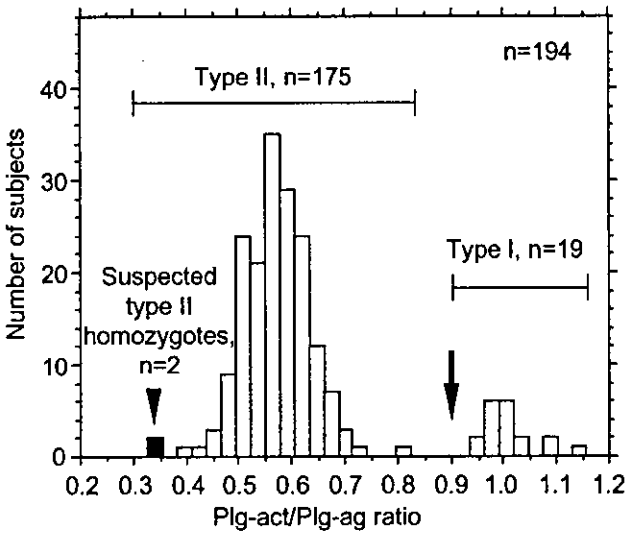

Fig. 3. Distribution of activity/antigen ratio of plasminogen (Plg-act/Plg-ag) in 194 individuals with plasminogen deficiency. The arrow indicates the cut-off point, 0.9. Nineteen individuals showed ratios over 0.9, indicating type I plasminogen deficiency, and 175 individuals showed ratios less than 0.9, indicating type II plasminogen deficiency. Among type II deficiency, two showed very low ratios indicating homozygous type II deficiency.

and Standardization Committee of the International Society on Thrombosis and Haemostasis [24]. In our study, we calculated the ratio of plasminogen activity to antithrombin activity (Fig. 2b). As a result, the two peaks were clearly separated. The mean −2 SD of the ratio was 0.69. To distinguish the plasminogen deficiency from the normal plasminogen, we used the ratio 0.69 as the cut-off. Only 192 among 4517 showed ratios less than 0.69.

Of the individuals with ratios higher than 0.69, two showed low plasminogen and antithrombin activities with normal levels of plasminogen antigen and protein C, indicating a combined deficiency of plasminogen and antithrombin (subject 1: male, age 70 years, plasminogen activity 59.4%, plasminogen antigen 110.6%, antithrombin activity 65.8%, protein C 110.8%; subject 2: male, age 75 years, plasminogen activity 69.8%, plasminogen antigen 118.0%, antithrombin activity 74.2%, protein C 123.8%). Thus, a total of 194 individuals (194/4517, 4.30%) were considered to be plasminogen-deficient. Among 194 individuals, two showed extremely low plasminogen activity, indicating a homozygous plasminogen deficiency (subject 3: male, age 60 years, plasminogen activity 24.4%, plasminogen antigen 74.0%, antithrombin activity 97.2%, protein C 120.1%; subject 4: male, age 80 years, plasminogen activity 26.2%, plasminogen antigen 78.4%, antithrombin activity 67.2%, protein C 61.3%).

Plasminogen deficiency (194 individuals) can be divided into two groups, type I (hypoplasminogenemia) or type II (dysplasminogenemia), and can be judged by the ratio of activity and antigen of plasminogen (Plg-act/Plg-ag). Figure 3 shows the distribution of the Plg-act/Plg-ag ratio. The Plg-act/Plg-ag ratio exhibited two peaks. We set the cut-off of the ratio to be 0.90. Nineteen out of 194 individuals were classified into type I plasminogen deficiency, and the remaining 175 were type II plasminogen deficiency, including two suspected homozygous plasminogen deficiency.

Consequently, in the Japanese general population, the overall prevalence of heterozygous type I plasminogen deficiency was 0.42% ($n = 19$; men $n = 8$; women $n = 11$) and heterozygous and homozygous type II plasminogen deficiency were 3.83% ($n = 173$; men $n = 82$; women $n = 91$) and 0.04% ($n = 2$; men $n = 2$; women $n = 0$), respectively (Fig. 4).

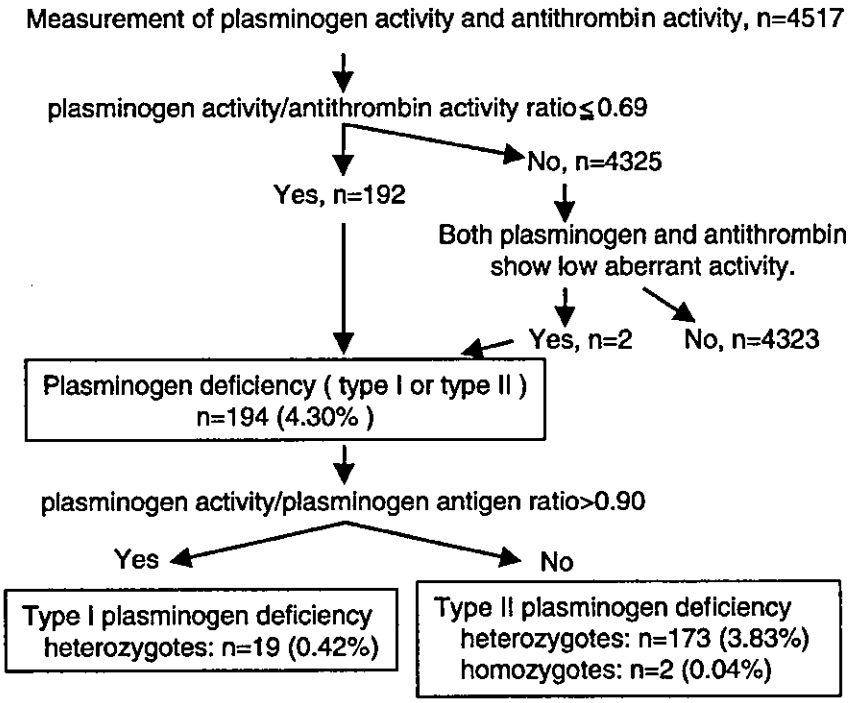

Fig. 4. Flow chart of selection of individuals with plasminogen deficiency. To identify the plasminogen deficiency in 4715 individuals, we performed a two-stage selection. The first used a 0.69 plasminogen/antithrombin ratio as the cut-off to distinguish the deficients from the normals, and the second used a 0.9 activity/antigen ratio of plasminogen for discrimination of plasminogen deficiency type I and type II.

Table 1 Comparison of prevalence of plasminogen deficiency between diseased groups and age-matched and sex-matched controls

	Number of heterozygote (prevalence)	Odds ratio (95% CI) (vs. controls)	*P*-value
Patients with DVT ($n = 108$)	3 (2.78%)		
Controls ($n = 324$)	13 (4.01%)	0.65 (0.21–2.07)	0.62
Patients with cardioembolic stroke due to NVAF ($n = 110$)	6 (5.55%)		
Controls ($n = 330$)	13 (3.94%)	1.31 (0.57–3.03)	0.52

CI, confidence interval; DVT, deep vein thrombosis; NVAF, non-valvular atrial fibrillation.

Table 2 Characteristics of homozygous type I plasminogen deficiency identified in National Cardiovascular Center

Patient number	Age (sex)	Plg-act (%)	Plg-ag (%)	Age at onset for thrombosis	Diagnosis	Family study phenotypic*
1	84 (F)	16.0	146.5	70	Stroke	nt
2	85 (M)	14.0	97.7	74	Stroke, arteriosclerotic obliterance	nt
3	72 (M)	10.8	98.2	63	Acute myocardial infarction, double aortic arch	9/9
4	73 (F)	15.2	89.3	55	Myocardial infarction	nt
5	15 (M)	8.2	61.6	–	Tetralogy of Fallot	nt
6	53 (M)	5.5	95.0	–	Aortic regurgitation	3/3
7	78 (M)	8.3	83.1	–	Cerebral hemorrhage	2/2
8	69 (M)	14.1	103.0	–	Arteriosclerotic obliteranse, chronic renal failure	nt
9	6 (F)	10.5	102.3	–	Ventricular septal defect	2/2
10	71 (F)	4.8	85.8	66	Angina pectoris, atrial septal defect	nt
11	74 (M)	11.6	78.7	63	Acute myocardial infarction	nt
12	73 (F)	1.5	107.0	–	Mitral stenosis	nt
13	13 (F)	11.7	104.4	–	Atrial septal defect, ventricular septal defect	3/3
14	55 (M)	13.6	95.7	50	Angina pectoris	nt
15	53 (M)	6.7	84.3	–	Cerebral hemorrhage	nt
16	56 (M)	17.7	79.8	55	Acute myocardial infarction	nt
17	72 (F)	7.3	73.1	65	Mitral stenosis	nt
18	71 (M)	9.7	88.3	64	Angina pectoris	nt
19	65 (M)	8.4	78.0	65	Stroke	nt

Plg-act, plasminogen activity; Plg-ag, plasminogen antigen. *The number of homozygotes or heterozygotes in the pedigree in which plasminogen activity was studied is indicated in the denominator. Within the numerator the total number of tested family members. M, male; F, female; nt, not tested.

Relevance of plasminogen deficiency to deep vein thrombosis and cardioembolic stroke due to NVAF

To establish whether plasminogen deficiency is a risk factor for thrombotic disorders, we identified plasminogen deficiency in 3 patients with DVT ($n = 108$) and in 6 patients with cardioembolic stroke due to NVAF ($n = 110$) by using the cut-off ratio of 0.69. Table 1 shows the prevalence of the plasminogen deficiency between the patient group and the age- and sex-matched controls. The results indicate that the prevalence was not different, indicating that plasminogen deficiency is not a risk factor for these thrombotic disorders.

Clinical phenotype of homozygous plasminogen deficiency

In an independent study, we screened for plasminogen activity in patients admitted to our hospital over 8 years, and identified 19 patients with extremely low plasminogen activity. Table 2 shows the characteristics and disease phenotypes of these patients. Most of the patients showed around 10% plasminogen activity (10.3% in average), except for patient 12 with only 1.5% activity. All of the patients had a normal level of antigen, indicating type II plasminogen deficiency, probably carrying plasminogen Tochigi mutation. Some of the patients had arterial thrombotic complications such as stroke ($n = 3$), MI ($n = 4$), or angina pectoris ($n = 3$). However, the disease occurred at advanced age, suggesting that homozygous plasminogen deficiency would not be a primary cause of these thrombotic diseases. None of the patients showed DVT.

