are expressed as the mean ± SD. Changes between the pre- and postoperative NAA/Cr or Cho/Cr ratio were evaluated by using the Wilcoxon signed rank test. Differences in the change of each neuropsychological test score among the controls and patients were evaluated by using the Mann-Whitney *U* test. Differences or incidences of each baseline characteristic among the 3 groups (patients with postoperatively improved, unchanged, or impaired cognition) were evaluated by using the χ^2 test followed by the Bonferroni inequality correction or the Scheffe F test. A multivariate statistical analysis of factors related to postoperatively improved or impaired cognition relative to postoperatively unchanged cognition was also performed by using a logistic regression model with odds ratios with 95% CIs calculated. Variables with $P < .2$ in the univariate analyses were selected for analysis in the final model. For all statistical analyses, significance was set at the $P < .05$ level. The only exception was the χ^2 test followed by the Bonferroni inequality correction, in which differences were deemed statistically significance if $P < .05/3 = 0.0167$.

RESULTS

During 36 months, 136 patients underwent CEA. Of these, 104 patients preoperatively satisfied the inclusion criteria. However, 2 patients experienced new major neurologic deficits that lasted 2 weeks after surgery; another 2 patients developed additional asymptomatic ischemic lesions on MR imaging, including T2-weighted and FLAIR sequences performed 1 month after surgery, compared with preoperative MR imaging. These 4 patients did not undergo MR spectroscopy and neuropsychological testing after surgery and were excluded. Thus, the remaining 100 patients were analyzed in the present study. None of these patients experienced further ischemic symptoms between initial evaluation and surgical intervention.

Table 1: NAA/Cr and Cho/Cr ratios before and after surgery[a]

	Before Surgery	After Surgery	*P* Value
NAA/Cr ratio			
Ipsilateral hemisphere	1.654 ± 0.195	1.660 ± 0.228	.2819
Contralateral hemisphere	1.720 ± 0.177	1.718 ± 0.190	.9694
Cho/Cr ratio			
Ipsilateral hemisphere	0.895 ± 0.109	0.888 ± 0.104	.3132
Contralateral hemisphere	0.868 ± 0.093	0.867 ± 0.095	.8760

[a] Values are expressed as means.

The mean age of the 100 patients (89 men, 11 women) studied was 68 ± 6 years (range, 52–75 years). Concomitant disease states and symptoms were recorded, including 88 patients with hypertension, 37 patients with diabetes mellitus, and 53 patients with dyslipidemia. Sixty-four patients had ipsilateral symptomatic ICA stenosis, and the remaining 36 patients had asymptomatic ICA stenosis. Preoperative MR imaging demonstrated infarction in the hemisphere ipsilateral to the ICA stenosis in 54 patients and no infarction in 46 patients. The overall average degree of ICA stenosis was 87.3 ± 8.8% with a range of 70%–99% according to the method of the North American Symptomatic Carotid Endarterectomy Trial.[1] The contralateral ICA was occluded in 6 patients, and 8 additional patients had 60%–99% stenosis.

The mean ± SD of NAA/Cr and Cho/Cr ratios in each cerebral hemisphere before and after CEA among 100 patients is summarized in Table 1. When we analyzed them as a group, none of these values differed between measurements before and after surgery.

Figure 2 shows the distribution of ΔNAA/Cr and ΔCho/Cr ratios in each patient in each cerebral hemisphere. The ΔNAA/Cr ratio ranged from −0.370 to 0.402 or from −0.190 to 0.303 in the cerebral hemisphere ipsilateral or contralateral to the operative site, respectively. The ΔCho/Cr ratio ranged from −0.170 to 0.204 or from −0.130 to 0.140 in the cerebral hemisphere ipsilateral or contralateral to the operative site, respectively.

On the basis of the neuropsychological assessments performed before and after surgery, 10 (10%), 80 (80%), and 10 (10%) patients were regarded as having postoperatively improved, unchanged, and impaired cognition, respectively. Table 2 shows differences in each neuropsychological test score between the 2 tests (the second test score–the first test score) in controls and patients. While none of these discrepancies differed between the controls and all patients or between controls and patients without a postoperative change in cognition, all the differences were higher in patients with postoperatively improved cognition and lower in those with postoperatively impaired cognition compared with the controls.

