Cerebral blood flow can be measured quantitatively or qualitatively using several methods, including single photon emission computed tomography (SPECT). However, since individual measurements of CBF alone do not adequately assess cerebral hemodynamic status, paired blood flow measurements are taken with resting values being established, followed by measurements after stimulation of cerebral vasodilation. The CBF response to acetazolamide (ACZ) challenge determined as cerebrovascular reserve (CVR) in SPECT, transcranial Doppler (TCD), and magnetic resonance angiography (MRA), is frequently used as an index of autoregulatory vasodilation in clinical settings to evaluate hemodynamic impairment. However, relationships between CVR measured by PET and other hemodynamic parameters must be clarified before CVR can be widely used as an index of cerebral hemodynamic impairment. The present study attempts to establish the CVR to ACZ as a reliable index of Stage I and Stage II hemodynamic compromise in patients with chronic occlusive cerebrovascular disease using both Gas-PET and H_2O-PET.

MATERIALS AND METHODS

Patients

We used simultaneous Gas-PET and ACZ challenge H_2O-PET to examine 25 consecutive patients with cerebrovascular disease at Osaka University Medical School Hospital. Immediately before the PET study, each patient underwent neurological and neuro-radiological evaluations, including an evaluation for occlusive cerebrovascular disease by Doppler ultrasonography, MRI, magnetic resonance angiography (MRA), and cerebral angiography. MRI was performed in the orbito-meatal plane with 5-mm-thick sections using a 1.5-T unit (1.5-T Sigma Horizon; GE Medical System; 1.5-T Magnetome Vision; Siemens). Infarction was defined as a focal area with prolonged T1 and T2 relaxation times. The maximal interval between the MRI and PET studies was 30 days. Patients underwent the PET study at least 4 weeks after their most recent clinical episode, when their neurological condition had stabilized. Cerebral angiography was performed in all patients. The maximum percentage stenosis and the presence of ulceration, which can cause artery-to-artery embolism, were evaluated according to the recommendations of the North American Symptomatic Carotid Endarterectomy Trial (NACET).[7] We studied severe stenosis (70%–99%) and cerebrovascular artery occlusion. And, the mechanism of stroke in each patient was clinically diagnosed and classified according to the National Institute of Neurological Disorders and Stroke (NINDS) Classification of cerebrovascular disease III.[8] Patients with embolic infarction (large infarction) who had cardioembolic risk factors such as atrial fibrillation, valvular heart disease, or myocardial infarction and with acute phase were excluded from this study. We finally studied 17 consecutive patients [8 men, 9 women; mean age 56.5 ± 16.7 yr (mean ± SD)]. Five patients had cerebral infarction (lacuna infarction and/or minimal infarction in the territory of the affected arteries), eight had transient ischemic attacks, and four had asymptomatic carotid artery disease. Table 1 summarizes the clinical features and the angiographic and MRI findings.

Table 1 Patient characteristics

Patient No.	Age/Sex	Neurological Deficits	Disease	Angiographic Findings	MRI Findings
1	31/F	Rt hemiparesis	TIA	Bil ICAO	Rt BG*
2	71/M	Lt hemiparesis	TIA	Rt ICAO	Bil BG*
3	59/M	Headache	TIA	Rt MCAS	Bil BG & CR*
4	66/M	Rt hemiparesis & Dysarthria	CI	Lt MCAO	Lt MCA territory**
5	29/F	Lt hemiparesis & Visual field defect	CI	Bil ICAO	Rt parieto-occipital lobe**
6	62/M	Rt sensory disturbance	CI	Lt ICAS	Bil BG*
7	69/F	Lt hemiparesis	TIA	Rt MCAO	None
8	19/M	None	Asymptomatic	Bil MCAO	Lt parietal lobe**
9	39/F	Lt hemiparesis & Dysarthria	TIA	Rt MCAO	None
10	67/M	Dizziness	Asymptomatic	Lt ICAO	Lt BG*
11	74/M	Lt ischemic retinopathy	Asymptomatic	Lt ICAO	None
12	72/M	None	Asymptomatic	Lt ICAS	Lt CR*
13	63/F	None	TIA	Lt ICAS	None
14	56/F	Lt hemiparesis	TIA	Rt MCAO	Rt MCA territory**
15	59/F	Dysarthria	CI	Bil ICAO	Lt CR*
16	69/F	Lt sensory disturbance	CI	Rt MCAO	Rt CR*
17	56/F	Lt hemiparesis	TIA	Rt ICAS	None

Rt, right; Lt, left; Bil, bilateral; TIA, transient ischemic attack; CI, cerebral infarction; Asymptomatic; asymptomatic carotid artery disease; MCA, middle cerebral artery; MCAS, middle cerebral artery stenosis; MCAO, middle cerebral artery occlusion; ICAS, internal carotid artery stenosis; ICAO, internal carotid artery occlusion; BG, basal ganglia; CR, corona radiata: *lacuna infarction; **small infarction (<15 mm)

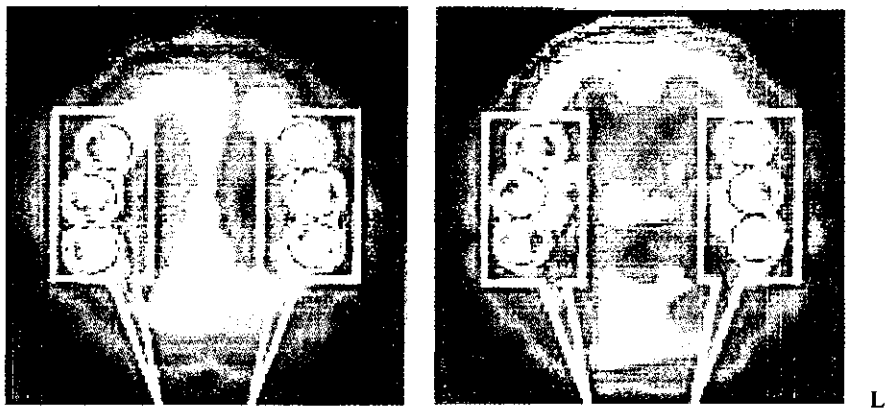

Fig. 1 Comparison of CVR and gas-PET parameters. A comparison of CVR and gas-PET parameters in 68 ROI in all MCA territories revealed significant negative correlations between CVR and OEF ($r = -0.559$; $p < 0.0001$) and between CVR and CBV/CBF ($r = -0.331$; $p < 0.0095$). Values of CBF and $CMRO_2$ were not correlated with CVR.

PET Imaging

All patients were scanned with a Headtome V/SET 2400W system (Shimadzu Co., Ltd., Kyoto, Japan), which acquires 63 slices with an interslice distance of 3.1 mm. All scans were performed at a resolution of 3.7 mm FWHM in the transaxial direction and at 5 mm in the axial direction. The patient's head was immobilized in a holder and positioned using light beams to obtain transaxial slices parallel to the orbito-meatal line. Before the PET study, we performed germanium 68-gallium 68 transmission scanning over 10 minutes for attenuation correction. Images were reconstructed using an ordered-subset expectation maximization (OS-EM) algorithm (12 iterations with 4 ordered subsets). The [^{15}O]gas steady state method required the patients to inhale a mixture of $C^{15}O_2$ (550 MBq/min) and $^{15}O_2$ (1,300 MBq/min) through a mask. The scan time was nine minutes, and four samples of blood were manually obtained from the radial artery during each scan. We measured the concentration of the radiotracer activity in whole blood and plasma using a well counter, as well as the arterial blood hematocrit, hemoglobin concentration, PaO_2 and $PaCO_2$ values. The CBV was measured after inhalation of 2,000 MBq of $C^{15}O$ and a 9-minute scanning period. We manually sampled arterial blood three times during the scan and measured radiotracer activity in whole blood. The values for CBF, $CMRO_2$, and OEF were calculated based on the steady state method, and $CMRO_2$ and OEF were corrected according to the CBV.

After the Gas-PET examination, patients received a 36-sec intravenous bolus of 1,110 MBq $H_2{}^{15}O$ at a flow rate of 30 ml/min through a cannula placed in the antecubital vein to initiate ACZ challenge [^{15}O]H_2O PET. Data were acquired over a scanning period of 160 s using a 128 × 128 matrix. Regional CBF was determined from the $H_2{}^{15}O$ bolus injection and autoradiographic methods while the participants were in a resting state and 10 minutes after the injection of ACZ. Input function was evaluated for 4 minutes at a rate of 5 ml/min by continuous arterial blood sampling via a catheter needle inserted in the radial artery, and ^{15}O radioactivity was concurrently measured using a beta-detector (Shimadzu Corp., Kyoto, Japan). The study protocol complied with the standard ethical guidelines of Osaka University Medical School, and written informed consent was obtained from all participants.

Data Analysis

All PET data were analyzed using the "Dr. View pro5.0" image analysis software system (Asahi Kasei Joho System Co., Ltd., Tokyo, Japan) running on a UNIX system and an Indigo 2 station (Silicon Graphics, Mountain View, CA, USA). Circular regions-of-interest (ROI), 20 mm in diameter, were placed over the cortex at the levels of the basal ganglia (lower MCA territory) and parietal lobe (upper MCA territory) on PET images from each patient (Fig. 1). All ROI generated on the resting CBF and ACZ-challenge CBF images measured using H_2O-PET were transferred to the CBF, OEF, $CMRO_2$, and CBV images measured using Gas-PET. All ROI on PET images can be made to exactly coincide using this system. Sixty-eight regions were finally investigated in images from 17 patients (four regions per patient: right, left, upper and lower MCA). The following equation estimated the percentage increase in rCBF induced by the ACZ challenge in the form of the CVR: CVR = (ACZ challenge CBF − Resting CBF)/Resting CBF.