Discussion

Here, we reported age- and gender-related changes of plasminogen activity using a Japanese general population comprising 4517 adults aged 32–89 years. Changes of the plasminogen activity have been reported in a healthy Scottish population comprising 9811 adults aged 17–65 years [25]. Comparing these two studies, our population covered an elderly population and the Scotland study covered a younger population. Age-related changes of plasminogen activity in the age groups

overlapping between the two studies were well consistent. In addition, we observed a decrease of plasminogen activity in the elderly, between 70–79 years and 80–89 years (Fig. 1). The low plasminogen activity in the elderly is probably affected by liver's ability to generate protein, because a decrease of albumin level with age also was observed ($r = -0.35$, $P < 0.0001$).

In the present study, we found the prevalence of plasminogen deficiency to be 4.30% in the Japanese general population ($n = 4517$), the prevalences of type I and II heterozygous plasminogen deficiency being 0.42% and 3.87%, respectively. The prevalence of type II plasminogen deficiency in the present study agreed with previously reported results in Japanese [6–9]. We also obtained the prevalence of plasminogen deficiency in patients with DVT and in patients with cardioembolic stroke and found that those prevalences were not different from those obtained from age-matched and sex-matched control groups selected from the general population. Most of these patients were residents in the northern Osaka area where the cohort study took place. Therefore, our study indicated that heterozygous plasminogen deficiency is not a risk factor for thrombotic complications.

We identified 173 heterozygotes and two homozygotes in 4517 individuals. If we assume that all those with type II deficiency carried the plasminogen Tochigi mutation, the Ala→Thr substitution at position 601, we can calculate the allele frequency of plasminogen Tochigi to be 1.96% in the Japanese general population. This allele frequency is similar to those of factor V Leiden mutation (2–7%) [26] and prothrombin 20210 A mutation (0.35–2.0%) [27] found in the Caucasian general population. We also identified 19 heterozygotes of type I plasminogen deficiency. The prevalence of type I plasminogen deficiency thus obtained (0.42%) showed quite good agreement with the previously observed prevalence in the Scotland population (28/9,611, 0.29%) [18].

There are several reports addressing a phenotype of mice with homozygous type I plasminogen deficiency [28,29]. The plasminogen gene in these mice was abnormal so that no plasminogen activity was present in plasma, resulting in spontaneous fibrin deposition due to impaired thrombolysis. One of the useful features of engineered mice is that although a transgenic or knockout gene may have no phenotype, a phenotype may become apparent with a physiological or pathological challenge. For example, mice deficient in plasminogen exacerbated renal injury in experimental crescentic glomerulonephritis [30]. Those mice also abolished wound healing after myocardial infarction [31]. These studies suggest that even though individuals with plasminogen deficiency did not show venous thrombosis, they may express a certain phenotype after a challenge or insult. Therefore, careful continuous observation in individuals with plasminogen deficiency is required for assessment of relation of plasminogen deficiency with its phenotype.

In conclusion, the prevalence of heterozygous plasminogen deficiency is about 4% in Japanese, and plasminogen deficiency is not a primary cause of thrombosis. This conclusion was also supported by the phenotypes of 19 patients with homozygous type II plasminogen deficiency.

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Addendum of the roles of authors

Drs Okamoto, Sakata, Mannami, and Miyata were responsible for the study design, interpretation of the data and preparation of the article. Drs Baba, Katayama, Matsuo, Yasaka, Minematsu, and Tomoike were responsible for sample collection, steering and discussion.

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Extracorporeal Double Filtration Plasmapheresis in Acute Atherothrombotic Brain Infarction Caused by Major Artery Occlusive Lesion

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Extracorporeal double filtration plasmapheresis (EDFP) can quickly lower plasma viscosity and fibrinogen concentration. EDFP has the potential to improve cerebral microcirculation in acute ischemic stroke and ultimately to salvage penumbral tissue. However, no evidence is available to show that EDFP can increase cerebra blood flow (CBF). Therefore, we investigated whether EDFP could increase CBF by quantitative CBF measurements and documented the clinical effects of EDFP in acute ischemic stroke. EDFP was performed ten times in seven patients diagnosed as having acute atherothrombotic brain infarction caused by major artery occlusive lesion. They also fulfilled one of the following entry criteria: 1) diffusion/perfusion mismatch demonstrated by MRI on admission; 2) a hemispheric syndrome, but only a small lesion on diffusion weighted MRI (< 25% of MCA territory); or 3) progressing stroke. Exclusion criteria were 1) contraindication of heparin or 2) spontaneous improvement of symptoms. Time from stroke onset to EDFP varied from 5 hr to 7 days. Plasma viscosity was quickly lowered by EDFP without affecting RBC counts, Hb, or Hct in all patients. Positron emission tomography (PET) with 15-O labeled H_2O measurements revealed a significant CBF increase from 36.4 ± 8.3 ml/100 g/min to 40.7 ± 6.8 ml/100 g/min in the affected hemisphere ($P = 0.048$). Definite CBF improvement was also demonstrated by single photon emission computed tomography (SPECT) in one of two patients who had severe stenosis of the middle cerebral artery. Furthermore, this patient showed remarkable improvement of hemiplegia immediately following EDFP (NIHSS score: 18 to 13). In conclusion, EDFP can increase CBF in ischemic brain tissue in acute atherothrombotic brain infarction. Further clinical studies should focus on the efficacy of EDFP on outcome of patients with this stroke subtype. J. Clin. Apheresis 18:167–174, 2003. © 2003 Wiley-Liss, Inc.

Key words: plasmapheresis; brain ischemia; cerebral blood flow; recanalization

INTRODUCTION

The ischemic penumbra is a major target in the treatment of acute brain infarction. A reversal of ischemic neurological deficits can be achieved by blood flow restoration in the penumbral tissue. In fact, striking improvements of ischemic neurological deficits have been documented after the early reopening of occluded vessel by spontaneous clot lysis [1–3] or thrombolytic therapy [4–6]. Recent progress in magnetic resonance imaging has enabled us to identify a diffusion/perfusion mismatch area as the ischemic area with a high risk of progression to irreversible damage [7,8]. If local perfusion does not improve within a certain time frame, a significant part of the penumbra or the mismatch area will deteriorate to infarct [9]. Injection of recombinant tissue-type plasminogen activator (rt-PA) can significantly improve outcome if it is initiated within 3 hr of stroke onset [4]. However, fewer than 5% of stroke patients receive rt-PA therapy because of this time constraint and other limiting criteria [10]. Even when the occluded vessel can not be opened, the blood flow restoration in penumbral tissue could theoretically be

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achieved by a rheological modification. Based on the Hagen-Poiseulle equation ($Q = \Delta Pr^4/8\ L\eta$ where Q is blood flow, ΔP is the pressure gradient, r is the vessel radius, L is length, and η is the viscosity), blood flow can be increased by lowering the blood viscosity [11]. Effects of hematocrit (Hct) lowering by venesection and infusion on acute stroke have been investigated by several randomized trials [12–16]. However, no clinical studies have succeeded in confirming any favorable effects of these hemodilution therapies. Recently, extracorporeal plasmapheresis was applied to ischemic stroke patients to quickly lower their plasma viscosity by the removal of macromolecules from plasma without removal of red blood cells (RBC), so as not to reduce oxygen transport capacity [17–20]. This process also lowers the fibrinogen concentration. It is known that a high level of fibrinogen concentration is an independent risk factor for development of stroke. Thus extracorporeal plasmapheresis would appear to be an ideal method for rheological intervention in the acute stage of ischemic stroke. However randomized study failed to demonstrate any beneficial effects of extracorporeal double filtration plasmapheresis (EDFP) in patients with sudden onset of ischemic hemispheric stroke within 6 hr [18]. We suspects that this failure might be the result of patient selection. Because sudden onset in seconds or minutes of a maximum neurological deficit is one of the characteristic features of embolic stroke, patients with embolic occlusion of a major cerebral artery would have been included in the previous study. In embolic infarction, there is usually not time for collateral circulation to become established, and the cerebral blood flow (CBF) decline is usually greater than in atherothrombotic infarction. Therefore, the beneficial effects of rheological modification by EDFP would be better demonstrated in patients with atherothrombotic brain infarction caused by major artery occlusive lesion that shows a diffusion/perfusion mismatch or in patients with a progressing stroke. In such patients, a large amount of hemodynamically compromised tissue including ischemic penumbra would be present. We undertook an investigation of such patients to determine if EDFP increased CBF in ischemic brain tissue and whether any reversal of ischemic neurologic deficits could be observed after EDFP.

MATERIAL AND METHODS

We studied seven patients with a mean age of 65.4 ± 6.6 years, who were admitted to our Stroke Care Unit from September 1999 to December 2000. They were diagnosed as having an acute atherothrombotic brain infarction caused by occlusive disease of a major cerebral artery, which was confirmed by cerebral angiography or magnetic resonance angiography (MRA) on admission. They also fulfilled one of the following entry criteria: 1) diffusion/perfusion mismatch demonstrated by MRI on admission; 2) a hemispheric syndrome, but only a small lesion on diffusion weighted MRI (<25% of MCA territory); or 3) progressing stroke. A hemispheric syndrome was defined as a complex of neurological deficits that consisted of various degrees of hemiparesis, consciousness disturbance, conjugate ocular deviation, and cortical signs such as aphasia or neglect syndrome. Patients were excluded if they had 1) cardioembolic stroke, 2) contraindication of heparin, or 3) spontaneous improvement of symptoms. The vascular lesions and clinical picture of the seven patients are listed in Table I. Diffusion/perfusion mismatch was observed in three patients (patients 1, 3, and 4 in Table I). A 1.5 T EPI-equipped whole body scanner was used (MAGNETOM VISION, Siemens Medical Systems, Inc., South Iselin, NJ) and isotropic diffusion-weighted images were obtained by using a multislice, single-shot spin-echo EPI sequence (b value = 1,000). Perfusion-weighted images were obtained after a bolus of gadolinium-DTPA and time-to-peak images were created. Three patients were enrolled because of a hemispheric syndrome with only a small infarct volume on the initial diffusion weighted MRI (patients 2, 6, and 7 in Table I). The other patient was also enrolled because of a gradual progression of ischemic symptoms resulting from basilar artery occlusion (patient 5).