Table 3 shows the univariate analysis of patient characteristics among the 3 groups. The ΔNAA/Cr and ΔCho/Cr ratios in the cerebral hemisphere ipsilateral to the operative site statistically differentiated patients with postoperatively improved, unchanged, and impaired cognition. Specifically, ΔNAA/Cr and ΔCho/Cr ratios were higher in patients with postoperatively improved cognition and lower in those with postoperatively impaired cognition compared with patients without a postoperative change in cognition (Fig 2*A*). In addition, the ΔNAA/Cr ratio in the contralateral cerebral hemisphere was higher in patients with postoperatively improved cognition than in those with postoper-

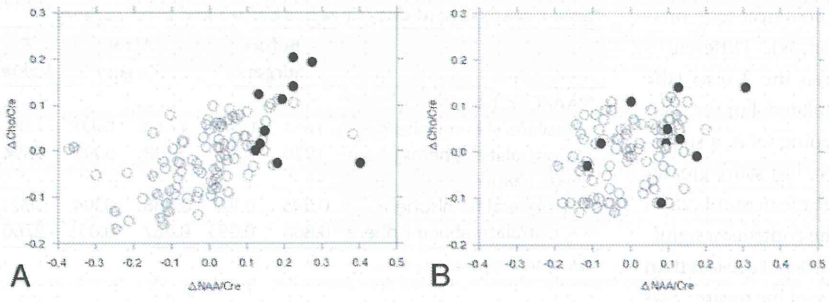

FIG 2. Distribution of ΔNAA/Cr and ΔCho/Cr ratios in each patient in the cerebral hemisphere ipsilateral (*A*) or contralateral (*B*) to the operative site. Closed, open, and half-tone circles indicate patients with postoperatively improved, unchanged, and impaired cognition, respectively.

atively unchanged or impaired cognition (Fig 2*B*). Other variables did not differ when comparing patients with postoperatively improved, unchanged, or impaired cognition. After we eliminated closely related variables in the univariate analyses, the following confounders ($P < .2$) were adopted in the logistic regression model for the multivariate analysis: age; ΔNAA/Cr ratio in each cerebral hemisphere; ΔCho/Cr ratio in the ipsilateral cerebral hemisphere for postoperatively improved cognition relative to postoperatively unchanged cognition; and ΔNAA/Cr and ΔCho/Cr ratios in the ipsilateral cerebral hemisphere for postoperatively impaired cognition relative to postoperatively unchanged cognition. Subsequent multivariate analysis revealed that positive and high ΔNAA/Cr ratios in the ipsilateral cerebral hemisphere significantly correlated with postoperatively improved cognition (95% CI, 13.3–21.3; $P = .0016$) and that negative and high absolute values of the ΔNAA/Cr ratio (95% CI, 0.018–0.101; $P = .0039$) and the ΔCho/Cr ratio (95% CI, 0.042–0.135; $P = .0046$) in the ipsilateral cerebral hemisphere significantly correlated with postoperatively impaired cognition.

Figures 3 and 4 show proton MR spectra in each patient with postoperative improvement or impairment in cognitive function, respectively.

DISCUSSION

The present study demonstrated that postoperative changes in cerebral metabolites measured by using proton MR spectroscopy were associated with changes in cognitive function following CEA.

Several studies have investigated changes in NAA/Cr and/or Cho/Cr ratios following CEA by using proton MR spectroscopy. While most of these studies reported increases in these values after CEA,[23-26] the remainder found no changes.[30] The variation in results between these studies may be from differences in methodologic factors, including the number of patients, types of patient, types of MR scanners, and the timing of postoperative assessment. In particular, previous studies have been performed by using devices operating at 1.5T and included a relatively smaller sample size (≤20 subjects).[23-26,30] The main advantages of proton MR spectroscopy at 3T over that at 1.5T include a higher signal-to-noise ratio, higher spatial and temporal resolutions, and better spectral resolution.[11] These advantages allow acquisitions of higher quality and result in a higher sensitivity for the detection of nervous system metabolites.[11] In the present study using a 3T imager and a large sample size, NAA/Cr and Cho/Cr ratios did not differ between measurements before and after CEA when they were analyzed as a group. Thus, NAA/Cr and Cho/Cr ratios usually do not change after CEA.