Seven age-matched normal volunteers with non-focal neurological symptoms and without evidence of ischemic lesions after MRI or stenotic lesions in the major cerebral arteries after MRA, underwent H_2O-PET to determine

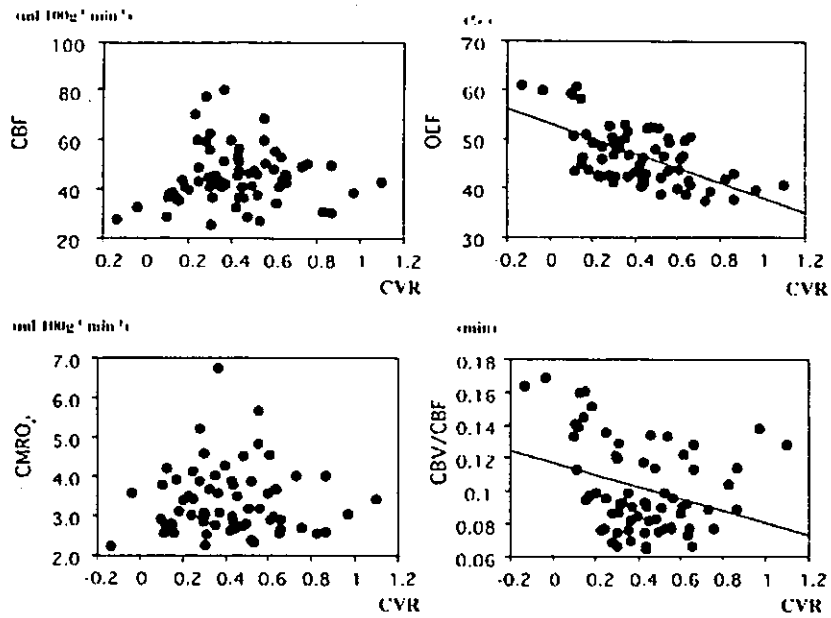

Fig. 2 ROI setting. Circular regions-of-interest (ROIs), 20 mm in diameter, were placed on cerebral cortices of middle cerebral artery territories on the levels of basal ganglia and parietal lobe in PET images of each patient.

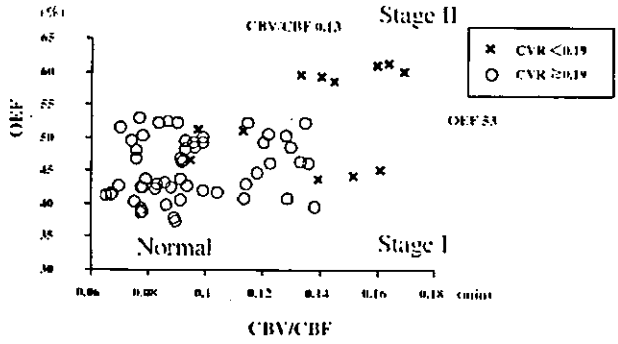

Fig. 3 CVR measured by ACZ challenge H_2O-PET in normal, Stage I and Stage II regions. Plot of values of OEF versus CBV/CBF ratio for all 68 ROI with complete quantitative PET studies. Abnormal values (mean + 2SD) for OEF and CBV/CBF ratio are indicated by dotted lines. All six regions diagnosed as Stage II by Gas-PET (*upper right quadrant*) corresponded perfectly with reduced CVR (CVR < 0.19). CVR values were decreased in three of 54 regions diagnosed as normal (*lower left quadrant*) and in three of eight regions diagnosed as Stage I (*lower right quadrant*).

normal control values. Normal Gas-PET values from healthy controls were not available. We therefore utilized Gas-PET parameter values from seven patients with no infarction and no severe stenosis or occlusion (<50%) who had non-specific brain symptoms without focal signs (pre-operation for cerebral aneurysm, headache, dizziness and syncope) as the normal values. We assessed the relationship between the CVR measured by H_2O PET and the Gas-PET parameters in the MCA territories using linear-regression analysis and Pearson's correlation coefficient. All data are expressed as means ± SD. Differences in data between groups were statistically evaluated using an unpaired t-test. Differences with p values of <0.05 were considered statistically significant.

RESULTS

The normal control values for the CBF and CVR measured by H_2O PET for the MCA territories were 45.5 ± 8.5 m*l* 100 g^{-1} min^{-1} and 0.49 ± 0.15 (mean ± SD). The CVR values were judged abnormal when beyond the mean − 2SD of the normal controls. All 68 regions were classified into two groups according to the CVR values measured by H_2O PET: reduced group (N = 12) with a CVR value of <0.19 (mean − 2SD) and a normal group (N = 56) with a CVR of 0.19 or higher. We used normal Gas-PET parameter values as follows: CBF, 46.9 ± 11.3 m*l* 100 g^{-1} min^{-1}; OEF, 44.1 ± 4.62%; $CMRO_2$, 3.39 ± 0.82 m*l* 100 g^{-1} min^{-1}; CBV, 4.22 ± 0.75 m*l* 100 g^{-1}; and CBV/CBF ratio, 0.09 ± 0.02 min. All regions were classified into three groups based on their OEF values: normal, OEF < 48.7% (mean + 1SD of the mean OEF value); slightly increased, 48.7% ≤ OEF < 53.3% (mean + 2SD of the mean OEF value) and increased, OEF ≥ 53.3%. We classified 68 regions into three groups compared with the base of our normal ranges of CBV/CBF and OEF. The three groups were normal [CBV/CBF < 0.13 min (mean + 2SD of the mean CBV/CBF ratio); OEF < 53.3%; N = 54], Stage I [CBV/CBF ≥ 0.13 min and OEF < 53.3%; N

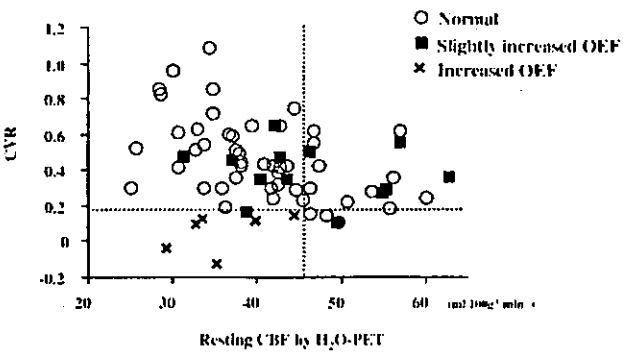

Fig. 4 Detection of Stage II cerebral hemodynamic failure using ACZ challenge H_2O-PET. Plot of CVR versus resting CBF values for all 68 ROI with quantitative ACZ challenge H_2O-PET studies. Cut-off values for CVR and CBF of 0.19 (mean – 2SD) and 46.9 m*l* 100 g^{-1} min^{-1} (normal value) respectively (*lower left quadrant*), led to sensitivity and specificity values of 86% (6/7) and 98% (61/62), respectively, for detecting misery perfusion (OEF ≥ 53.3%).

= 8] and Stage II [OEF ≥ 53.3%; N = 6].

Comparison of CVR with other PET parameters

We compared the CVR and PET parameters in the 68 ROI of all MCA territories and found a significant negative correlation between CVR and OEF ($r = -0.559$; $p < 0.0001$; Fig. 2) and between CVR and CBV/CBF ($r = -0.331$; $p < 0.0095$; Fig. 2). The CBF and $CMRO_2$ did not correlate with the CVR (Fig. 2).

CVR in normal, Stage I and Stage II regions

The CVR was reduced in all six regions diagnosed as Stage II by Gas-PET (Fig. 3). However, CVR values were decreased in three of the 54 regions diagnosed as normal and in 3 of the 8 regions diagnosed Stage I (Fig. 3). Therefore, CVR was actually variable in the Stage I and normal groups.

Detection of Stage II cerebral hemodynamic failure using resting CBF and CVR

The three groups classified based on their OEF values were plotted according to CBF at rest and the CVR values obtained from the ACZ challenge H_2O-PET study (Fig. 4). A CVR cut-off value of 0.19 (mean – 2SD) combined with a CBF cut-off value of 46.9 m*l* 100 g^{-1} min^{-1} (normal value) resulted in sensitivity and specificity for detecting misery perfusion of 86% (6/7) and 98% (61/62), respectively (OEF ≥ 53.3%). Therefore, the combination of the CVR and CBF appears to be a reliable index with which to accurately detect Stage II hemodynamic compromise in occlusive carotid diseases.