After obtaining a written informed consent from the next of kin of each patient, EDFP was started. A double lumen catheter (Flexxicon dual lumen DLC-4010, Vas-cath Inc., Ontario, Canada) was inserted into the femoral vein. For the primary plasma separation and the plasma differential separation, a Plasmaflo OP-05W and a Cascadeflo AC-1760 (Asahi Medical Co., Tokyo) were used, respectively. Heparin was continuously injected at 2,500 IU/hr. The procedure lasted about 90 min and 1.0–2.0L of plasma were filtered. Blood sampling was performed before and after EDFP to measure blood viscosity and other various rheological and biochemical parameters including LDL-cholesterol, fibrinogen, and α2-macroglobulin. It is known that plasma viscosity can be quickly lowered after EDFP but will gradually return to the initial hemorheological condition. To maintain low plasma viscosity level, EDPF should be repeated every 12 to 24 hr [18]. We repeated EDFP when fluctuation of symptoms and signs was observed more than 24 hr after the initial EDFP.

Quantitative CBF measurements were performed by using positron emission tomography (PET) (ECAT EXACT47, Siemens Medical Systems, Inc.) with the

TABLE I. Patient Profiles and Changes in Plasma Viscosity, Fibrinogen Concentration, and Neurological Deficits Before and Immediately After Initial Extracorporeal Double Filtration Plasmapheresis (EDFP)*

Patients	Vascular lesion	EDFP	Timing	Plasma viscosity (mPa)	Fibrinogen (mg/dl)	NIHSS score before and after EDFP[a]
1.54, M	Lt. IC occlusion	1st	14 h	1.503 → 1.289	423 → 248	12 → 12 (4)
2.62, M	Rt. M1 occlusion	1st	4 days	1.673 → 1.493	385 → 278	13 → 13 (5)
3.62, M	Lt. M1 stenosis	1st	8 h	1.369 → 1.188	243 → 138	18 → 13 (6)
4.74, M	Rt IC stenosis	1st	7 days	1.576 → 1.436	439 → 254	5 → 5 (3)
5.70 M	BA stenosis	1st	4 days	1.464 → 1.287	297 → 221	19 → 19 (16)
6.69, M	Rt. IC stenosis	1st	11 h	1.394 → 1.197	234 → 164	17 → 17 (13)
7.67, M	Rt. IC stenosis	1st	15 h	1.288 → 1.184	255 → 191	17 → 17 (0)
	Mean ± SD		2.4 ± 2.6 day	1.467 ±0.131 → 1.296 ± 0.124	325.1 ± 88.4 → 213.4 ± 51.2	14.4 ± 4.9 → 13.7 ± 4.6
	P value			*P* < 0.0001	*P* = 0.001	n.s.

*IC, internal carotid artery; MI, stem of the middle cerebral artery; BA, Basilar artery; NIHSS, NIH Stroke Scale.
[a]() NIHSS score at one month after onset.

TABLE II. Cerebral Blood Flow (CBF) Change Before and Immediately After Extracorporeal Double Filtration Plasmapheresis (EDFP)*

Patients	Vascular lesion	EDFP	Timing	Plasma viscosity (mPa)	Fibrinogen (mg/dl)	CBF change (ml/100 g/min) Affected hemisphere	Non-affected hemisphere
1.54, M	Lt. IC occlusion	2nd	6 days	1.514 → 1.391	321 → 248	Lt: 37.9 → 45.8	Rt: 52.3 → 52.4
2.62, M	Rt. M1 occlusion	1st	4 days	1.673 → 1.493	385 → 278	Rt: 28.7 → 32.6	Lt: 45.3 → 44.9
3.62, M	Lt. M1 stenosis	1st	8 h	1.369 → 1.188	243 → 138	(SPECT: significant improvement)	
4.74, M	Rt. IC stenosis	1st	7 days	1.576 → 1.436	439 → 254	Rt: 47.9 → 48.9	Lt: 42.0 → 41.5
5.70 M	BA, Lt M1 stenosis	2nd	9 days	1.479 → 1.370	291 → 220	Lt: 28.9 → 35.4	Rt: 27.9 → 35.7
6.69, M	Rt IC stenosis	2nd	7 days	1.607 → 1.281	371 → 236	Rt: 39.4 → 40.4	Lt: 34.3 → 34.3
7.67, M	Rt. IC stenosis	1st	15 h	1.288 → 1.184	255 → 191	(SPECT: no significant change)	
	Mean ± SD		4.9 ± 3.3 day	1.501 ± 0.135 →1.335 ± 0.120	329.3 ± 72.3 → 223.6 ± 46.6	36.4 ± 8.3 → 40.7 ± 6.8	40.6 ± 9.2 → 41.7 ± 7.4
	P value			*P* = 0.0013	*P* = 0.0006	*P* = 0.048	n.s.

*IC, internal carotid artery; M1, stem of the middle cerebral artery; BA, Basilar artery; NIHSS, NIH Stroke Scale.

^{15}O-labeled water bolus injection method before and after the first or second EDFP in five patients. The spatial resolution of the PET scanner was 4.5 mm in-plane at full width at half maximum and 4.5 mm axially. Patients were positioned on the PET couch in the supine position and the right brachial artery was catheterized for obtaining the blood counts and monitoring the blood pressure. The head was firmly fixed in a headrest restraint with a plastic collar (Philadelphia Collar, Canada). During the acquisition of PET scans, arterial blood pressure and ECG were continuously monitored. Using a power injection system (CYPRIS®, ^{15}O-water injection module, O1-C, Sumitomo Heavy Industries, Ltd.), a 943.5 MBq of $H_2{}^{15}O$ was injected via a 22-gauge Teflon cannula inserted into the left antecubital vein through a 0.22 μm filter (MILLEX-GV, Millipore Co., Ltd., Billerica, MA) at a rate of 0.4 ml/sec for 50 sec with saline flushing. Arterial blood was continuously withdrawn at a constant speed of 3.82 ml/min using an infusion/withdrawal pump (Harvard Apparatus, South Natic, MA). The radioactivity concentration in the arterial blood was measured using a beta-ray detector (Hamamatsu Photonix, Japan) to calculate the input function. Blood withdrawal was started 10 sec prior to the ^{15}O-labeled water injection and continued until 180 sec after the injection. The acquisition time for each CBF measurement was 90 sec. Mean CBF values were calculated by placing circular regions of interest (ROIs) in the cortical area. Because of the constancy of arterial $PaCO_2$ levels before and after the EDFP, CBF values were not corrected by CO_2 levels. In the other two patients, PET measurement was not performed but qualitative CBF evaluations were performed using single photon emission computed tomography with ^{99m}Tc-hexa-methylpropylene amineoxime (^{99m}Tc-HMPAO-SPECT) before and after the initial EDFP.

RESULTS

EDFP was performed 10 times in seven patients. The initial EDFP was started from 5 hr to 4 days after the onset of ischemic symptoms. EDFP significantly

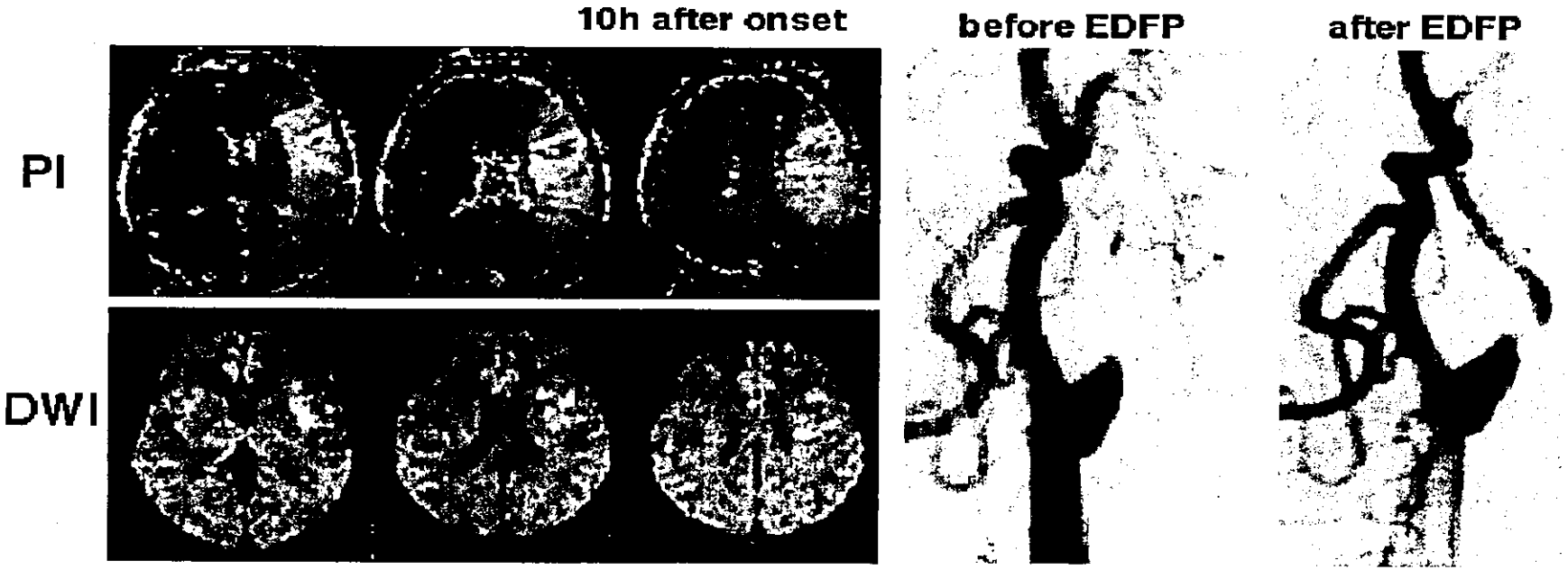

Fig. 1. MRI images and cerebral angiograms in Patient 1. PI, perfusion image (time-to-peak image); DWI, diffusion-weighted image.