While numerous studies investigated changes in cognitive functioning following CEA by using neuropsychological testing,[3-8] there were no clear guidelines for determining significant improvement or impairment in cognition. This is because such changes may, in part, reflect the "practice effect" (an improvement in scores when patients are repeatedly tested).[3,7] In contrast, physicians or patients' families or both often report subjective postoperative improvement or impairment in cognition for patients undergoing CEA.[9] A recent study demonstrated that the optimal cutoff point of the degree of postoperative increase or decrease in neuropsychological test scores, such as the WAIS-R verbal IQ, WAIS-R performance IQ, Wechsler Memory Scale, Rey copy, and Rey recall, in detecting subjective improvement or impairment in cognition after surgery is identical to the mean + 2SDs or the mean − 2SDs, respectively, of the control value obtained from healthy subjects.[9] Furthermore, of patients with a postoperative increase in test scores more than the upper cutoff point or of those with a postoperative decrease in test scores less than the lower cutoff point in ≥1 neuropsychological test, 90% of patients in each group exhibited subjectively improved or impaired cognition, respectively, after surgery.[9] All patients with postoperative increases or decreases in test scores between the upper and lower cutoff points in all neuropsychological tests exhibited subjectively unchanged cognition after surgery.[9] Thus, in the present study, we determined significant postoperative improvement and impairment in cognition for each patient by using the same definition. As a result, 10% of patients who underwent CEA were defined as having postoperative improvement in cognition, and another 10% of patients who underwent CEA were defined as having postoperative impairment in cognition. These incidences were consistent with those from previous studies.[9]

The present study demonstrated that a postoperative increase and decrease in NAA/Cr ratios in the cerebral hemisphere ipsilateral to operative site were significantly associated with postoperative improvement and impairment in cognition, respectively. NAA is an amino acid found almost exclusively in neuronal cells, and the level of NAA in the brain may be an index of neuronal viability.[12] The NAA/Cr ratio also correlates with cerebral oxygen metabolism of the gray matter in patients with steno-occlusive carotid artery disease.[16] In addition, a decrease in the NAA/Cr ratio is associated with a decrease in cognitive function in elderly populations,[18,21] patients with cerebral infarction,[17,22] and those with Alzheimer disease.[20] Thus, the present data suggested that neuronal damage caused by CEA results in postoperative cognitive impairment. In contrast, in patients with idiopathic normal pressure hydrocephalus, a significant increase in the NAA/Cr ra-

Table 2: Differences in each neuropsychological test score between the 2 tests (the second test score–the first test score) in controls and patients[a]

Test	Controls[9] (n = 40)	All Patients (n = 100)	Subgroup A) Improved Cognition (n = 10)	Subgroup B) Unchanged Cognition (n = 80)	Subgroup C) Impaired Cognition (n = 10)	*P* Value Controls vs All Patients	*P* Value Controls vs A	*P* Value Controls vs B	*P* Value Controls vs C
WAIS-R verbal IQ	3.4 ± 4.5	4.0 ± 6.2	11.4 ± 4.6	4.3 ± 5.1	−5.1 ± 4.0	.2377	<.0001	0.1117	<.0001
WAIS-R performance IQ	4.9 ± 5.0	4.7 ± 7.0	11.9 ± 4.3	5.2 ± 5.7	−6.7 ± 5.1	.5791	.0001	0.3582	<.0001
WMS	4.7 ± 6.1	3.7 ± 7.2	11.9 ± 4.8	4.3 ± 5.1	−9.4 ± 7.7	.4560	.0002	0.2678	<.0001
Rey copy	0.4 ± 1.1	0.8 ± 1.8	4.1 ± 1.4	0.7 ± 1.2	−1.9 ± 1.7	.0599	<.0001	0.0845	<.0001
Rey recall	2.9 ± 3.5	2.6 ± 4.3	7.5 ± 3.3	2.7 ± 3.6	−3.6 ± 2.8	.9796	.0007	0.7578	<.0001

Note:—WMS indicates Wechsler Memory Scale.
[a] Values are expressed as means.