DISCUSSION

The present study revealed a significant negative correlation between the CVR and the OEF and between the CVR and the CBV/CBF ratio in the MCA territories of patients with occlusive cerebrovascular disease. The CVR value of all regions with an elevated OEF was below 0.19. Additionally, the combination of CVR and CBF was highly sensitive and specific for detecting misery perfusion (OEF ≥ 53.3%). However, when the CBV/CBF ratio was elevated and OEF was normal (Stage I), the CVR was highly variable. These results support the notion that [^{123}I]*N*-isopropyl-*p*-iodoamphetamine SPECT (^{123}I-IMP SPECT) with ACZ challenge is clinically useful.[9,10]

Gibbs et al. applied the CBF/CBV ratio instead of vasodilative capacity as an indicator of autoregulatory vasodilation in brain tissue with decreased cerebral perfusion pressure.[11] The CBF/CBV ratio (or its inverse, the CBV/CBF ratio) is mathematically equivalent to the vascular mean transit time (MTT)[5] and could be more accurate than CBV alone as an index of cerebral perfusion pressure.[12] Although correlated with the CBV/CBF ratio, the CVR value was quite variable in the Stage I and normal groups. Some investigators have reported that CBV increases within the autoregulatory range,[13–15] while others have found minimal or no increases in CBV until autoregulatory capacity is exceeded.[12,16] CBV may be a complex physiological parameter, since it is composed of arterial, capillary and venous compartments.[17]

Based upon Powers' previous theory,[4,5] we originally surmised that CVR to ACZ would gradually decrease in the Stage I and would fall below zero in Stage II. However, the correlation between CVR and OEF in the present study was negative, suggesting that OEF may increase even at the stage when CVR in response to ACZ is still maintained. The correlation between CVR and OEF ($r = -0.559$) was closer than that between CVR and CBV/CBF ($r = -0.331$), suggesting that CVR can be an index reflecting both reduced perfusion pressure and increased OEF. In the modified model of hemodynamic and metabolic responses to a reduction in cerebral perfusion pressure after Powers et al.,[4] OEF can increase with reductions in CBF through autoregulatory ranges while CBV may not change.[18,19] The risk of ischemic stroke with increased OEF and increased CBV being higher than that with increased OEF and normal CBV suggests that OEF together with CBV can reflect hemodynamic compromise more precisely than OEF alone.[19] Recent studies have demonstrated the usefulness of the CVR to ACZ for predicting ischemic stroke.[20] Therefore, the CVR to ACZ challenge in H_2O-PET combined with OEF in Gas-PET may be a better index with which to predict future stroke than OEF alone since more information about cerebral perfusion pressure is added.

In conclusion, the CVR to ACZ challenge could be influenced by both autoregulatory capacity and OEF.

Whether the combination of CVR with OEF may indeed add more predictive information about future ischemic stroke should be examined in a prospective study. The present results supported the notion that SPECT with ACZ challenge can be applied in clinical practice to detect patients with misery perfusion.

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Significance of serum soluble thrombomodulin level in acute cerebral infarction

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The purpose of the present study was to investigate sequential changes in serum soluble thrombomodulin (sTM) concentrations in patients with acute cerebral infarction (ACI), and to correlate sTM concentrations with the severity of ACI evaluated by Japan Stroke Scale. Eighty-three consecutive patients with ACI were enrolled, and blood examinations were carried out soon after admission and 1 month after. sTM concentrations at admission in patients with cardioembolic infarction (3.2 ± 1.2 ng/ml) were significantly lower than those of lacunar infarction (3.9 ± 1.2) ($P < 0.05$). Serial examinations revealed that sTM concentrations increased significantly 1 month after admission (3.8 ± 1.2), compared with those at admission (3.6 ± 1.2) ($P = 0.02$). Of three ACI subtypes, sTM concentrations during 1 month significantly increased in atherothrombotic infarction ($P = 0.002$) or, not significantly, in cardioembolic infarction ($P = 0.09$). The sTM concentrations at admission showed a significant inverse correlation with the severity of ACI ($P = 0.04$). Although sTM concentrations serve as a useful marker for endothelial cell damage, they are decreased in patients with severe ACI, especially in atherothrombotic and cardioembolic infarctions. Lower sTM concentrations may play some important role in disease progression or in the recurrence following ACI, although the exact mechanism of this unique result should be clarified.

Introduction

Acute cerebral infarction (ACI) is produced by thrombosis of the cerebral artery that leads to a reduced regional cerebral blood flow and neuronal ischemia, followed by neuronal cell death. Endothelial dysfunction plays an important role in thrombus formation, and cerebral thrombosis causes further endothelial damage, so that good markers for endothelial dysfunction/damage are at a high premium.

Thrombomodulin (TM), a receptor for thrombin, is a transmembrane glycoprotein located on the surface of vascular and lymphatic endothelium (Maruyama *et al.*, 1985; Esmon, 1989). When thrombin is bound to TM, fibrin formation, platelet activation (Esmon, 1989) and thrombin-mediated protein S cleavage (Mitchell *et al.*, 1986) are inhibited, and protein C is activated (Walker *et al.*, 1979). In addition to the membrane TM, this glycoprotein was reported to exist in soluble circulating forms in plasma. Interestingly, these fragments together with membrane TM have been shown to exhibit an anticoagulant activity (Dittman, 1991). Soluble TM (sTM) levels increase in several pathologies when endothelial cells are damaged. Increased sTM levels have been reported amongst patients with peripheral and coronary artery disease (Blann and Seigneur, 1997). However, it has not been established whether acute ischemic stroke can affect sTM levels in the peripheral blood. The relation is not definite between sTM levels and severity or outcome of the acute ischemic stroke.

Recently, we reported in preliminary studies the several endothelial markers and adhesion molecules in acute ischemic stroke. In these studies, sTM concentrations during the acute stage did not increase, but became lower compared with the subacute (Kozuka *et al.*, 2002) or chronic stage (Nomura *et al.*, 2001).

The present study was performed with increased numbers of enrolled patients to exhibit the sequential change in sTM concentrations in each ACI subtype. Furthermore, we correlated these markers with the severity of stroke at admission, mortality within 1 month and the functional outcome 1 month after admission to elucidate the significance of sTM concentrations in ACI.

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Subjects and methods

Patients

Eighty-three consecutive patients with ACI admitted to the neurologic section in our hospital within 48 h from the onset were enrolled in the present study and followed up for 1 month. ACI was defined as a rapidly progressing focal neurologic deficit of vascular origin lasting more than 24 h, excluding hemorrhagic stroke by computed tomography performed at admission. Magnetic resonance imaging of the brain was immediately performed to detect the infarcted area. Patients were classified according to the Classification of Cerebrovascular Disease III (CVD III) of the National Institute of Neurological Disorders and Stroke (Committee Established by the Director of the NINDS, 1990), and consisted of three subtypes: atherothrombotic, cardioembolic and lacunar infarctions. Exclusion criteria were acute infection, malignancy and acute or chronic liver or kidney disease at admission. We selected controls without a past history of vascular events as references. Informed consent was obtained from all patients and control subjects.

Clinical details

Blood examinations were carried out soon after admission and 1 month after admission. We tested the Japan Stroke Scale (JSS) (Gotoh *et al.*, 2001) at admission to evaluate the severity of ACI and the Functional Independence Measure (FIM) to evaluate the functional outcome of the patients 1 month after admission. JSS, which has been developed by the Japan Stroke Society, is the only weighted, parametric stroke impairment scale offering a quantitative measure of the severity of stroke. Reliability and responsiveness were proved to be excellent (Gotoh *et al.*, 2001). Patients were assigned as hypertension, if they are taking antihypertensive agents, or the systolic blood pressure is >160 mmHg or diastolic >95 mmHg, or both. Patients were diagnosed as diabetes mellitus, if they are either treated with oral hypoglycemic agents or insulin, or the serum fasting blood sugar level exceeds 140 mg/dl, or both. Hyperlipidemia was diagnosed, if they are taking antihyperlipidemic agents, or the serum cholesterol level exceeds 220 mg/dl, or both. All patients were followed up for 1 month except six patients who died as a direct consequence of ACI within 1 month after admission. We defined the patients scoring ≥ 100 on FIM 1 month after admission as good functional outcome ($n = 45$) and <100 as poor functional outcome ($n = 32$).

Blood sampling and laboratory procedure

The serum sTM concentrations were determined at admission and 1 month after admission. Venous blood samples were drawn from the cubital vein of each subject, and were centrifuged for 10 min at 1200 *g* to obtain serum. Serum sTM concentration was measured by a sandwich enzyme immunoassay method using commercial monoclonal antibody-based enzyme-linked immunosorbent assay kits (TM Panacera; Fuji Chemical Industries Ltd, Tokyo, Japan). The monoclonal antibodies to human TM consisted of two types, recognizing different TM sites, and had been precoated onto a microplate. Standard samples and sera were pipetted into the wells, together with horseradish peroxidase (HRP)-conjugated anti-TM antibodies. After removal of unbound conjugated antibody, a substrate solution reactive to HRP was added to the wells. The absorbance of each well was measured with a spectrophotometer (Poseidon II; Aloka Co. Ltd, Tokyo, Japan). The concentration of sTM was obtained from the standard curve, plotting the optical density against the standard TM concentrations. The assay coefficients of variation between and within runs for TM were 4.2 and 3.0%, respectively.