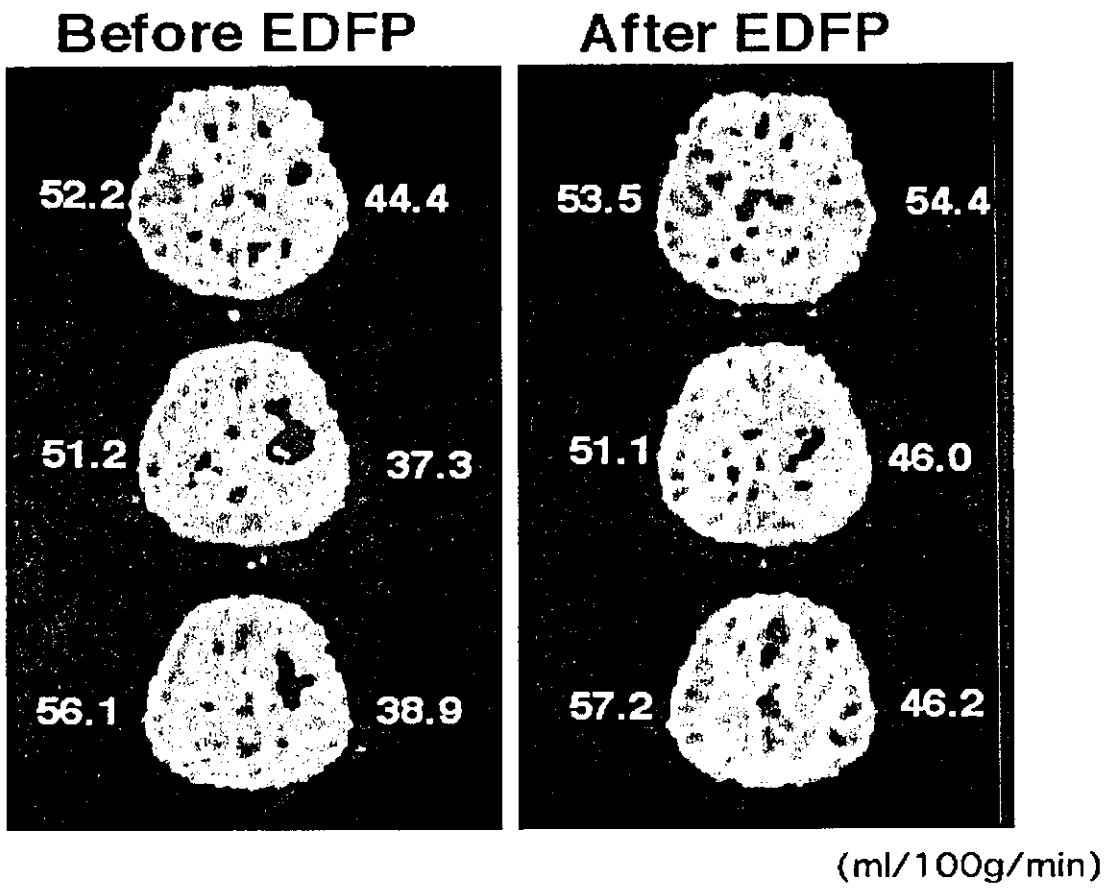

Fig. 2. PET images just before and after the plasmapheresis in Patient 1. A CBF increase of up to 10 ml/100 g/min was observed in the left hemisphere ipsilateral to the occlusion. No significant change was observed in CBF in the contralateral hemisphere.

lowered LDL-cholesterol, fibrinogen, and α2-macroglobulin (354.3±134.8 to 209.3±72.4 mg/dl, 325.1±88.4 to 213.4±51.2 mg/dl, and 152.4±73.3 to 107.0±48.3 mg/dl, respectively). Plasma viscosity also significantly decreased from 1.467±0.131 to 1.296±0.124 mPas ($P<0.0001$) but there was no significant change in NIH Stroke Scale score before and just after the initial EDFP (Table I). There were no significant changes in whole blood viscosity, RBC counts, hemoglobin, Hct, serum electrolyte concentrations, prothrombin time, active prothrombin time, plasminogen, anti-thrombin III level, or D-dimer (data not shown). PET measurements revealed a CBF increase up to 10 ml/100 g/min in the ischemic hemisphere (36.4±8.3 to 40.7±6.8 ml/100g/min, $P=0.048$). However, no significant increase was observed in the non-affected hemisphere (Table II). Definite CBF improvement after EDFP was also demonstrated on SPECT images in one of the other two patients who had severe stenosis of the MCA (patient 3 in Table II). This patient's hemiplegia showed definite improvement immediately after EDFP (NIH stroke scale score: 18 to 13). Repeated vascular examination revealed recanalization of the occluded internal carotid artery in one patient (patient 1 at 10 days after EDFP) and improvement of perfusion abnormality in MRA in one patient (patient 3 at 20 hr after EDFP).

Illustrative Cases

Patient 1. A 54-year-old man, with a 5-year history of treated hypertension, was admitted for the right hemiparesis and aphasia. Blood pressure was 196/116 mmHg and routine blood tests were normal. NIH stroke scale score was 12. Cerebral angiography revealed an occlusion of the left internal carotid artery (ICA), and diffusion/perfusion mismatch was demonstrated in the ipsilateral hemisphere 10 hr after the onset of ischemic symptoms (Fig. 1). Transesophageal echocardiography and transoral carotid ultrasound did not reveal any specific embolic source nor the findings of carotid dissection [21,22]. We diagnosed the occlusion was atherothrombotic in nature. EDFP was initiated 14 hr after onset. Plasma viscosity and fibrinogen concentration were decreased from 1.503 to 1.289 mPa and from 423 to 248 mg/dl, respectively. Aphasia was slightly improved but NIH stroke scale score was not changed after EDFP. The second EDFP was performed 6

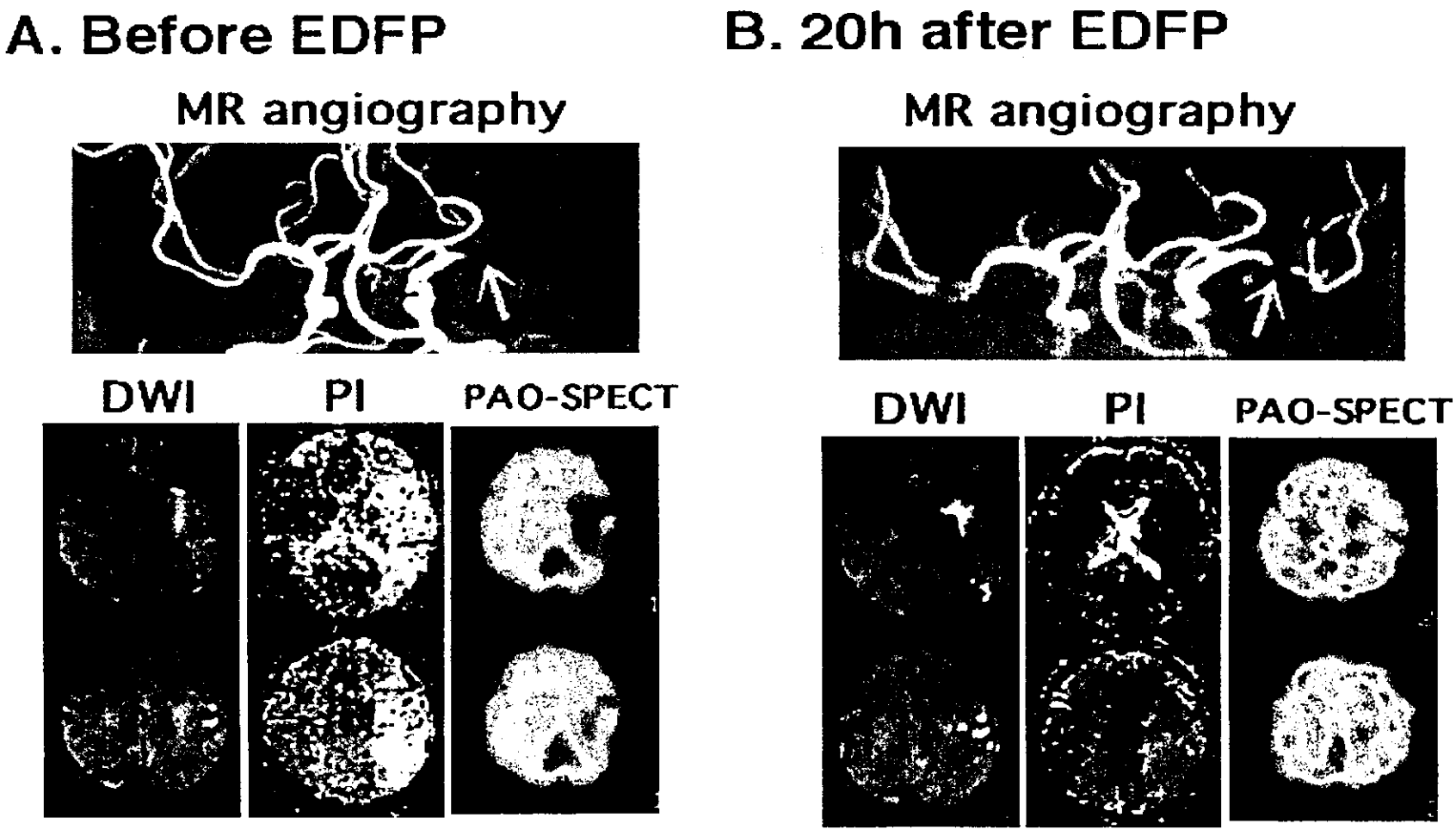

Fig. 3. Neuroradiological assessment before and after the plasmapheresis in Patient 3. **A:** Before the extracorporeal double filtration plasmapheresis (EDFP), MR angiography revealed severe stenosis in the middle cerebral artery (arrow) and diffusion/perfusion mismatch was observed in diffusion-weighted image (DWI), perfusion image (PI), and ^{99m}Tc-HMPAO-SPECT. **B:** Twenty hr after the EDFP, improvement of distal opacification of the middle cerebral artery was demonstrated by MR angiography (arrow). A significant CBF improvement was observed both in ^{99m}Tc-HMPAO-SPECT and perfusion image (PI), and consequently a diffusion/perfusion mismatch was not observed.

days after onset with the monitoring of CBF by quantitative PET imaging. After the second EDFP, plasma viscosity and fibrinogen concentration were decreased from 1.514 to 1.391 mPa and from 321 to 248 mg/dl, respectively. The baseline CBF study showed an area of significant hypoperfusion over the left MCA territory. A significant increase of CBF was observed after EDFP in the ischemic hemisphere from 37.3 to 46.0 ml/100 g/min (Fig. 2). Ten days after the onset of stroke, a duplex ultrasonographic examination detected recanalization of the occluded left ICA. This was confirmed by cerebral angiography performed before the carotid endarterectomy, which was performed 1.5 months after the onset (Fig. 1). After the carotid endarterectomy, the patient was discharged with an NIH stroke scale score of 4 and Rankin scale of 1.