Table 3: Comparison of characteristics among patients with postoperatively improved, unchanged, or impaired cognition

	Group A) Improved Cognition (n = 10)	Group B) Unchanged Cognition (n = 80)	Group C) Impaired Cognition (n = 10)	*P* Value A vs B	*P* Value B vs C	*P* Value C vs A
Age (yr) (mean)	64.9 ± 3.5	68.9 ± 6.2	67.9 ± 6.0	.1378	.8751	.5357
Male sex	90% (9/10)	89% (71/80)	90% (9/10)	>.9999	>.9999	>.9999
Hypertension	100% (10/10)	88% (70/80)	80% (8/10)	.5946	.6175	.4737
Diabetes mellitus	50% (5/10)	36% (29/80)	30% (3/10)	.4942	>.9999	.6499
Dyslipidemia	70% (7/10)	51% (41/80)	50% (5/10)	.3268	>.9999	.6499
Symptomatic lesion	60% (6/10)	63% (50/80)	80% (8/10)	>.9999	.4908	.6285
Infarction on preoperative MRI	40% (4/10)	55% (44/80)	60% (6/10)	.5053	>.9999	.6563
Degree of ICA stenosis (%) (mean)	88.0 ± 10.6	87.7 ± 8.2	83.0 ± 10.3	.9942	.2739	.4348
Bilateral lesions	20% (2/10)	13% (10/80)	20% (2/10)	.6175	.6175	>.9999
Duration of ICA clamping (min) (mean)	37.0 ± 5.0	37.2 ± 5.8	36.9 ± 6.2	.9940	.9870	.9992
ΔNAA/Cr ratio in ipsilateral hemisphere (mean)	0.201 ± 0.085	0.011 ± 0.112	−0.171 ± 0.085	<.0001	<.0001	<.0001
ΔNAA/Cr ratio in contralateral hemisphere (mean)	0.081 ± 0.122	−0.001 ± 0.092	−0.044 ± 0.098	.0428	.4108	.0169
ΔCho/Cr ratio in ipsilateral hemisphere (mean)	0.077 ± 0.088	−0.005 ± 0.063	−0.108 ± 0.053	.0013	<.0001	<.0001
ΔCho/Cr ratio in contralateral hemisphere (mean)	0.036 ± 0.079	−0.003 ± 0.070	−0.011 ± 0.082	.2786	.9430	.3465

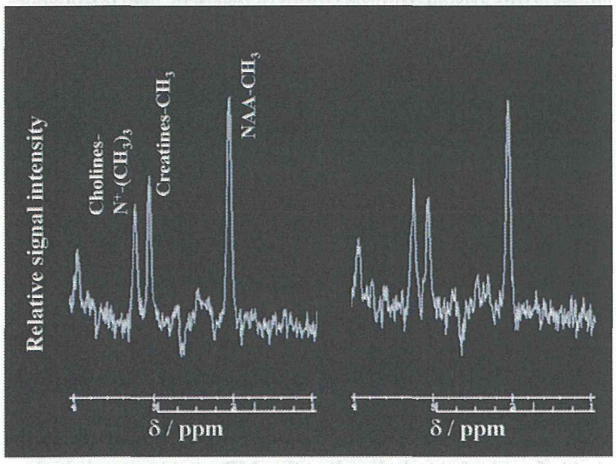

FIG 3. Proton MR spectra obtained by using point-resolved spectroscopy in the region of interest in the cerebral hemisphere ipsilateral to the operative site in a 62-year-old man with improved cognition after endarterectomy for symptomatic right internal carotid artery stenosis. Area for the NAA or choline peak to the area for the creatine peak is relatively increased after surgery (NAA/creatine, 1.83; choline/creatine, 1.06) (right graph) compared with the preoperative spectrum (NAA/creatine, 1.56; choline/creatine, 0.86) (left graph).

