Table 3. Cumulative risk of death by baseline characteristics

Variable	Number	One-year cumulative risk of death, %	All-cause mortality HR	p value
Age				
≤59 years	1,800 (16.4)	1.5±0.3	1	
60–69 years	2,987 (27.2)	3.4±0.4	2.2 [1.39–3.58]	0.0008
70–79 years	3,857 (35.1)	6.4±0.5	4.5 [2.87–6.92]	<0.0001
≥80 years	2,337 (21.3)	16.0±1.0	11.5 [7.45–17.72]	<0.0001
Gender				
Male	6,945 (63.2)	6.6±0.4	0.9 [0.78–1.08]	0.28
Female	4,036 (36.8)	7.1±0.5	1	
Hypertension				
Yes	6,811 (62.0)	6.3±0.4	0.8 [0.68–0.94]	0.0072
No	4,170 (38.0)	7.5±0.5	1	
DM				
Yes	2,634 (24.0)	7.4±0.6	1.1 [0.95–1.37]	0.1565
No	8,347 (76.0)	6.5±0.3	1	
Hypercholesterolemia				
Yes	1,988 (18.1)	4.2±0.5	0.6 [0.43–0.72]	<0.0001
No	8,993 (81.9)	7.3±0.3	1	
Smoking				
Yes	1,973 (18.0)	4.4±0.7	0.6 [0.43–0.72]	<0.0001
No	9,008 (82.0)	7.3±0.3	1	
AF				
Yes	2,008 (18.3)	12.6±1.0	2.4 [1.99–2.80]	<0.0001
No	8,973 (81.7)	5.5±0.3	1	
History of stroke				
Yes	3,263 (29.7)	8.8±0.6	1.6 [1.32–1.85]	<0.0001
No	7,454 (70.3)	5.8±0.3	1	
Stroke subtype				
Lacunar	4,341 (39.5)	4.0±0.4	1	
Atherothrombotic	3,430 (31.2)	7.8±0.5	2.3 [1.84–2.82]	<0.0001
Cardioembolic	1,877 (17.1)	12.5±1.0	3.6 [1.83–2.82]	<0.0001
Other	586 (5.3)	8.1±1.4	2.2 [1.51–3.10]	<0.0001
TIA	747 (6.8)	3.5±0.8	0.9 [0.59–1.46]	0.7587
mRS				
Score 0–2	7,410 (67.6)	2.8±0.2	1	
Score 3–5	3,553 (32.4)	15.7±0.8	6.7 [5.59–8.04]	<0.0001
Place of residence after discharge				
Own home	7,583 (69.2)	3.3±0.3	1	
Institution	3,379 (30.8)	15.1±0.8	5.6 [4.74–6.71]	<0.0001

HR = Hazard ratio. Figures in parentheses are percentages, those in brackets indicate 95% confidence intervals.

Univariate analysis showed that factors associated with death included being elderly, normotensive, nonhypercholesterolemic, nonsmoker, having AF, a history of stroke, nonlacunar stroke, mRS scores of 3–5 and discharge to an institution (table 3). Multivariate analysis demonstrated that significant independent predictors of death after stroke included male gender, older age, DM, AF, history of stroke, nonlacunar stroke and residence in an institution (table 4).

Figure 3 shows the cumulative survival rates for patients residing in their own home and for those residing in institutions with mRS scores of 0–2 and those with mRS scores of 3–5. The cumulative mortality rate was lower in patients residing in their own homes than in those in insti-