Patient 3. A 62-year-old man, with a 3-year history of treated diabetes and hypertension, presented with right hemiplegia, conjugate ocular deviation to the left, and global aphasia. On admission, CT scan showed only a slight cerebral atrophy. Blood pressure was 140/80 mmHg and routine blood tests were normal. NIH stroke scale score was 18. Four hr after the onset of symptoms, MRA and MRI studies revealed a severe stenosis of the left MCA trunk and diffusion/perfusion mismatch in the left hemisphere; ^{99m}Tc-HMPAO-SPECT also demonstrated severe hypoperfusion over the left MCA territory. EDFP was started 8 hr after onset. Plasma viscosity and fibrinogen concentration were decreased from 1.369 to 1.188 mPa and from 243 to 138 mg/dl, respectively. During EDFP, his neurological deficits gradually improved. At the end of the EDFP, he could raise his right arm and leg. NIH stroke scale score improved from 18 to 13. Twenty hr after the EDFP, MRI studies and ^{99m}Tc-HMPAO-SPECT were repeated. An improvement of the stenotic lesion of the left MCA was revealed by MRA. The ischemic lesion on the diffusion MRI was not enlarged and the diffusion/perfusion mismatch had vanished. The ^{99m}Tc-HMPAO-SPECT also demonstrated a dramatic CBF improvement in the MCA territory (Fig. 3). He was discharged with an NIH stroke scale score of 6 and Rankin scale of 1.

DISCUSSION

The therapeutic principle of EDFP in acute ischemic stroke is to increase CBF in penumbral tissues by reducing plasma viscosity and ultimately to minimize ischemic neurological deficits. However, little work has been done to study whether EDFP can increase CBF in acute stroke patients. As far as we know, the present study is the first designed to elucidate the quantitative change in CBF after EDFP in acute ischemic stroke. Quantitative CBF can be repeatedly measured by PET with $H_2{}^{15}O$ within 2 min. It is ideal to monitor CBF change by PET during all EDFP procedures. However, PET measurement was performed in five of ten EDFP because of the technical complexity. The observed maximum CBF increase in our study was 10 ml/100 g/min. Previous human PET studies have demonstrated that a region with CBF between 12 and 22 ml/100 g/min can be characterized as an ischemic penumbra [23,24]. Thus the extent of the CBF increase we have demonstrated is sufficient to salvage the penumbral tissue. It is of great interest that the CBF increase was observed only in the ischemic hemisphere ipsilateral to the major cerebral artery that had the occlusive lesion. This observation concurs with the results of an experimental study using rats. Lenz et al. [25] demonstrated that changes in blood viscosity do not result in changes in the CBF as long as the blood vessels retain their capacity to compensate for the changes in viscosity by vasodilating with increased viscosity and vasoconstricting with decreased viscosity. It is well known that, even in the normal brain, a significant increase in CBF can be induced by lowering the hematocrit. A recent experimental study demonstrated that 40–60% of the increased CBF after venesection and infusion could be the result of compensatory vasodilation due to reduction of arterial oxygen content. This would imply that hematocrit lowering, although widely accepted as a method of hemodilution, cannot achieve a penumbra rescue [26].

In a clinical setting, EDFP should be used in patients who have an ischemic penumbra and who will thus benefit from EDFP and show definite recovery from ischemic neurological deficits. Therefore we only selected patients with lesions on DWI smaller than the ischemic area that would be suspected, based on neurological signs or perfusion images, or patients with progressing stroke caused by atherothrombotic major cerebral artery occlusion. We succeeded here in demonstrating a favorable recovery in four patients. In these patients, the NIH Stroke Scale score improved more than 8 points from baseline (5–17 points) in the first month after EDFP (Table I). A definite reversal of neurological deficits was demonstrated immediately following EDFP in patient 3. In this patient, we observed dramatic improvement of MRA finding as well as CBF improvement. Recanalization of an occluded vessel was observed in one patient 10 days after EDFP. This might be merely the natural course. On the other hand, we could hypothesize that significant reduction of fibrinogen concentration achieved with EDFP accelerate the recanalization of occluded vessels by the lysis of fresh thrombi that were superimposed on an atherosclerotic arterial lesion. It has been shown that the reduction of fibrinogen concentration may be ben-

eficial in ischemic stroke. In one study, a significant improvement of functional status at 90 days after stroke onset was demonstrated in patients with intravenous ancrod, a purified fraction of venom from the Malaysian pit viper (*Calloselasma rhodostoma*) to achieve rapid defibrinogenation [27]. In addition, increased cerebral CO_2 reactivity after defibrinogenation by ancrod or heparin-mediated extracorporeal LDL precipitation (HELP) has also been demonstrated in humans [28,29].

In conclusion, EDFP has the potential to improve CBF in ischemic tissues and to ultimately salvage penumbral tissues. Given the positive findings of this study, further prospective studies are required, especially in patients with atherothrombotic infarction.

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SHORT REPORT

Isolated pulmonary arteriovenous fistula without Rendu-Osler-Weber disease as a cause of cryptogenic stroke

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There has been uncertainty as to whether a right to left shunt through an isolated pulmonary arteriovenous fistula (P-AVF) without Rendu-Osler-Weber (ROW) disease can cause paradoxical brain embolism. A population of 747 acute ischaemic stroke patients was examined to determine the frequency and clinical characteristics of those patients who had an isolated P-AVF. The presence of a P-AVF was determined as follows. On patients with a stroke of undetermined cause, both transoesophageal echocardiography and transcranial Doppler with saline contrast medium was performed to detect a right to left shunt. If a P-AVF was then suspected, selective pulmonary angiography and enhanced chest CT was performed to confirm the presence of the P-AVF. Four patients (0.5%) were diagnosed as having a stroke associated with an isolated P-AVF. All the patients were middle-aged women (mean age 61 years). In all these patients, the P-AVF could not have been suspected on physical findings or chest *x* ray. The P-AVF was always single and located in the lower lobe. All the patients had asymptomatic deep venous thrombosis, and three patients developed pulmonary embolism. As D-dimer and thrombin–antithrombin complex were elevated in all patients, this indicated an activation of both fibrinolytic and thrombin activity. Our results show that an isolated P-AVF without ROW disease can cause paradoxical brain embolism. Thus, the existence of an isolated P-AVF as a right to left shunt in patients with a stroke of unknown origin should not be overlooked, even if a P-AVF is not suggested by the initial physical findings or chest *x* ray.

Rendu-Osler-Weber (ROW) disease is characterised by multiple dermal, mucosal, and visceral telangiectasia that are associated with recurrent bleeding, and by a pulmonary arteriovenous fistula (P-AVF) in 15% of patients.[1] The right to left shunt caused by P-AVF with ROW disease can cause paradoxical brain embolism.[1 2] To the best of our knowledge, however, only four cases of paradoxical brain embolism associated with an isolated P-AVF without ROW disease, including our one previous case, have been reported.[3–6] Therefore, the question remains as to whether an isolated P-AVF is associated with ischaemic stroke, and, in particular, with paradoxical brain embolism. The aims of this study were to investigate the frequency of brain infarction associated with an isolated P-AVF, to evaluate the salient clinical characteristics of this condition, and to elucidate a mechanism for the development of ischaemic stroke in these patients.

SUBJECTS AND METHODS

We reviewed the records of 747 consecutive ischaemic stroke patients who were admitted to our division within 7 days of stroke onset between August 1998 and December 2002. We identified those patients with a brain infarction that was associated with an isolated P-AVF without ROW disease. The diagnosis of ROW disease was made clinically based on the "classic triad" of telangiectasia, recurrent epistaxis, and a family history of the disorder. The presence of a P-AVF was determined as follows. When we had a patient with a stroke of undetermined cause, we always performed both a transoesophageal echocardiography (TOE) with saline contrast medium and a transcranial Doppler (TCD) with saline contrast medium so as to detect a right to left shunt, such as a patent foramen ovale (PFO) or a P-AVF. A patient with a stroke of undetermined cause was defined as a patient who did not have a lacunar stroke; did not have more than a 50% stenosis in the cerebral artery irrigating the affected lesions; and had no potential cardiac sources of emboli (such as atrial fibrillation, acute myocardial infarction, old myocardial infarction with intraventricular thrombus, mitral valve disease, prosthetic valve, implantation of a pacemaker, or a dilated cardiomyopathy). If a P-AVF was suspected based on the findings of the TCD study with saline contrast medium that detected microembolic signals (MES) through the middle cerebral artery or the basilar artery, and the TEE did not demonstrate a PFO,[7] we then always performed selective pulmonary angiography and enhanced chest CT so as to confirm the presence of the P-AVF. Pulmonary embolism was diagnosed by lung perfusion scintigraphy, and deep venous thrombosis (DVT) was diagnosed by venography and/or ultrasonography.