Statistical analysis

The data were analyzed with Stat View, version 5.0 (SAS Institute Inc., Cary, NC, USA), for Macintosh. Values were expressed as mean ± standard deviation. The statistical significance of difference between two groups was tested with one-way ANOVA for continuous data or chi-square test for frequency data. Differences amongst three groups were tested with one-way ANOVA for continuous data, chi-square test for frequency data or Kruskal–Wallis test for ordinal data (FIM score). *Post hoc* test using Fisher's PLSD was used for continuous data. Sequential studies were analyzed by repeated measures ANOVA. Correlation between the sTM concentrations and the scores of JSS was calculated with the Spearman rank correlation coefficient. Values at $P < 0.05$ were considered statistically significant.

Results

Table 1 summarizes the clinical features of the patients and controls. Although the frequencies of hypertension and diabetes mellitus were significantly higher in patients compared with controls, the frequency of hyperlipidemia was not different between the two groups.

Table 2 summarizes the clinical features of the patients in each ACI subtype. The proportion of male and

Table 1 Characteristics of patients and controls enrolled in the present study

	Controls	Patients	*P*-value
Number of subjects	66	83	
Age (mean ± SD)	65.8 ± 14.6	68.5 ± 10.2	0.2
Gender (male/female)	42/24	54/29	0.9
Death within 1 month		6	
Hypertension	20	45	0.004
Diabetes mellitus	7	24	0.006
Hyperlipidemia	12	22	0.2
JSS at admission (mean ± SD)		7.9 ± 7.0	
FIM 1 month after admission (median)		108	
sTM concentration (ng/ml)	3.5 ± 1.2		
At admission		3.6 ± 1.2	0.8
1 month after		3.8 ± 1.2	0.1

Differences between controls and patients were tested with one-way ANOVA for continuous data or the chi-square test for frequency data. JSS, Japan Stroke Scale; FIM, Functional Independence Measure.

female was not identical amongst three ACI subtypes, using chi-square test ($P = 0.01$). Mean age and the frequencies of hypertension, diabetes mellitus and hyperlipidemia were not different amongst three groups. JSS score at admission (by one-way ANOVA) and FIM score 1 month after admission (by Kruskal–Wallis test) were not identical amongst three groups. *Post hoc* test revealed that the patients with lacunar infarction had significantly lower JSS scores at admission compared with those with atherothrombotic and cardioembolic infarction.

No significant difference in sTM concentrations was observed between controls and patients at admission or 1 month after admission (Table 1). sTM concentrations at admission in patients with cardioembolic infarction (3.2 ± 1.2 ng/ml) were significantly lower than those of lacunar infarction (3.9 ± 1.2 ng/ml), using *post hoc* test (Table 2). Serial examinations revealed that sTM concentrations increased significantly 1 month after admission (3.8 ± 1.2 ng/ml), compared with those at admission (3.6 ± 1.2 ng/ml), using repeated measures ANOVA ($F = 6.1$, $P = 0.02$). Of three ischemic stroke subtypes, sTM concentrations during 1 month were significantly increased in atherothrombotic infarction ($F = 11.5$, $P = 0.002$) or, not significantly, in cardioembolic infarction ($F = 3.4$, $P = 0.09$) (Fig. 1).

There was no significant difference in sTM concentrations at admission between the deceased group (3.2 ± 0.9 ng/ml) and survivor group (3.6 ± 1.2 ng/ml), using one-way ANOVA ($F = 0.6$, $P = 0.5$).

The sTM concentrations at admission in patients with poor outcome (3.3 ± 1.1 ng/ml) appeared to be lower than those with good outcome (3.8 ± 1.3 ng/ml), using one-way ANOVA ($F = 3.5$, $P = 0.07$). The sTM concentrations in patients with poor outcome significantly increased 1 month after admission (3.9 ± 1.3 ng/ml) and became almost equivalent to those of good outcome (3.8 ± 1.1 ng/ml), using one-way ANOVA ($F = 0.03$, $P = 0.9$) (Fig. 2).

The sTM concentrations at admission showed a significant inverse correlation with the JSS scores at admission, calculated with a Spearman rank correlation coefficient ($z = -2.1$, $P = 0.04$) (Fig. 3).

Discussion

In the present study, we found that sTM concentrations were almost similar in patients with ACI as a whole compared with controls, and unexpectedly

Table 2 Characteristics of patients in each cerebral infarction subtype

	Atherothrombotic infarction	Cardioembolic infarction	Lacunar Infarction	*P*-value
Number of subjects	27	21	35	
Age (mean ± SD)	69.3 ± 10.2	68.4 ± 13.2	67.8 ± 8.0	0.8
Gender (male/female)	20/7	8/13	26/9	0.01
Death within 1 month	0	6	0	
Hypertension	16	12	17	0.7
Diabetes mellitus	7	4	13	0.3
Hyperlipidemia	7	4	11	0.6
JSS at admission (mean ± SD)	10.8 ± 5.6	13.3 ± 7.7	2.5 ± 2.0[a]	<0.001
FIM 1 month after admission (median)	67	86	119	0.001
sTM concentration (ng/ml)				
At admission	3.4 ± 1.0	3.2 ± 1.2[b]	3.9 ± 1.2	0.04
1 month after	4.0 ± 1.2	3.5 ± 1.3	3.8 ± 1.0	0.5

Differences amongst three groups were tested with one-way ANOVA for continuous data, chi-square test for frequency data or Kruskal–Wallis test for ordinal data (FIM score). *Post hoc* test was used for continuous data. JSS, Japan Stroke Scale; FIM, Functional Independence Measure. [a]Significantly lower score compared with both atherothrombotic and cardioembolic infarction. [b]Significantly lower value compared with lacunar infarction.

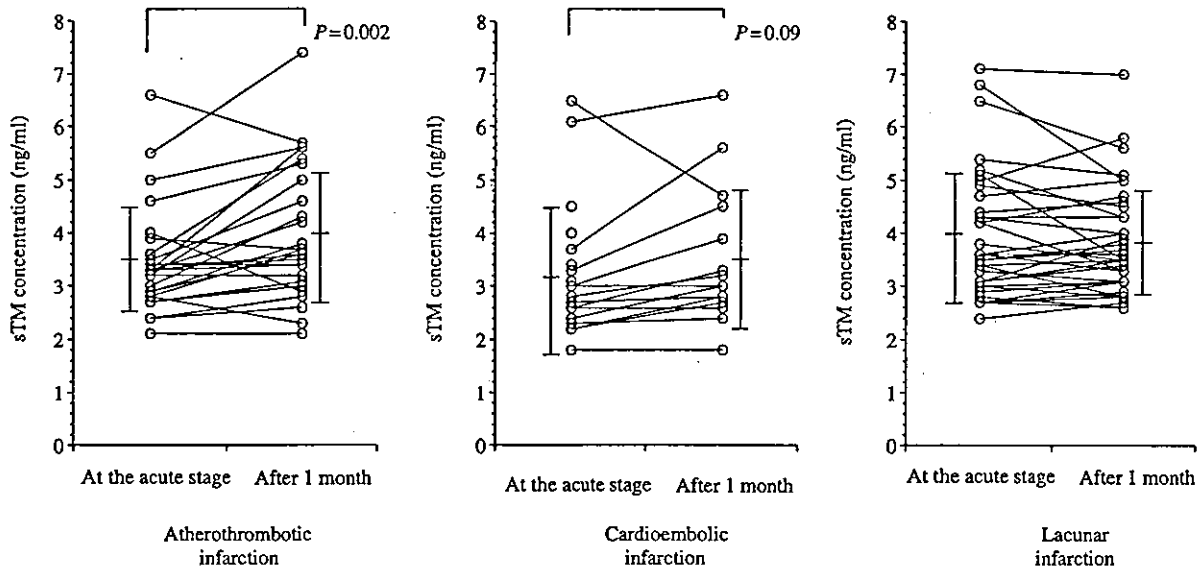

Figure 1 Sequential changes in sTM concentrations in each ACI subtype. sTM concentrations during 1 month were significantly increased in atherothrombotic infarction ($F = 11.5$, $P = 0.002$) or, not significantly, in cardioembolic infarction ($F = 3.4$, $P = 0.09$) using repeated measures ANOVA.

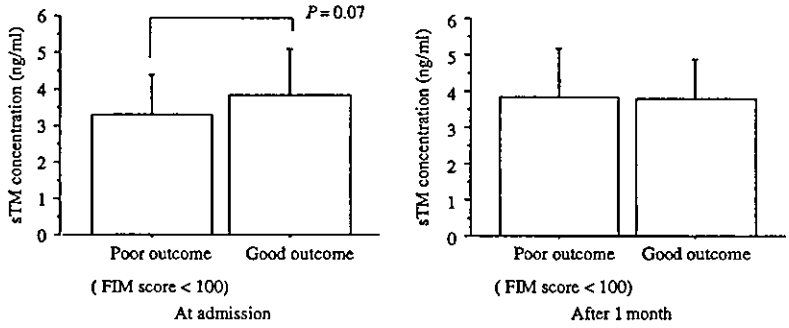

Figure 2 Comparisons of sTM concentrations between poor and good functional outcome groups. sTM concentrations at admission in patients with poor functional outcome appeared lower than those of good functional outcome ($n = 77$, $F = 3.5$, $P = 0.07$), and sTM concentrations between the two groups became similar 1 month after admission ($n = 77$, $z = -0.03$, $P = 0.9$). Differences were tested using one-way ANOVA.

lowered at the acute stage in patients with serious condition.