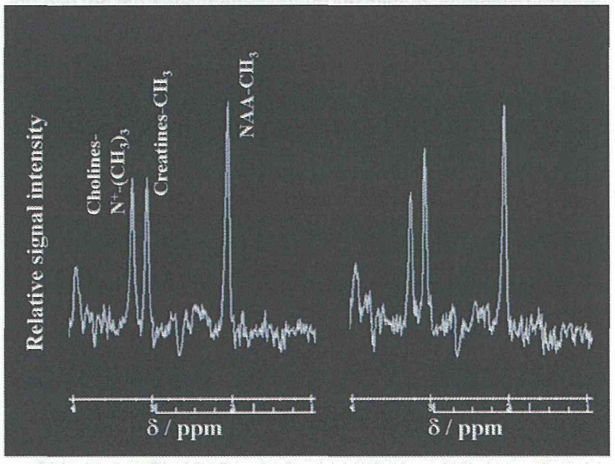

FIG 4. Proton MR spectra obtained by using point-resolved spectroscopy in the region of interest in the cerebral hemisphere ipsilateral to the operative site in a 72-year-old man with impaired cognition after endarterectomy for symptomatic left internal carotid artery stenosis. The area for the NAA or choline peak to the area for the creatine peak is relatively reduced after surgery (NAA/creatine, 1.33; choline/creatine, 0.83) (right graph) compared with the preoperative spectrum (NAA/creatine, 1.57; choline/creatine, 0.99) (left graph).

tio following shunting is related to postoperative cognitive improvement,[19] which is consistent with observations from the present study. Therefore, a reduction in NAA may be reversible, and the level of NAA can recover with cognitive improvement, probably resulting from restoration of brain perfusion following surgery.

A postoperative decrease in Cho/Cr ratios in the ipsilateral cerebral hemisphere was significantly associated with postoperative impairment in cognition. The level of choline in the brain may be associated with membrane synthesis or degeneration in neural tissue.[13] Elevation of the choline signal has been demonstrated in patients with multiple sclerosis,[15] in those with severe vasospasm after subarachnoid hemorrhage,[31] and in patients with acute ischemic stroke.[32] In such pathologic conditions, damage to neural tissue may be ongoing, resulting in an increase in myelin membrane degeneration products and leading to elevation of the choline signal. In contrast, the choline level detected on proton MR spectroscopy is decreased in the ischemic cores and in the surrounding tissue that otherwise appears normal on MR imaging performed from 5 to 30 days after the onset of massive cerebral infarction.[33] In this subacute stage of ischemic stroke, damage to the neural tissue is probably complete, and myelin membrane synthesis and degeneration may be reduced, resulting in a decrease in the choline signal. Cho/Cr ratios correlated positively with scores of neuropsychological testing in the elderly population[18,21] and in patients with preclinical Huntington disease.[34] In the latter, investigators have suggested that a decrease in Cho indicates a reduction of myelin membrane turnover that precedes neuronal death that may be responsible for the neuropsychological deficits.[34]

Thus, a postoperative decrease in the Cho/Cr ratio as well as a postoperative decrease in the NAA/Cr ratio may imply neuronal damage caused by CEA, thereby resulting in postoperative cognitive impairment. On the other hand, the univariate analyses showed that the ΔCho/Cr ratio in the cerebral hemisphere ipsilateral to the operative site was significantly higher in patients with postoperatively improved cognition than in those with postoperatively unchanged or impaired cognition, though the multivariate analysis did not demonstrate a significant difference. Considering the correlation between Cho/Cr ratios and cognitive functioning in the elderly population,[18,21] the postoperative increase in Cho may imply recovery of abnormally reduced myelin membrane turnover, resulting in cognitive improvement after surgery.

The relationship between increases or decreases in cerebral metabolite and cognitive changes following CEA remains poorly defined. A recent study suggested that normalization of cerebral metabolism via improvement in cerebral hemodynamics after CEA may result in cognitive improvement.[9] In contrast, cognitive impairment after CEA may result from various mechanisms.[9] First, to perform CEA, the ICA and common carotid arteries are cross-clamped. There is a transient decrease in perfusion in the ipsilateral middle cerebral artery territory in some patients. When the reduction in hemispheric perfusion is significant enough to damage neuronal tissues, it may cause postoperative impairment of cognitive function accompanied by a reduction in cerebral metabolism. Second, a large percentage of patients exhibit evidence of gaseous and particulate emboli in the middle cerebral artery during CEA. The particulate embolization during surgery may result in decreases in cerebral metabolism and neuropsychological deterioration. Third, cerebral hyperperfusion after CEA is defined as a major increase in ipsilateral cerebral blood flow after surgical repair of carotid stenosis that is well above the metabolic demands of the brain tissue. This phenomenon often manifests with unilateral headache, face and eye pain, seizure, and focal symptoms that occur secondary to cerebral edema or intracerebral hemorrhage. Post-CEA hyperperfusion, even when asymptomatic, also causes postoperative cortical neural damage that results in postoperative cognitive impairment.