RESULTS

ROW disease was not observed in the 747 patients studied. However, seven patients were suspected as having a P-AVF by TCD and TEE studies. Pulmonary angiography and chest CT studies could not confirm a P-AVF in three of these patients. Therefore, four patients (0.5%), including our previously reported patient (case 1),[6] were diagnosed as having an embolic stroke associated with an isolated P-AVF. TCD studies in all these patients showed that MES were detected during normal breathing without having to perform a Valsalva manoeuvre or a cough. The clinical characteristics of all four patients are shown in the table. None of the patients had clinical evidence of hypoxia, such as cyanosis, dyspnea, and erythrocytosis. We could not auscultate vascular sounds in the lung fields, and the chest

Abbreviations: DVT, deep venous thrombosis; MES, microembolic signals; P-AVF, pulmonary arteriovenous fistula; PFO, patent foramen ovale; ROW, Rendu-Osler-Weber; TCD, transcranial Doppler; TOE, transoesophageal echocardiography

Table 1 Clinical characteristics of four patients with P-AVF without Rendu-Osler-Weber disease

	Case 1	Case 2	Case 3	Case 4
Gender	Female	Female	Female	Female
Age (years)	62	59	50	68
Location of brain infarction	Left MCA Territory	Thalamus	Cerebellum	Cerebellum
Medication before stroke onset	–	Ticlopidine (200 mg)	–	Aspirin (80 mg)
History of TIA or brain infarction	+	+	+	+
Risk factor	–	HT, HL	–	HT, HL
Chest *x* ray	Normal	Normal	Normal	Normal
Location of P-AVF in lung	Right lower	Right lower	Right lower	Left lower
Single or multiple P-AVF	Single	Single	Single	Single
Size of P-AVF (mm) on CT	5	7	2	4
DVT	+	+	+	+
Pulmonary embolism	+	+	+	–
RBC count×10 000/μl	377	404	383	327
Hg (g/dL)	11.1	12.0	8.2	10.6
Ht (%)	30.2	38.0	26.8	32.3
PaO_2 (mmHg)	76	85	88	83
TAT μg/L	4.2	3.7	2.7	2.3
D-dimer μg/L	2.4	1.5	3.9	1.1
After embolisation of P-AVF				
Medication	Free	Warfarin	Warfarin	Warfarin
Recurrent stroke	–	–	–	BH
Observation interval (months)	57	45	14	13

MCA, middle cerebral artery; TIA, transient ischaemic attack; HT, hypertension; HL, hyperlipidaemia; BH, brain haemorrhage; TAT, thrombin–antithrombin III complex; P-AVF, pulmonary arteriovenous fistula; DVT, deep venous thrombosis; RBC, red blood cell

x rays did not demonstrate any abnormality, such as a nodular density in the bilateral lung lobes, which would have led us to suspect P-AVF. The P-AVF found on lung CT and pulmonary angiography was always single and located in the lower lobe. The mean size of the P-AVF on CT was 4.5 mm. All patients had pulmonary embolism, and three had asymptomatic deep venous thrombosis. D-dimer and thrombin–antithrombin complex were elevated in all patients, indicating the activation of both fibrinolytic and thrombin activity.

The P-AVFs were occluded with catheter embolisation within 2 months of stroke onset. Our follow up was conducted for an average of 32 months (range 13–57 months), and no recurrent ischaemic strokes were documented, but one patient (case 4) had a thalamic haemorrhage 8 months after catheter embolisation of the P-AVF. This patient had been treated with warfarin and her international normalised ratio was 2.2 at the onset of the brain haemorrhage.

Fig 1 shows the findings of case 2: a pulmonary angiography with a P-AVF; a venogram of the lower limbs showing a DVT; and lung perfusion scintigraphy showing pulmonary embolism.

DISCUSSION

We have presented four acute ischaemic stroke patients with P-AVF not associated with ROW disease. None of the patients had cardiac or arterial sources for the emboli. Furthermore, all the patients had DVTs and three patients developed pulmonary embolism. Therefore, we diagnosed all these patients as having had paradoxical brain embolism through a P-AVF. We conclude that an isolated P-AVF can cause paradoxical brain embolism.

It is of interest to note that the size of the P-AVFs differed between previously reported cases and our patients. The previously reported cases, due to the large size of the P-AVFs,[1–5] had physical findings, such as auscultate vascular sounds, or abnormalities on chest *x* ray. In contrast, in all of our patients the P-AVFs could not be suspected on physical findings or chest *x* ray, because of their small size. Therefore, the presence of P-AVF in stroke patients who have normal physical findings and no chest *x* ray abnormalities cannot be excluded.

A TCD with saline contrast medium performed on all patients was useful in identifying the presence of a P-AVF during the acute phase of the stroke. As a persistent right to left shunt occurs in a P-AVF, micro air bubbles can be detected in the cerebral arteries using TCD with saline contrast medium during normal breathing without the need to use provocative methods, such as a Valsalva manoeuvre or a cough.[7] Therefore, in patients who have had an embolic

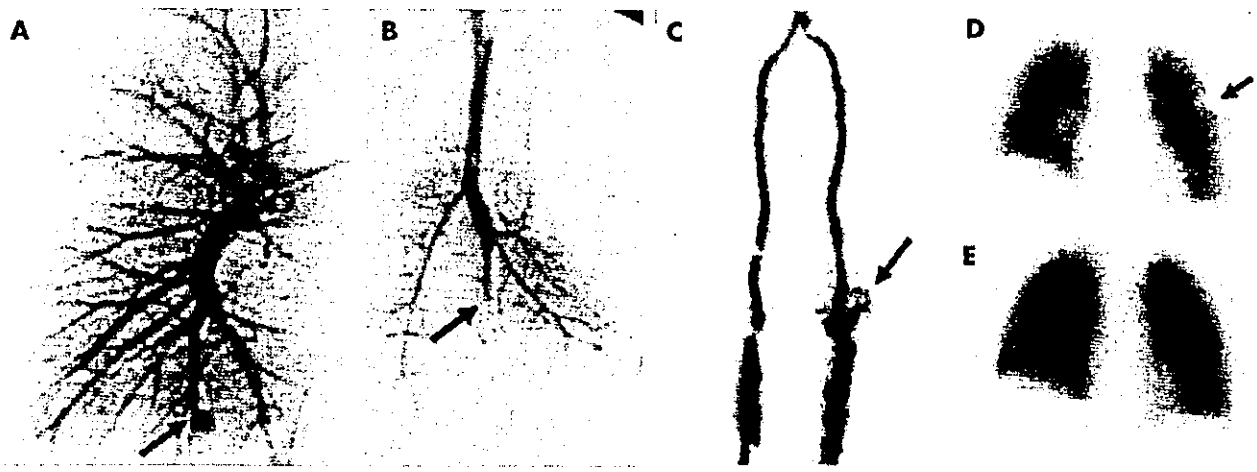

Figure 1 The pulmonary angiography (A, B), venogram of the lower limbs (C), and the ventilation–perfusion lung scintigraphy (D, E) of case 2. (A) Selective pulmonary angiography 30 days after stroke onset shows a P-AVF (arrow) in the right lower lobe. (B) After embolisation therapy with a metal coil, the feeding vessels to the P-AVF are completely occluded. (C) Venogram of the left lower limbs 4 days after stroke onset shows the abnormal collateral flow (arrow) in the leg veins, indicating a deep venous thrombus. (D) Lung perfusion scintigram 4 days after stroke onset shows the defect (arrow) in the left upper lung, indicating a pulmonary embolism. (E) The follow-up study taken 24 days after stroke onset shows no perfusion defect in the left upper lung.

stroke of undetermined cause and cannot perform a Valsalva manoeuvrer or a cough because of aphasia or a disturbance of consciousness, TCD with saline contrast medium can help detect a P-AVF in the acute phase of stroke.

Catheter embolisation is a safe and effective treatment for P-AVF.[6] All four patients had a history of ischaemic stroke or TIA prior to the present stroke. After catheter embolisation of their P-AVF, none of these patients had a recurrent ischaemic stroke. Thus, catheter embolisation of a P-AVF appears to be an effective method of preventing recurrent ischaemic stroke in such patients.

In conclusion, an isolated P-AVF without ROW disease can cause cryptogenic stroke. Thus, one should not overlook an isolated P-AVF as a right to left shunt in patients with a stroke of unknown origin, even when physical findings or chest *x* ray findings are not suggestive of a P-AVF.

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A case of frequently recurring amaurosis fugax with atherothrombotic ophthalmic artery occlusion

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Abstract—A 49-year-old woman with diabetes mellitus and hyperlipidemia experienced frequent transient monocular blindness in the right eye during a 3-week period. Examination revealed atherothrombotic occlusion of the right ophthalmic artery (OA) without embolic sources. After treatment with aspirin, attacks resolved completely. Her symptoms were attributed to microembolism from the occluded OA.

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Amaurosis fugax, or transient monocular blindness, is an abrupt loss of vision in one eye usually lasting seconds or minutes. Visual loss is predominantly attributed to microembolism from atherosclerotic lesions of the ipsilateral internal carotid artery (ICA)[1,2] or other lesions of structures, including the aortic arch and heart.[3] We encountered a patient experiencing frequent episodes of right-sided visual loss for 3 weeks before medication. She displayed atherothrombotic occlusion of the right ophthalmic artery (OA) without embolic sources in the ipsilateral carotid artery, aortic arch, or heart. To the best of our knowledge, this is the first reported case of frequently recurring amaurosis fugax associated with OA occlusion.

Case report. A 49-year-old woman with diabetes mellitus and hyperlipidemia medicated with insulin, oral hypoglycemic agents, and antihyperlipidemic agents for 10 years was referred to our hospital with transient right-side monocular blindness. She had not been taking contraceptive medication. Symptoms first appeared 1 month before admission and occurred once or twice daily for 3 weeks. She complained that the symptom began spontaneously with faint purple speckles, and gradually her vision was lost. Attacks tended to last less than 1 minute, and the patient reported no problems in the left eye. She visited an ophthalmologist and was prescribed oral aspirin, 100 mg/d, for 2 days before being admitted to our hospital.

Physical examination on admission revealed no bruits over the carotid artery or in the orbits. Blood pressure was 110/72 mm Hg, and pulse rate was 64 beats/min and regular. No neurologic deficit was identified. Funduscopy by an ophthalmologist revealed no abnormal findings on admission. During hospitalization, immediately after she complained of faint purple speckles, the finding of funduscopy by an ophthalmologist was also normal. Laboratory data revealed no abnormalities except for fasting blood glucose (174 mg/dL), glycosylated hemoglobin (7.0%), and total cholesterol (227 mg/dL). Hematocrit (35.5%) and platelets (195,000/mm^3) were within normal ranges. Coagulation parameters, including antithrombin III (96.4%), fibrinogen (332 mg/dL), thrombin–antithrombin complex (0.86 μg/mL), fibrin–fibrinogen degradation products (9 μg/mL), D-dimer (0.5 μg/mL), and protein C activity (132.6%), were within normal limits. Erythrocyte segmentation rate (23 mm/h) was slightly increased, but C-reactive protein was <0.06 mg/dL. Other circulating immune complexes, including C3 (95.9 mg/dL), C4 (18.7 mg/mL), CH50 (47 U/mL), antinuclear antibodies (1:40), anticardiolipin antibody (2.1 U/mL), myeloperoxidase antineutrophil cytoplasmic antibodies (MPO-ANCA) (<10.0 EU), and proteinase-3 antineutrophil cytoplasmic antibodies (PR3-ANCA) (<10.0 EU), were also within normal limits. Chest radiography, brain CT, and brain MRI revealed no abnormalities. Transthoracic echocardiography, transesophageal echocardiography, 12-lead EKG, and 24-hour EKG monitoring revealed no findings indicating emboligenic heart disease or right-to-left shunt. EEG and visual evoked potential test results were also normal. Carotid duplex sonography did not demonstrate any stenosis or plaques in bilateral carotid arteries.