The sTM levels increase in diseases caused by endothelial cell damage, including disseminated intravascular coagulation syndrome (Wada *et al.*, 1992), pulmonary embolism, respiratory distress syndrome, chronic renal insufficiency, acute hepatic insufficiency (Iwabuchi *et al.*, 1990; Takano *et al.*, 1990), diabetic angiopathy (Tanaka *et al.*, 1991), systemic lupus erythematosus (Takaya *et al.*, 1991), chronic myelogenous leukemia (Morishita *et al.*, 1992) and cancer (Lindahl *et al.*, 1993). Although increased sTM levels have also been reported amongst patients with peripheral and coronary artery disease (Blann and Seigneur, 1997), it has not been established that sTM levels are elevated in patients with ACI. In the present and preliminary (Nomura *et al.*, 2001; Kozuka *et al.*, 2002) studies, the sTM levels in patients with ACI at admission did not increase, compared with controls. These results indicate that endothelial damage to the brain vessels may hardly cause elevated sTM levels. These may be supported by the finding that TM is absent in the brain parenchyma (Ishii *et al.*, 1986; Wang *et al.*, 1997) and is low (Ishii *et al.*, 1986) or distributed differently (Wang *et al.*, 1997) in the cerebral vessels.

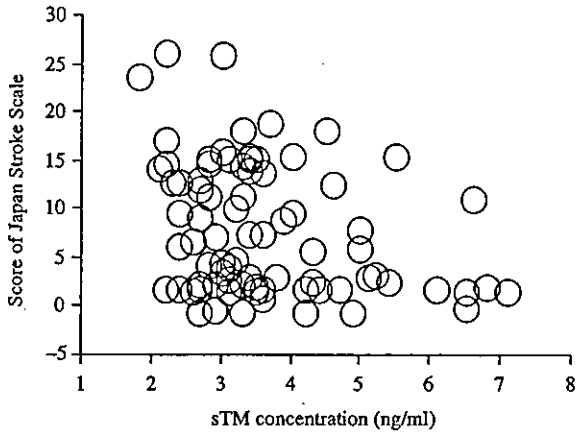

Figure 3 Relationship between Japan Stroke Scale (JSS) and sTM concentrations at admission. sTM concentrations at admission showed a significant inverse correlation with the JSS score at admission, calculated using the Spearman rank correlation coefficient ($n = 83$, $z = -2.1$, $P = 0.04$).

We demonstrated lower sTM concentrations in patients with cardioembolic infarction than in patients with lacunar one at admission. Serial examination showed that sTM concentrations during 1 month increased significantly in atherothrombotic infarction and, not significantly, in cardioembolic infarction. Furthermore, sTM concentrations at admission showed a significant inverse association with the severity of the stroke measured by JSS, and the poor functional outcome group (scoring <100 on FIM) tended to have lower sTM concentrations at admission than the good functional outcome group (scoring ≥ 100 on FIM). Consequently, sTM levels seem possibly to be decreased during the acute stage, especially in serious patients with ACI.

We could postulate two possible mechanisms for the decrease in sTM concentrations of patients with ACI; one is consumption, the other, which is more probable, down-regulation. A marked elevation of plasma concentration of the thrombin–antithrombin III complex, reflecting the generation of thrombin, was reported in cardioembolic stroke (Takano *et al.*, 1992). Authors also revealed modest but significant decreases in the concentrations of antithrombin III and protein C, suggesting that these procoagulant inhibitors may have been consumed and consumption coagulopathy occurs in cardioembolic stroke (Takano *et al.*, 1991). The present study prompted us to consider that generated excess thrombin following ACI might cause significant decreases in sTM concentrations by consumption.

Thrombomodulin on cultured endothelial cells is up-regulated by cyclic adenosine monophosphate analogs (Hirokawa and Aoki, 1990), and down-regulated by tumor necrosis factor-alpha (TNF-α) (Conway and Rosenberg, 1988), interleukin-1 (IL-1) (Nawroth *et al.*, 1986) or endotoxin (Moore *et al.*, 1987). It has been reported that TNF-α and IL-1 significantly increase in the rat ischemic cortex after temporary occlusion of the middle cerebral artery followed by reperfusion (Wang *et al.*, 1994). In addition, a recent study elucidated that an elevation in these proinflammatory cytokines in the peripheral blood was observed soon after ischemic stroke (Fassbender *et al.*, 1994; Ferrarese *et al.*, 1999; Vila *et al.*, 2000). Thus, sTM concentrations could conceivably be down regulated by these cytokines especially in acute ischemic stroke patients with large infarcts.

We thought that sTM levels could be decreased by similar mechanisms (down-regulation and/or consumption) in both atherothrombotic and cardioembolic infarctions. However, the number of enrolled patients with cardioembolic infarction was limited, and six patients with cardioembolic infarction (28.6%) died as a direct consequence of cerebral infarction before blood examination 1 month after. Further study of increased number of patients with cardioembolic infarction should be needed, and investigating the association between sTM and thrombin–antithrombin III complex, protein C, TNF-α, or IL-1 may be helpful to corroborate the hypothesis. Low sTM level plays an important role, accelerating the hemostatic unbalance toward a very potent prothrombotic state. Therefore, decreased sTM levels in ACI patients might cause the enlargement of the infarct area or the further development of vascular events. Conversely, we cannot deny another possibility that sTM concentration fluctuated physiologically and lower sTM concentration, triggering the ACI, was detected at the time of onset. However, the inverse association between sTM concentration and the stroke severity more strongly suggests that sTM concentration was affected by the incidence of cerebral infarction.

In conclusion, sTM levels did not increase in ACI, but rather decreased in atherothrombotic and cardioembolic infarctions. Lower sTM values were correlated with the severity at admission and seemed to associate with a poor functional outcome. The significance of these results should be investigated by further studies to clarify its mechanism.

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Short communication

Cerebral ischemia in 5-lipoxygenase knockout mice

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Abstract

Cerebral ischemia induces 5-lipoxygenase translocation and leukotriene production in the brain. We tried to clarify the pathological significance of 5-lipoxygenase on cerebral ischemia using 5-lipoxygenase knockout mice. No significant difference was observed in the infarct size following permanent and transient ischemia for 60 min between both types of mice. The present study did not support the idea that leukotriene production is involved in infarct expansion in focal cerebral ischemia.

Theme: Disorders of the nervous system
Topic: Ischemia

Keywords: Cerebral ischemia; 5-Lipoxygenase; Knockout mouse; Leukotriene

Arachidonic acid is released from membrane phospholipids by phospholipase A2 after cerebral ischemia and metabolized through three major enzymatic pathways [4,21,22]: cyclooxygenase (COX), lipoxygenase, and cytochrome P450 oxygenase. 5-Lipoxygenase together with five lipoxygenase activating protein (FLAP) [9] converts arachidonic acid to LTA4, which is hydrolyzed to LTB4 or conjugated to glutathione to yield LTC4, LTD4 and LTE4. These LTs induce a variety of responses including chemotaxis of leukocytes, smooth muscle contraction, and an increase in vascular permeability. In experimental cerebral ischemia, arachidonic acid is released from membrane phospholipid and converted enzymatically to products of the COX and lipoxygenase pathway [2,17]. We previously demonstrated the intracellular redistribution of 5-lipoxygenase from cytosolic to membranous fractions, which could make the enzymatic activity full [20]. Although the implication of 5-lipoxygenase on the brain edema after transient global ischemia has been investigated with lipoxygenase inhibitor [3,13,16], the pathological significance of 5-lipoxygenase in focal cerebral ischemia has not been fully examined. Ciceri et al. [6] demonstrated recently that LT biosynthesis inhibitor MK-886 significantly reduced the cortical infarct size by 30% in permanent occlusion of middle cerebral artery in rats. In human autopsied brains, upregulation of 5-lipoxygenase has been shown in neurons, glial cells and mononuclear leukocytes during focal ischemic damage [23]. For a better understanding of the involvement of LT in focal cerebral ischemia, we used 5-lipoxygenase knockout mice and compared the infarct size after permanent and transient MCA occlusion between knockout and littermate mice.

5-Lipoxygenase knockout mice, originally produced by Chen et al. [5], were purchased from the Jackson Laboratory (Bar Harbor, ME, USA). We first backcrossed them with the C57BL/6 mice (Charles River, Japan) more than five times to have genetic background of the homozygous and wild-type mice, and used them in the subsequent experiment. After mating heterozygotes, we selected the homozygous and wild-type mice by PCR amplification of genomic DNA extracted from the tail. All homozygous and wild-type mice used in the present study were mature males aged 12–16 weeks. Mice were given free access to food and water prior to surgery. The genotype was again confirmed after killing mice by immunoblotting of the lung homegenate using an antibody against 5-lipoxygenase (Cayman Chemical, Ann Arbor, USA). The experimental procedures involving labo-

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ratory animals have been approved by the Institutional Animal Care and Use Committee of the Osaka University Medical School.