The univariate analyses in the present study showed that the ΔNAA/Cr ratio in the cerebral hemisphere contralateral to the operative site was significantly higher in patients with postoperatively improved cognition than in those with postoperatively unchanged or impaired cognition. However, multivariate analysis did not demonstrate the significance of this relationship. In the former patients, improvement in cerebral hemodynamics in the contralateral cerebral hemisphere after CEA may result in postoperative increases in cerebral metabolism. When there is contralateral ICA stenosis or occlusion in addition to ipsilateral ICA stenosis, perfusion in the contralateral cerebral hemisphere is often reduced before surgery and may be increased via collateral circulation from the ipsilateral ICA after surgery. However, the incidence of the contralateral ICA stenosis or occlusion was not different among the 3 subgroups of patients with different cognitive changes after surgery. Another possible mechanism may be related to improvement in brain perfusion in the contralateral anterior cerebral artery territory via the anterior communicating artery from the ipsilateral ICA after surgery when the A1 portion of the contralateral anterior cerebral artery is hypoplastic. However, this possibility was not investigated in the present study.

The present study has several limitations that require discussion. First, area ratios for NAA or choline were expressed as a ratio relative to creatine. The absolute value of each area can be obtained from MR spectroscopy. However, the absolute value includes errors arising from variations of magnetic field homogeneity, and converting to the ratio reduces such errors.[35] Creatine concentration is relatively constant in each region of the brain, even in the context of metabolic disease or rapid fluctuation in energy metabolism.[17,24] Muñoz Maniega et al[33] demonstrated that while creatine concentration was significantly reduced in the ischemic cores, it did not change in surrounding tissue that otherwise appeared normal on MR imaging. Because the present study did not include patients with new postoperative ischemic lesions on MR imaging, postoperative changes of total creatine concentration may minimally affect our results. Second, the cerebral hemisphere ipsilateral to the ICA stenosis often exhibits brain atrophy even when massive cortical infarction is not detected on MR imaging in the cerebral hemisphere.[36] In that situation, the proportion of CSF occupying the region of interest for measurement of cerebral metabolites by using MR spectroscopy is higher, thereby reducing the accuracy of metabolic ratios measured by MR spectroscopy.[36] Finally, a single-voxel region of interest for measurement of metabolites was placed on the section through the centrum semiovale. The value in the single-voxel region of interest does not always reflect the metabolic condition in the whole cerebral hemisphere. Although a topographic map of cerebral metabolism can be obtained by using a multivoxel method, metabolic values acquired from a single voxel may provide more accurate quantification of cerebral metabolic ratios than those acquired from multiple voxels.[11]

CONCLUSIONS

The present study by using 3T proton MR spectroscopy in a relatively large number of patients demonstrated that postoperative changes in cerebral metabolites are associated with changes in cognitive function after CEA. Further investigations by using other modalities, such as oxygen 15 gas or [^{18}F] fluorodeoxyglucose positron-emission tomography, would be of benefit to confirm the relationship between changes in cerebral metabolism and change in cognitive function after CEA.

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ORIGINAL ARTICLE
Renal Disease

Comparison of Predictability of Future Cardiovascular Events Between Chronic Kidney Disease (CKD) Stage Based on CKD Epidemiology Collaboration Equation and That Based on Modification of Diet in Renal Disease Equation in the Japanese General Population

– Iwate KENCO Study –

Masaki Ohsawa, MD; Kozo Tanno, MD; Kazuyoshi Itai, PhD; Tanvir Chowdhury Turin, PhD; Tomonori Okamura, MD; Akira Ogawa, MD; Kuniaki Ogasawara, MD; Tomoaki Fujioka, MD; Toshiyuki Onoda, MD; Yuki Yoshida, MD; Shin-ichi Omama, MD; Yasuhiro Ishibashi, MD; Motoyuki Nakamura, MD; Shinji Makita, MD; Fumitaka Tanaka, MD; Toru Kuribayashi, PhD; Tomiko Koyama, PhD; Kiyomi Sakata, MD; Akira Okayama, MD

Background: Whether estimated glomerular filtration rate (eGFR) calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) Study equation ($eGFR_{CKDEPI}$) improves risk prediction compared to that calculated using the Modification of Diet in Renal Disease (MDRD) study equation ($eGFR_{MDRD}$) has not been examined in a prospective study in Japanese people.