Cerebral digital subtraction angiography (DSA) was performed on the day of admission. Right selective internal carotid angiography revealed that the right OA branching from the ICA was occluded in the intracranial segment. Right selective external carotid angiography revealed that the middle meningeal artery perfused the distal site of the OA, including the optic branches. These findings indicate that the arteries in the right orbit were supplied not by the ICA system but from a branch of the external carotid artery (ECA). No other abnormalities were observed in bilateral carotid arteries, intracranial cerebral arteries, or left OA (figure). Head-up tilting test did not reveal orthostatic hypotension or orthostatic visual loss. The day after treatment with 200 mg/d oral aspirin was initiated, symptoms resolved completely.

Discussion. Amaurosis fugax has predominantly been attributed to thromboembolism originating from atheromatous lesions of the ipsilateral ICA.[1,2] Other potential causes include embolism from atrial fibrillation or myocardial infarction,[3] ulcerated aortic atheromatous plaque,[4] microthromboembolism from the ECA, or dural arteriovenous fistula from the OA. Arterial spasm[5] or hemodynamic retinal vascular insufficiency[6] is also considered to be a less frequent etiology of amaurosis fugax. Some investigators have described a relationship between OA stenosis and amaurosis fugax.[7] In our case, cerebral angiography revealed complete occlusion of the right OA. To the best of our knowledge, however, amaurosis fugax associated with OA occlusion has not previously been

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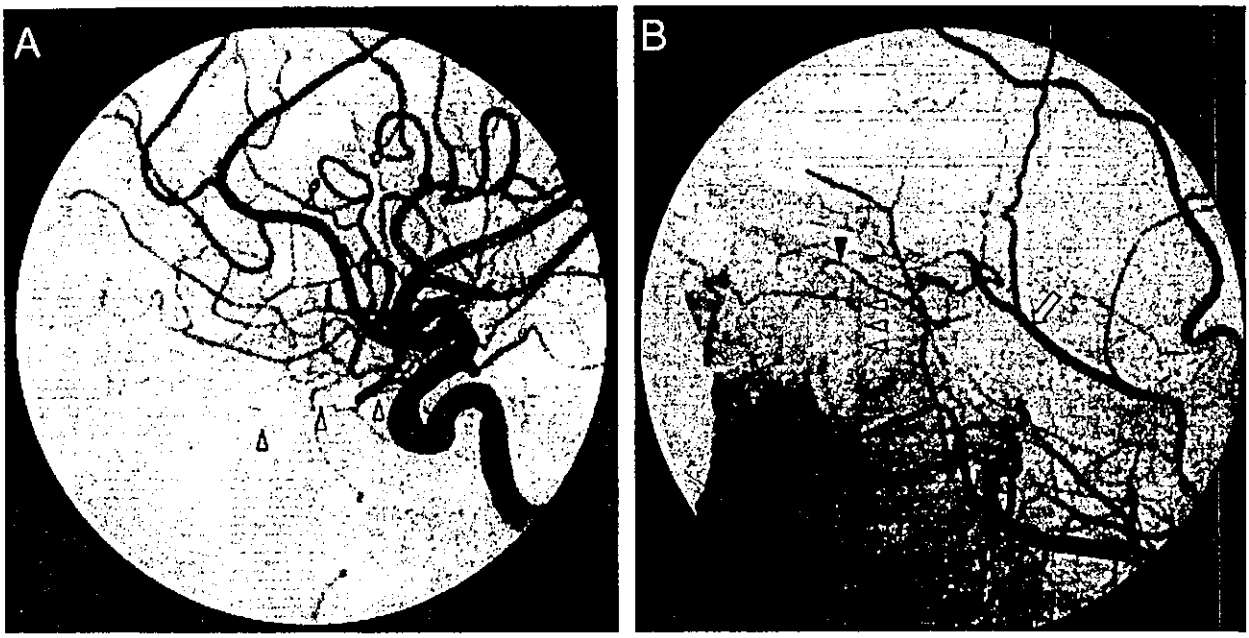

Figure. Intra-arterial digital subtraction angiography. Right selective internal carotid angiography (A) demonstrates the right ophthalmic artery faintly in the intracranial segment (white arrowhead), and the intracanalicular segment is not seen. Right selective external carotid angiography (B) shows the middle meningeal artery (white arrow) perfusing the ophthalmic artery distal to the supraoptic portion in the intraorbital segment (black arrowhead), demonstrating a subtle choroidal crescent (white arrowhead). These findings indicate a branch of the external carotid artery, not the internal carotid artery, perfuses the right ocular branches.

reported. In 1 to 2% of the population, the OA originates from the middle meningeal artery.[8] If OA branching in the present case were anomalous, the proximal part of the OA branching from the ICA would not be displayed on angiography. No obvious episodes of orthostatic visual loss were reported, nor did head-up tilting test show orthostatic hypotension or any attacks. Furthermore, treatment with aspirin resolved attacks. Therefore, transient monocular blindness was attributed to microembolism from the occluded OA rather than to OA anomaly.

The patient in the present case experienced unusually frequent attacks of transient monocular blindness. Recurrent attacks of amaurosis fugax reported in the literature have usually been limited to up to three occurrences.[6,9] The high frequency of attacks in our patient might be explained by platelet hyperaggregability because aspirin proved so effective in preventing attacks.

Bruno et al.[10] classified amaurosis fugax into four groups according to pattern of visual loss: 1) altitudinal or lateralized—the peripheral visual field was involved and was demarcated from intact vision by a horizontal or vertical line; 2) diffuse—visual loss involved the entire field of vision at all times; 3) constricting—vision constricted concentrically; and 4) miscellaneous—other unusual patterns of visual loss. The pattern in the present case was not completely compatible with the initial three patterns and was considered to be miscellaneous. Patients displaying the miscellaneous pattern demonstrate no cardiac sources of emboli, no carotid artery ulcerations, and no visible retinal emboli. Therefore, the results of neuroimaging in our patient are compatible with this hypothesis.

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Post-ischemic cyclooxygenase-2 expression is regulated by the extent of cerebral blood flow reduction in non-human primates

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Abstract

We determined whether up to 24 h of ischemia could induce the expression of cyclooxygenase-2 (COX-2) in the brain of nonhuman primates. Randomized animals were subjected to either a 2 h ischemia (group II; $n = 3$) or a 24 h ischemia (group III; $n = 3$). Three animals in group I served as controls. In group III, regional cerebral blood flow (CBF) and the cerebral glucose metabolic rate (CMRglc) were evaluated using positron emission tomography. Upregulation of COX-2 mRNA expression was observed after 2 h of ischemia, but disappeared by 24 h in the ischemic temporal cortex, in which both CMRglc and CBF were markedly reduced. In the ischemic parietal cortex, where CMRglc was preserved, COX-2 expression persisted even 24 h after ischemia. This study is the first to demonstrate neuronal COX-2 induction within potentially viable hypoperfused brain areas in nonhuman primates.

Keywords: Cyclooxygenase-2; Focal brain ischemia; Positron emission tomography; Primate; Cerebral blood flow; Cerebral glucose metabolic rate

Cyclooxygenase-2 (COX-2) is expressed in the brain in discrete neuronal populations in the cortex in response to activation of *N*-methyl-D-aspartate (NMDA) receptors [13]. Several reports, using various rodent models, suggested that COX-2 played role in the development of ischemic injury during focal brain ischemia [1,10]. Supporting these data are the findings in COX-2 deficient mice of a significant reduction in brain injury that was produced by occlusion of the middle cerebral artery, compared to control animals [4]. While these data support the notion that COX-2 inhibition might represent a possible therapeutic target for ischemic stroke, it is still not clear from the few postmortem studies that have been reported [3,11], whether COX-2 is expressed during the acute phase of a stroke. To begin to address this question, we developed a primate model of thromboembolic stroke [7] in which we measured serial changes in cerebral blood flow (CBF) before and after arterial occlusion using positron emission tomography (PET) [8]. The temporal cortex and basal ganglia ipsilateral to the arterial embolization were regarded as the ischemic core, while the ipsilateral parietal cortex was regarded as the peri-infarct area where CBF-cerebral glucose metabolic rate (CMRglc) uncoupling was observed 24 h after embolization. In this paper, we examined the topography and temporal profile of COX-2 expression within 24 h of ischemia in a primate model of thromboembolic stroke using high-resolution PET.

Nine adult male cynomolgus monkeys weighing 3.4–4.3 kg were used in this study. All procedures were approved by our Institutional Animal Care and Use Committee, and were performed in accordance with the standards published by

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the National Research Council (Guide for the Care and Use of Laboratory Animals). Permanent focal ischemia was produced by carotid arterial embolization as previously described [7]. The monkeys were randomized into three groups of three animals. Animals in group I (normal control) did not undergo arterial embolization. Animals in group II underwent permanent focal ischemia for 2 h, while those in group III underwent permanent focal ischemia for 24 h. COX-2 expression in postmortem brains obtained from each group was investigated by biochemical means. In group III, CBF and the CMRglc were evaluated using high-resolution PET. Each animal was anesthetized by sevoflurane inhalation [0.5–2.0% sevoflurane delivered in a N_2O/O_2 (70%/30%) gas mixture] and was artificially ventilated. Body temperature was monitored and maintained at around 37 °C with the aid of heating pads.