Twelve animals each of the wild-type and knockout mice were used for evaluation of infarct size. General anesthesia was induced with 2.0% halothane and maintained with 0.5% halothane by means of an open face mask. A polyacrylamide column with an inner diameter of 0.8 mm for measurement of cortical microperfusion by laser Doppler flowmetry (LDF, Advanced Laser Flowmetry, Model ALF-21, Advance) was attached to the intact skull, 3.5 mm lateral to the bregma with dental cement. A metal plate-type thermometer with a diameter of 3.0 mm was also attached to the skull over the parietal cortex to record skull temperature. A femoral artery was cannulated with a PE-10 polyethylene tube which was connected to a pressure transducer attached to a calibrated polygraph, and body temperature was monitored with a rectal thermometer. Body and skull temperature was monitored and maintained at 36.0–37.5 °C and 35.0–36.5 °C, respectively, using a heat lamp.

The left MCA was exposed as described previously [12]. In brief, mice were placed in the recumbent position, and a vertical skin incision was made in the midpoint between the left orbit and the external auditory canal. The massecter muscles were dissected at the inferior edge of the zygoma, and the mandible was pulled downward to expose the skull base. Using a dental drill, a small burr hole was made in the skull over the left MCA. The dura was opened and the MCA was picked up. For permanent focal ischemia, the MCA was occluded using a microbipolar electrocoagulator just proximal to the point where

Table 1
Physiologic variables and neurological scores after MCA occlusion

Permanent MCA occlusion			During ischemia				
Mouse strain		Preischemic	5 min	15 min	30 min	2 h	24 h
Wild type	Change in LDF (%)	100	22.8±7.1	23.5±5.0	22.0±4.9	–	–
	MABP (mm Hg)	75.8±5.3	72.0±4.3	70.5±3.5	73.0±5.7	–	–
	Body temperature (°C)	36.5±0.6	36.7±0.5	37.0±0.4	37.2±0.4	36.4±0.5	36.2±0.4
	Skull temperature (°C)	35.6±0.9	35.9±1.0	35.4±0.5	35.3±0.3	–	–
	Neurological Score	0	–	–	–	2.5±0.5	0.8±0.4
5-LO K/O	Change in LDF (%)	100	19.2±4.9	24.0±5.7	23.1±4.4	–	–
	MABP (mm Hg)	83.0±8.5	69.0±3.4	69.7±9.5	68.0±5.3	–	–
	Body temperature (°C)	36.2±0.6	36.8±0.2	37.0±0.3	37.2±0.3	36.4±0.2	36.8±0.4
	Skull temperature (°C)	35.2±0.4	36.2±0.9	35.6±0.5	35.9±0.7	–	–
	Neurological Score	0	–	–	–	2.8±0.4	1.3±0.8

Transient MCA occlusion			During ischemia				
Mouse strain		Preischemic	5 min	15 min	30 min	45 min	60 min
Wild type	Change in LDF (%)	100	21.3±7.3	25.2±5.7	24.2±5.4	24.2±7.4	22.4±5.3
	MABP (mm Hg)	76.2±8.9	74.0±7.6	69.7±7.6	68.7±6.1	71.0±3.3	69.3±6.2
	Body temperature (°C)	36.5±0.4	36.6±0.5	36.9±0.6	36.8±0.4	36.9±0.5	37.0±0.5
	Skull temperature (°C)	35.6±0.7	35.9±0.5	35.6±0.8	35.6±0.4	35.8±0.5	35.5±0.4
	Neurological Score	0	–	–	–	–	–
5-LO K/O	Change in LDF (%)	100	21.2±7.8	22.8±5.0	22.7±5.5	22.1±4.9	20.4±4.4
	MABP (mm Hg)	79.2±7.3	68.8±6.6	65.0±4.6	65.5±8.7	66.8±8.8	67.3±8.1
	Body temperature (°C)	36.0±0.6	36.2±0.8	36.7±0.5	36.9±0.8	36.9±0.8	36.7±0.6
	Skull temperature (°C)	35.3±0.4	35.8±0.4	35.5±0.4	35.9±0.6	35.9±0.9	35.7±0.4
	Neurological Score	0	–	–	–	–	–

Transient MCA occlusion		Recirculation			
Mouse strain		5 min	15 min	2 h	24 h
Wild type	Change in LDF (%)	72.5±17.8	81.7±13.2	–	–
	MABP (mm Hg)	68.2±2.4	69.8±5.5	–	–
	Body temperature (°C)	36.7±0.5	37.3±0.4	36.1±0.2	36.0±0.7
	Skull temperature (°C)	35.9±0.8	36.1±0.7	–	–
	Neurological Score	–	–	2.5±0.8	0.8±0.4
5-LO K/O	Change in LDF (%)	82.1±39.1	94.8±27.7	–	–
	MABP (mm Hg)	69.7±5.8	69.7±4.4	–	–
	Body temperature (°C)	36.7±0.4	36.8±0.5	36.4±0.4	36.3±0.8
	Skull temperature (°C)	35.5±0.4	35.8±0.5	–	–
	Neurological Score	–	–	2.7±0.8	0.8±0.4

Values are shown as mean±S.D. based on six animals. Laser Doppler flowmetry (LDF), mean arterial blood pressure (MABP), 5-lipoxygenase-knockout mice (5-LO K/O).

the olfactory branch came off. For transient focal ischemia, the MCA was occluded temporally with Zentype miniclip for 60 min under halothane anesthesia and recirculated by clip removal. Cortical microperfusion was monitored for 30 min after permanent MCA occlusion and for 15 min after clip removal in transient MCA occlusion. After disconnection of halothane anesthesia, each mouse was allowed to recover for 2 h in a chamber where the ambient temperature was maintained at 35 °C to prevent hypothermia, and then each mouse was kept at room temperature. At 2 h and 1 day after MCA occlusion, mice were evaluated by a blinded observer for their neurological deficits. The neurological deficit score assignment of 0–4 was based on the methods described previously by Yang et al. [24]: 0, no neurological deficit; 1, failed to extend right forepaw while held by tail; 2, circled to the right; 3, fell to the right; 4, unable to walk spontaneously.

Seven days after MCA occlusion, mice were killed with an overdose of pentobarbital. The whole brains were removed, fixed by immersion into an alcohol–5% acetic acid solution for 5 h at 4 °C, dehydrated, and embedded in paraffin. Tissue sections (5 μm) were obtained every 1 mm, beginning at the frontal pole, and were examined after conventional staining with hematoxylin and eosin. The volume of ischemic infarction was measured using an MCID image analysis system (Imaging Research, St. Catharine's, Ontario, Canada). The infarction areas were calculated by tracing the areas on a computer screen. The volume (mm^3) was determined by integrating the appropriate area and the section thickness.

All values reported here are expressed as mean±S.D. Statistical significance of differences among groups was tested by unpaired *t*-test.

Cortical microperfusion in the ischemic center after MCA occlusion was 15–30% of the baseline value in both permanent and transient occlusion. In transient MCA occlusion, cortical microperfusion recovered to 80–90% of the baseline value 15 min after clip removal. The values were similar between knockout and wild-type mice (Table 1). Other physiologic parameters, including blood pressure and body and skull temperatures, were maintained similarly between both types of mice (Table 1). Infarct areas after both permanent and transient MCA occlusion were almost the same for both types of mice on the coronal slices 3 mm from the frontal pole (Fig. 1). The total infarct volume also was similar between both types of mice in permanent (wild type, 33.0±3.3 mm^3, n=6; knockout mice, 33.6±4.4 mm^3, n=6; P=0.45) (Fig. 2) and transient ischemia (wild type, 13.8±6.3 mm^3, n=6; knockout mice, 14.4±5.6 mm^3, n=6; P=0.51) (Fig. 3). The neurological deficit score was also similar between both types of mice in permanent and transient ischemia (Table 1).

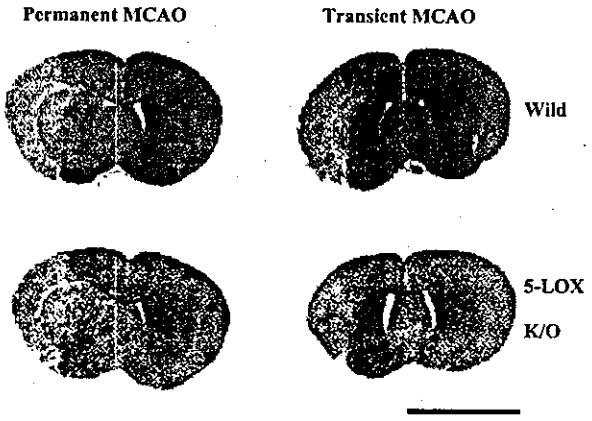

Fig. 1. Hematoxylin and eosin (HE) staining in the whole brain of wild-type (Wild) and 5-lipoxygenase knockout mice (5-LOX K/O). Permanent middle cerebral artery occlusion (MCAO) (left column). Transient middle cerebral artery occlusion. The extent of infarct area was similar in both types of mice (right column). Bar = 0.5 mm.