Methods and Results: Participants (n=24,560) were divided into 4 stages (1, ≥90; 2, 60–89 (reference); 3a, 45–59; 3b+ <45 $ml \cdot min^{-1} \cdot 1.73 m^{-2}$) according to $eGFR_{CKDEPI}$ or $eGFR_{MDRD}$. Endpoints were all-cause death, myocardial infarction (MI) and stroke. Area under the receiver operating characteristic curves (95% confidence intervals) for predicting all-cause death, MI and stroke by $eGFR_{CKDEPI}$ vs. $eGFR_{MDRD}$ were 0.680 (0.662–0.697) vs. 0.582 (0.562–0.602); 0.718 (0.665–0.771) vs. 0.642 (0.581–0.703); and 0.656 (0.636–0.676) vs. 0.576 (0.553–0.599), respectively. Multivariate-adjusted Cox regression and Poisson regression analysis results were similar for adjusted incidence rates and adjusted hazard ratios in each corresponding stage between the 2 models and no differences were found in model assessment parameters. Net reclassification improvement (NRI) for predicting all-cause death, MI and stroke were estimated to be 6.7% (P<0.001), –1.89% (P=0.029) and –0.20% (P=0.421), respectively.

Conclusions: Better discrimination was achieved using $eGFR_{CKDEPI}$ than $eGFR_{MDRD}$ on univariate analysis. NRI analysis indicated that the use of $eGFR_{CKDEPI}$ instead of $eGFR_{MDRD}$ offered a significant improvement in reclassification of death risk. (*Circ J* 2013; **77:** 1315–1325)

Key Words: Chronic kidney disease; CKD-EPI equation; Estimated glomerular filtration rate; MDRD equation; Model assessment

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Department of Hygiene and Preventive Medicine, Iwate Medical University, Iwate (M.O., K.T., T. Onoda, K.S.); Department of Neurosurgery (A. Ogawa, K.O., Y.Y., S.O.), Department of Urology (T.F.), Department of Internal Medicine (Y.I., M.N., S.M., F.T.), Iwate Medical University, Morioka; Department of Health and Physical Education, Faculty of Education, Iwate University, Morioka (T. Kuribayashi); Iwate Health Service Association, Morioka (T. Koyama); The First Institute of Health Service, Japan Anti-Tuberculosis Association, Tokyo (K.I., A. Okayama); Department of Preventive Medicine and Public Health, Keio University, Tokyo (T. Okamura), Japan; and Department of Medicine, University of Calgary, Calgary, Alberta (T.C.T.), Canada

Mailing address: Masaki Ohsawa, MD, Department of Hygiene and Preventive Medicine, Iwate Medical University, 2-1-1 Nishitokuta, Yahaba-cho, Iwate 020-3694, Japan. E-mail: masakio@iwate-med.ac.jp