The PET studies were performed with a multi-slice PET scanner (ECAT EXACT HR/47, Siemens/CTI, Knoxville, TN) [12], which provided 47 tomographic images at 3.1 mm intervals per frame. The spatial resolution at the center of the field of view was 3.7 mm in-plane at full width at half maximum and 4.1 mm axially. CBF and CMRglc were determined using ^{15}O-labeled water (^{15}O-H_2O) and 2-[^{18}F]fluoro-2-deoxy-D-glucose, respectively, and data analysis was performed according to the methods described previously [8]. To control for potential problems that arise as a result of the repeated withdrawal of blood during prolonged periods of experimentation, as a result of the effects associated with variations in levels of anesthesia, and as a result of partial volume effects [2], we expressed our results in terms of asymmetry index (AI), which was defined as the ratio of the value of regional CBF in the ROIs in the ischemic hemisphere to that in the contralateral homologous ROIs.

All animals were sacrificed by exsanguination following perfusion with ice-cold saline under pentobarbital anesthesia. Each brain was quickly removed and stereotaxically divided on ice into three slices corresponding to the coronal PET images as the same manner as the previous study [8]. Samples were extracted from the temporal cortices, parietal cortices, and basal ganglia, the size of which were 1 × 1 cm (width × height; Fig. 1A). Each sample was subdivided into two 4 mm (depth) pieces, which were dissected out from each slice from both ischemic and non-ischemic temporal lobe sections – one piece (>60 mg) was used for biochemical examination while the other was used for histopathological analysis.

RNA blot analysis was performed as previously described [6]. Total RNAs were prepared from samples derived from the second slice from both sides of animals in each group. In one animal in group II, total RNAs were also prepared from samples in each of the three slices from the ischemic side. The amount of COX-2 mRNA in each region was expressed as the ratio of the COX-2 mRNA signal to the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA signal in that region (expression ratio).

Paraffin-embedded brain sections (3 μm thick), which

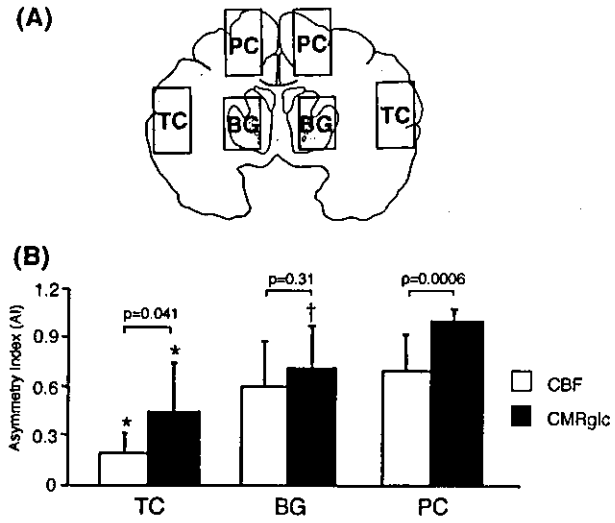
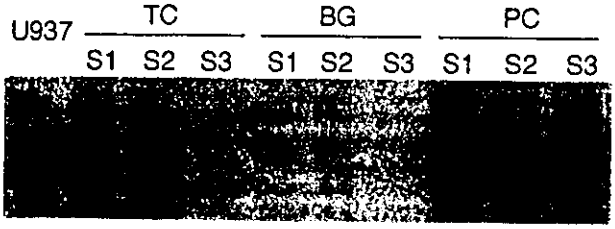


Fig. 1. (A) Manner of sample preparation. The brain was stereotaxically divided on ice into three slices corresponding to coronal PET images: the first rostral slice was a coronal cut at 23 mm from the frontal pole and another two slices were cut at 9 mm intervals parallel to the first slice. From each slice, both the ischemic and the non-ischemic temporal cortices (TC), parietal cortices (PC), and the basal ganglia (BG) were dissected out. (B)AIs for CBF and CMRglc following 24 h of focal ischemia. AIs for both CBF and CMRglc in the TC were reduced significantly (*) compared to those in the BG and PC. In the basal ganglia, AIs for CMRglc were also reduced significantly (†) compared to those in the parietal cortex. Results from a two-way analysis of variance that was used to assess the significance of differences in the AIs between CBF and CMRglc were: $P = 0.041$, $P = 0.31$, and $P = 0.0006$ for the temporal cortex, the basal ganglia, and the parietal cortex, respectively.

were taken from the second slice from the ischemic hemispheres in groups II and III, and second slice from the left hemispheres in group I, were deparaffinized and incubated with a polyclonal COX-2 antibody (Cayman Chemical, Ann Arbor, MI; dilution 1:100) for 2 h at room temperature. After sections were washed with phosphate-buffered saline, biotinylated goat serum against rabbit IgG (Vector Laboratories, Burlingame, CA) was applied and the sections were incubated for at least 30 min at 25 °C. Immunoreactive signals were visualized using diaminobenzidine as the chromogen in a peroxidase reaction (Vectastain Elite Kit, Vector Laboratories).

During the PET studies, physiological parameters including mean blood pressure, blood gases, and body temperature were maintained within the normal range. There were no significant differences in AIs for both CBF and CMRglc among the three slices from each site i.e. temporal cortex, parietal cortex, and basal ganglia. Significant reductions in AIs for CBF as well as CMRglc were demonstrated in the temporal cortex compared to those in the basal ganglia and parietal cortex (Fig. 1B). In the basal ganglia, AIs for CMRglc were also significantly reduced compared to those in the parietal cortex. On the other hand, the mean AI for CMRglc was preserved in the parietal cortex, whereas the mean AI for CBF was found to be reduced.



TC: temporal crtex, BG: basal ganglia, PC: parietal cortex
S1: slice 1, S2: slice 2, S3: slice 3

Fig. 2. RNA blot analysis of COX-2 expression following 2 h of ischemia in group II. COX-2 mRNA expression was detected in each region from the ischemic side. COX-2 mRNA expression in the ischemic parietal cortex was prominent, while expression in the ischemic basal ganglia was faint.

COX-2 mRNA expression following 2 h of ischemia (group II) was detected in each region from the ipsilateral hemisphere (Fig. 2). Expression of COX-2 mRNA in the first (slice1) and second (slice2) slices from the ischemic temporal cortex and in all slices (slice1, slice2, slice3) from the ischemic parietal cortex, was detected specifically as a single band (4.5 kb), the size of which was similar to that of human COX-2 mRNA extracted from lipopolysaccharide-treated U937 cells [5]; expression in the ischemic basal ganglia was faint. Expression of COX-2 mRNA in normal controls (group I) was as low as that on the side contralateral to the arterial embolization (Table 1). Expression ratios of COX-2 mRNA in group II were high in the ipsilateral cortices compared to those in group I. In group III, COX-2 mRNAs were undetectable in the ischemic temporal cortices and there were marked reductions in GAPDH mRNA expression, while expression ratios of COX-2 mRNA in the parietal cortex ipsilateral to the arterial embolization were prominently upregulated to the same levels as those seen in group II. Localized COX-2 immunoreactive neurons were detected predominantly in the ischemic parietal cortex in group III, while a few immunoreactive neurons were also observed in the ischemic temporal cortex and basal ganglia in group II. These immunoreactive neurons showed intense immunoreactivity in their cell bodies and apical dendrites (Fig. 3).

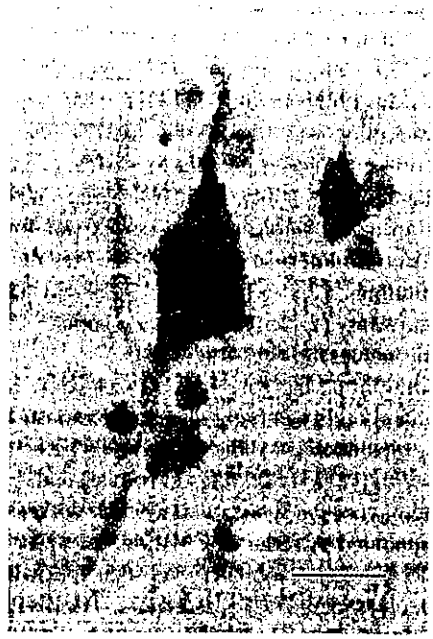


Fig. 3. Induction of COX-2 immunoreactive neurons in the parietal cortex in primate focal ischemia. Scale bar: 100 μm. Neuronal cell bodies and (apical) dendrites showed COX-2 immunoreactivity.

Table 1
Comparison of expression ratios of COX-2 mRNA between the three groups[a]

	Group I	Group II		Group III	
		ipsi	contra	ipsi	contra
Temporal cortex	0.83	11.83	0.69	–	0.60
	0.69	3.41	1.38	–	0.93
	0.45	6.97	1.57	–	0.38
Mean ± SD	0.66 ± 0.19	7.40* ± 4.23	1.21 ± 0.46	–	0.64 ± 0.28
Basal ganglia	0.33	1.03	0.66	–	0.55
	0.84	0.67	0.69	3.88	0.33
	0.13	3.91	0.93	2.07	0.28
Mean ± SD	0.43 ± 0.37	1.87 ± 1.78	0.76 ± 0.15	2.98 ± 1.28	0.39 ± 0.14
Parietal cortex	0.62	5.77	1.02	5.87	1.01
	0.65	4.44	1.49	2.62	0.87
	0.39	4.00	1.11	4.85	0.86
Mean ± SD	0.55 ± 0.14	4.74** ± 0.92	1.21 ± 0.25	4.45** ± 1.67	0.91 ± 0.08

[a] Group I indicates normal control. Animals in group II underwent permanent focal ischemia for 2 h, and those in group III underwent permanent focal ischemia for 24 h. Samples from three animals were used except in group III – ipsilateral (ipsi), basal ganglia – which were derived from only two animals because of a marked reduction in expression of both COX-2 and GAPDH mRNA. COX-2 transcripts were undetectable in all three samples from ipsilateral, temporal cortices in group III (–). A one-way analysis of variance was used to assess the significance of differences in the expression ratios between the three groups. *significantly higher than the temporal cortex in group I and temporal cortices contralateral (contra) to the arterial embolization in groups II and III ($P < 0.05$). **significantly higher than the parietal cortex in group I and parietal cortices contralateral (contra) to the arterial embolization in groups II and III ($P < 0.05$).