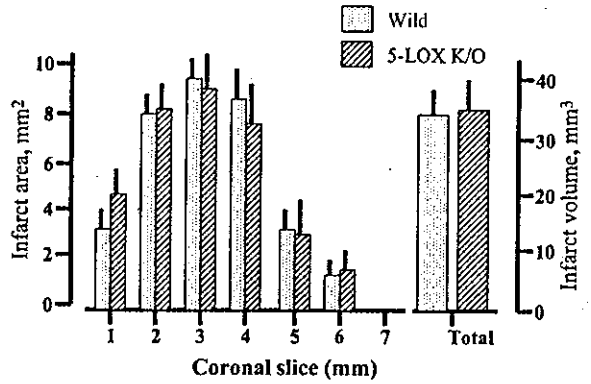

Fig. 2. Infarct area and size after permanent MCA occlusion. Infarct area in seven coronal sections from rostral to caudal (1–7 mm) and infarct volume shown for wild-type (stippled column) and 5-lipoxygenase knockout mice (hatched column). Infarct area and size was almost the same between the two types of mice.

After cerebral ischemia, arachidonic acid is released from membrane phospholipid and enzymatically converted to prostaglandin and thromboxane through cyclooxygenase, and to hydroperoxyeicotetraenoic acid (HPETE) through 5-, 12-, or 15-lipoxygenase. 5-Lipoxygenase is a key enzyme for production of LTs which constitute a class of potent biological mediators of inflammation and anaphylaxis. 5-Lipoxygenase knockout mice resist the lethal effects of shock by platelet-activating factor, and show reduced reaction to ear inflammation induced by arachidonic acid [5,10]. It was recently demonstrated that those mutant mice also exhibited a resistance to splanchnic artery occlusion shock [8]. Because the phenotype of 5-lipoxygenase knockout mice is useful to identify the role for leukotrienes in the pathophysiology under injurious insult, we used this mouse strain to investigate the role of 5-lipoxygenase and leukotriene in ischemic brain damage. In this study, we used both permanent and transient focal ischemia to mimic human stroke. The role of LT on ischemic brain damage has been examined with lipoxygenase inhibitor mostly in transient global ischemia model.

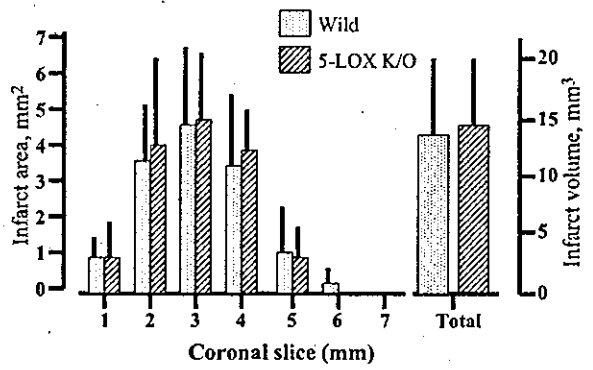

Fig. 3. Infarct area and size after transient MCA occlusion. Infarct area and infarct volume for wild-type (stippled column) and 5-lipoxygenase knockout mice (hatched column).

Several studies supported the involvement of LT on brain edema after ischemia [3,13,16]; however, the effect of lipoxygenase inhibitor on ischemic neuronal damage is controversial [1,7,18,21]. Furthermore, there are few studies examining the role of LT in focal cerebral ischemia. Ciceri et al. [6] demonstrated that LT biosynthesis inhibitor MK-886 decreased LT level and reduced the cortical infarct size by 30%, 24 h after permanent MCA occlusion. However, in our study, no significant difference was observed in the infarct size between wild type and 5-lipoxygenase knockout mice. Although we maintained blood pressure and body temperature and monitored change in cortical microperfusion after vessel occlusion, no information for such physiological parameters was shown in Ciceri et al.'s [6] study, making it difficult to compare our results with their study. Furthermore, infarction certainly expands during a few days after MCA occlusion in a rat [14,15]; therefore, evaluation of infarct size 24 h after occlusion in Ciceri et al.'s [6] study may look over infarct expansion. Although no protection against cerebral ischemia was found in 5-lipoxygenase knockout mice in this study, potential beneficial effect by suppression of LT production would be set off by increased substrate availability for cyclooxyagenase (COX) pathway. Among two COX isoforms, COX2 has been shown a key molecule for ischemic brain damage. Both inhibition of COX2 activity with COX2-specific inhibitor and deficiency of COX2 in knockout mice showed protection against ischemic brain damage [11,19]. Furthermore, 12-HPETE and 15-HPETE and their metabolites, lipoxins, produced by 12- and 15-lipoxygenase may increase and contribute to infarct expansion in 5-lipoxygenase knockout mice. However, our result does not exclude the possibility that inhibitor of 5-lipoxygenase or leukotriene antagonist may have potential as a therapeutic target against stroke. Inhibition of COX can increase substrate availability for 5-lipoxygenase and LT production which aggravate vascular permeability and brain edema. Combination of COX2 inhibitor with lipoxygenase inhibitor or LT antagonist in experimental stroke model may be worth consideration.

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脳血管障害の診断と治療の進歩*

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Ⅰ．脳血管障害の診断

脳血管障害とは，文字通り脳血管の障害すべてをさすが，一般的には表1の分類が用いられる[1]。脳血管障害の診断において，画像診断法の進歩が果たした役割は極めて大きく，なかでも頭部CTの登場は，出血性と虚血性病変の鑑別において決定的な役割を果たした。その後もさまざまな検査法が開発されているが，そのなかでも特に近年，急性期脳梗塞の診断にMRI拡散強調画像（diffusion weighted image：DWI）が用いられるようになり，有用性を発揮している[2]。すなわち，梗塞巣は発症後数十分よりDWIで高信号に描出さ

表1　NINDSによる脳血管障害の分類（NINDS-Ⅲ）

臨床病型
- A．無症候性
- B．局所性脳機能障害
  - 1．一過性脳虚血発作（TIA）
    - a．頸動脈系，b．椎骨脳底動脈系，c．両者，d：部位不明，e．TIAの疑い
  - 2．脳卒中
    - a．経過
      - 1）改善，2）悪化，3）不変
    - b．脳卒中の型
      - 1）脳出血
      - 2）クモ膜下出血
      - 3）脳動静脈奇形に伴う頭蓋内出血
      - 4）脳梗塞
        - a）機序
          - (1) 血栓性，(2) 塞栓性，(3) 血行力学性
        - b）臨床的カテゴリー
          - (1) アテローム血栓性脳梗塞，(2) 心原性脳塞栓症，(3) ラクナ梗塞，(4) その他
        - c）部位による症状
          - (1) 内頸動脈，(2) 中大脳動脈，(3) 前大脳動脈，(4) 椎骨脳底動脈系
- C．血管性痴呆
- D．高血圧性脳症

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表 2a　急性期虚血性脳卒中（血栓溶解療法非適応症例）の血圧管理

SBP＜220 または DBP＜120	大動脈解離，急性心筋梗塞，肺水腫，高血圧性脳症の合併例以外では降圧しない
SBP＞220 または DBP＞121～140	labetalol（10～20 mg）を 1～2 分以上かけて静注，10 分ごとに追加（最大量 300 mg） または nicardipine（5 mg/hr）を初期量として点滴静注，5 分ごとに 2.5 mg/hr ずつ増量し，最大 15 mg/hr まで可 血圧の 10～15%低下を目標
DBP＞140	nitroprusside（0.5 μg/kg/min）を初期量として点滴静注，持続的に血圧測定 血圧の 10～15%低下を目標

SBP：収縮期血圧，DBP：拡張期血圧（単位 mmHg）

れ，急性期病変の検知が容易であるばかりか，通常の MRI 画像で多発性に脳梗塞を認める症例でも陳旧性脳梗塞との鑑別が可能である。ただし，一過性脳虚血発作（TIA）では DWI で異常を認めないことも多く，DWI で異常所見を認めないからといって TIA は否定できない。

一方，出血性脳血管障害においても MRI の有用性は高く，慢性硬膜下血腫で血腫が等吸収を示す場合には CT で診断が困難な場合もあるが，このような例でも MRI による診断は容易である。また，クモ膜下出血では FLAIR 像でクモ膜下腔に高信号域を認めることが多い。

脳血管障害では，MRA（MR アンギオグラフィ）や頸動脈エコーで頭蓋内・外動脈を評価することも重要である。頸動脈狭窄症により虚血性脳血管障害を生じていることもあり，頸動脈内膜剝離術（carotid endarterectomy：CEA）の適応となる場合もある。また，クモ膜下出血では MRA により動脈瘤を同定することができる。ただし，動脈瘤の同定や手術方法の検討のために脳血管撮影や 3 D-CT アンギオを行う。脳出血のうち，脳動静脈奇形やモヤモヤ病などが疑われる場合には，MRA や脳血管撮影を行う。脳梗塞のうち，心原性脳塞栓症が疑われる場合にはホルター心電図や経胸壁心エコーによる心房細動，一過性心房細動などの不整脈や器質的疾患の検索，さらに経食道心エコーによる左房内血栓，モヤモヤエコー，卵円孔開存などの検索が必要である。さらに，超急性期の脳塞栓症では，後述する血栓溶解療法の適応を決めるために DWI と同時に灌流強調画像（perfusion weighted image：PWI）を施行し，脳組織障害と灌流障害の解離（diffusion-perfusion mismatch）を評価する。