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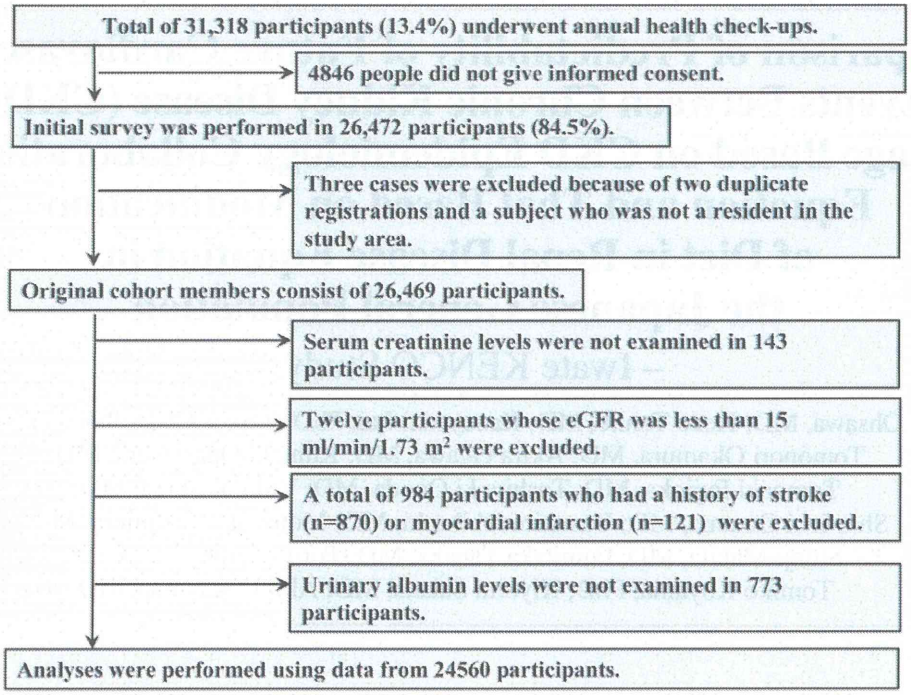

Figure 1. Flow chart of the procedure used to select participants for the Iwate KENCO study. The original cohort consisted of 26,469 participants. We excluded a total of 1,909 participants who lacked data for serum creatinine (n=143), 984 participants with a history of myocardial infarction or stroke (n=984), and 785 participants who lacked data used for analysis. eGFR, estimated glomerular filtration rate.

Chronic kidney disease (CKD) contributes not only to the risk for development of end-stage renal disease but also to the risk for cardiovascular morbidity and mortality.[1] CKD also increases risks for cardiovascular morbidity and mortality in Japanese people.[2–6] The National Kidney Foundation and the American Heart Association have proposed using CKD in cardiovascular risk stratification and treatment guidelines.[1,7] Defining and staging of kidney disease requires combining information on kidney damage, usually detected using albuminuria, and decreased renal function, usually based on glomerular filtration rate (GFR).[7]

The Modification of Diet in Renal Disease (MDRD) Study equation is the most widely used equation.[8] Recently, the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) developed a new equation.[9] The CKD-EPI Study equation was shown to be more accurate than the MDRD Study equation for the calculation of estimated GFR (eGFR).[9] Both the MDRD equation modified by a Japanese coefficient[10] and CKD-EPI equation modified by a Japanese coefficient[11] have recently been developed. The CKD-EPI equation modified by a Japanese coefficient was also shown to be more accurate than the MDRD equation modified by a Japanese coefficient using inulin clearance as a gold standard.[11]

The development of a more accurate equation for eGFR requires reclassification of CKD stage using the new equation instead of the old equation and confirmation of the concordance of CKD stage between the 2 models. Reclassification of CKD stage and correlations between the 2 equations were examined for US subjects.[9,12] Horio et al also examined proportions in each CKD stage separately for the 2 equations.[11]

The development of a more accurate equation for eGFR also requires reassessment of the predictability of CKD in prospective longitudinal studies using the new equation for eGFR. Comparisons of risk predictabilities have been widely used in the cardiovascular field, and new statistical methods have also been developed.[13–20] Recently, Matsushita et al used a new statistical method for comparing risk predictabilities between the 2 types of eGFR.[12] They showed that improved reclassification of CKD stage was observed using $eGFR_{CKDEPI}$ instead of $eGFR_{MDRD}$, with statistical significance in all 4 endpoints (death, myocardial infarction [MI], stroke and end-stage renal disease).

They noted, however, that considerable racial difference existed in correlations between the 2 types of eGFR and in risk predictabilities. They also noted that similar analyses should be performed for ethnicities other than white and African-American people.[12] It is necessary to compare correlations between the 2 types of eGFR and compare risk predictability for CKD stage between the 2 CKD stage models based on $eGFR_{CKDEPI}$ and $eGFR_{MDRD}$ in other ethnicities including Japanese people. Therefore, we compared correlations between the 2 types of eGFR and compared the risk predictability of models based on $eGFR_{CKDEPI}$ and on $eGFR_{MDRD}$ using traditional and newly developed techniques in statistics to examine model accuracy in univariate- and multivariate-adjusted analyses based on a prospective study in Japanese people.