II．脳血管障害の治療

脳血管障害では急性期の血圧管理が重要である。脳梗塞急性期には血圧が上昇するが，梗塞範囲が拡大する恐れもあり，むやみに降圧を行わないことが重要である。2003 年に発表された米国心臓協会（AHA）の勧告[3]では大動脈解離，急性心筋梗塞，肺水腫，高血圧性脳症を合併しなければ，収縮期血圧＜220 mmHg，または平均血圧＜130 mmHg であれば降圧しないとされている（表 2 a）。血栓溶解療法適応症例の血圧管理については別に推奨されている（表 2 b）。なお，Ca 拮抗薬の舌下投与は急激な血圧低下をきたす恐れがあり使用すべきではない。

脳出血の急性期にも血圧は上昇しているのが一般的であるが，積極的な降圧を行うべきか否かは依然明らかではなく，血腫増大予防のために降圧すべきとする意見もあれば，血腫増大と血圧の間には関連がなく，脳循環を保つために積極的な降圧は行わないほうがよいとする意見もある。血腫の増大は発症後 6 時間以内に起こることが多いため，この時期には収縮期血圧 160 mmHg を超えないように降圧し，その後は収縮期血圧 160～180 mmHg 以下にコントロールすべきとの意見が多い[4]。

脳動脈瘤の破裂によるクモ膜下出血でも，発症時に高度の血圧上昇をきたしていることが多い。急性期管理において重要なことは，再破裂の予防

表 2b　血栓溶解療法適応症例の血圧管理

開始前	
SBP＞185 または DBP＞110	labetalol(10～20 mg)を 1～2 分以上かけて静注，再度静注または nitropaste 1～2 インチ SBP≦185 および DBP≦110 が維持できなければ rtPA を使用しない
血栓溶解療法中および後	
血圧測定	開始後 2 時間は 15 分ごと，2～8 時間は 30 分ごと，6～16 時間は 1 時間ごと
DBP＞140	sodium nitroprusside（0.5 μg/kg/min）を初期量として点滴静注，不十分な時は追加
SBP＞230 または DBP 121～140	labetalol（10 mg）を 1～2 分以上かけて静注 labetalol を 10 分ごとに 10～20 mg 追加（最大量 300 mg），または初期量をボーラス投与後 2～8 mg/min で点滴静注，または nicardipine（5 mg/hr）を初期量として点滴静注，5 分ごとに 2.5 mg/hr ずつ増量し，最大 15 mg/hr まで可，不十分な時は sodium nitroprusside を考慮
SBP 180～230 または DBP 105～120	labetalol（10 mg）を 1～2 分以上かけて静注 labetalol を 10～20 分ごとに 10～20 mg 追加（最大量 300 mg），または初期量をボーラス投与後 2～8 mg/min で点滴静注

SBP：収縮期血圧，DBP：拡張期血圧（単位 mmHg）

である。どの程度まで降圧すべきかに明確な基準はないが，血圧上昇により動脈瘤への圧が高まり，再破裂の危険性が高まるため，積極的に降圧を行い，少なくとも収縮期血圧を 140 mmHg 以下に保つ必要があると思われる。また，激しい頭痛，不穏が更なる血圧上昇をきたすため鎮痛剤や鎮静剤を使用する。

脳梗塞の急性期治療としては，脳保護薬（エダラボン）の投与，抗凝固薬・抗血小板薬投与，酸素投与，抗脳浮腫薬の投与，血栓溶解療法，減圧術，リハビリテーションなどがあげられる。2001 年より日本で世界初の脳保護薬として使用可能となったエダラボンは，発症 24 時間以内の急性期脳梗塞のすべてに投与可能な薬物として注目されているが，特に高齢者における腎障害の副作用の報告があり注意を要する。

抗血小板療法としては，発症 5 日以内の脳血栓症に対してトロンボキサン A_2 合成酵素阻害薬（オザグレルナトリウム）の点滴静注が広く用いられている。また，発症後 48 時間以内の脳梗塞に対するアスピリン経口投与の有効性が大規模臨床試験により示され[5,6]，160～300 mg/日の投与が行われる。

抗凝固療法としては発症 48 時間以内のアテローム血栓性脳梗塞に対して抗トロンビン薬（アルガトロバン）の点滴静注の有効性が示され，広く用いられている。ヘパリン・低分子ヘパリンは脳梗塞急性期の画一的な使用には効果は確立していないが，心原性脳塞栓症の一部に使用されている。抗脳浮腫療法としてはグリセオールやマンニトールが使用され，グリセオールは発症後 14 日以内の死亡を減少させることが明らかとなっているが，マンニトールの有効性は明らかではない。なお，脳浮腫が著明な場合には減圧術を行うこともある。

脳塞栓症の超急性期，特に発症 3 時間以内の治療法として血栓溶解療法がある。前述した diffusion-perfusion mismatch は脳梗塞へと進展する可能性の高い部位と考えられ，超急性期の血栓溶解療法の適応となる領域として重要である。現在，日本において承認されている血栓溶解療法は発症 5 日以内の脳血栓症に対する低用量ウロキナーゼ（UK）（6 万単位/日）の 7 日間の静脈内投与のみであるが，一部の施設では高用量ウロキナーゼ動脈内投与による血栓溶解療法が行われている。そのほかに，日本で未承認の血栓溶解療法として発症 3 時間以内の脳梗塞に対する遺伝子組み換え組織プラスミノーゲンアクチベータ（rt-PA）による経

静脈的血栓溶解療法，発症6時間以内の中大脳動脈閉塞に対するプラスミノーゲンプロアクチベータ（pro-UK）局所動脈内投与があり，日本においてもt-PA静脈内血栓溶解療法の早期承認の可能性がある。

脳出血の急性期治療としては，前述した血圧管理を中心とした保存的治療と外科的治療がある。血腫量の多い例や意識障害例において定位的血腫吸引術，開頭血腫除去術などの外科的治療を行うこともある。また，脳出血による水頭症や脳室内血腫に対して脳室ドレナージを行うこともある。

脳梗塞および脳出血では急性期治療を行うとともに，慢性期治療として危険因子のコントロールや，虚血性脳卒中では再発予防のための抗血小板薬・抗凝固薬内服を行う。アテローム血栓症と総称される脳梗塞・TIAや心筋梗塞に罹患した高リスク群では抗血小板療法により脳卒中，心筋梗塞，血管死の発症が有意に減少することが証明され[7]，アスピリンで約25%，チクロピジンで33%のリスク低下が報告されている。なお，アスピリンの至適用量は75～150 mg/日が最も抑止効果が優れているとされている。さらに，シロスタゾールの脳梗塞再発予防への有効性が日本で行われた多施設ランダム化二重盲検比較試験で示され，2003年より脳梗塞（心原性脳塞栓症を除く）発症後の再発抑制に効能追加となっている[8]。

心原性脳塞栓症の二次予防にはワルファリンが用いられる。ワルファリンによる抗凝固療法はINR（international normalized ratio）を用いた用量調節が行われ，一般にINR 2.0～3.0（人工弁置換術後では2.5～3.5）が推奨されている。ただし，出血性合併症の問題から，日本では65歳以上の高齢者ではINR 1.6～2.6が安全かつ有効な領域であるとされている[9]。危険因子の高血圧治療に関しては，二次予防に積極的な降圧が有効であることが大規模試験PROGRESS（Perindopril Protection Against Stroke Study）によって証明されており[10]，高血圧患者の脳血管障害再発予防には厳格な血圧管理が必要であり，降圧自体が二次予防にとって重要であると考えられる。また，外科的な治療として，内頸動脈高度狭窄例に対してCEA・頸動脈ステント留置，内頸動脈閉塞例・中大脳動脈閉塞例・モヤモヤ病などでは浅側頭動脈—中大脳動脈吻合術（STA-MCA anastomosis）が行われる。なお，近年T_2*強調画像において認める微小出血が注目されており，微小出血の存在がsmall-artery disease，特に症候性脳出血と関連することが報告されており[11]，脳梗塞後の抗血小板療法・抗凝固療法の際に注意すべき所見と考えられる。

クモ膜下出血の治療としては，前述した血圧管理のほかに早期（72時間以内）の手術を行うが，重症例では手術の対象とならない例や安静待機後に慢性期の手術を行う例もある。手術法として脳動脈瘤クリッピング術，脳動脈瘤内塞栓術，親動脈閉塞術・トラッピング術が行われる。クモ膜下腔の出血が原因となり，出血後4～14日に脳血管攣縮が生じることがあるが，その予防的治療は確立された方法はなく，triple-H療法（hypertension, hypervolemia, hemodilution），塩酸ファスジルやCa拮抗薬投与，脳槽灌流が試みられている。症候性の脳血管攣縮への効果的な治療法はいまだなく，経皮経管血管形成術や選択的塩酸ファスジル動注・塩酸パパベリン動注が行われているが，いずれも保険適用はない。一方，急性期に生じる水頭症の治療として脳室ドレナージが行われ，慢性期に緩徐に進行する水頭症に対しては脳室腹腔短絡術（V-P shunt）を行う。

慢性硬膜下血腫の治療としては，血腫が少量の軽症例では保存的治療で血腫が減少・消失することもある。外科的治療として局所麻酔下に穿頭を行い，血腫を除去・洗浄する。通常，予後良好であるが再発を起こすことがあるので注意が必要である。

以上，脳血管障害の診断・治療についてまとめてみたが，脳血管障害と一言でいってもその範囲は非常に広範囲であり，限られた誌面でまとめるのは困難である。さらに透析という特殊な環境において発症する脳血管障害では，一般的な脳血管