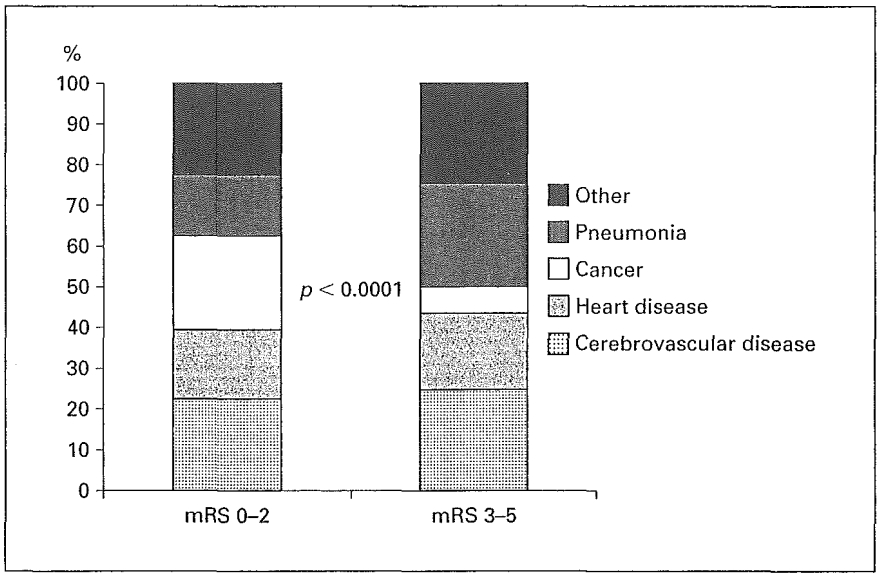

Fig. 2. The cause of death during follow-up for patients with mRS scores at hospital discharge of 0–2 and 3–5 ($p < 0.0001$). The most frequent cause of death was cancer (23.1%) in patients with mRS scores of 0–2, and pneumonia (25.5%) in those with mRS scores of 3–5.

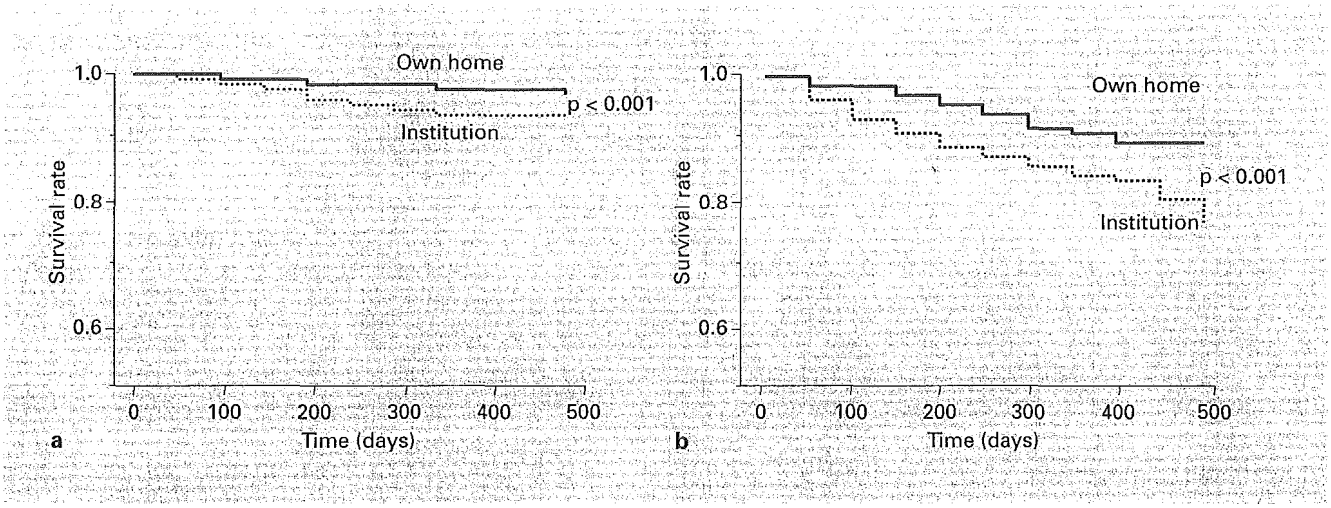

Fig. 3. The cumulative survival rates of patients discharged to their own homes and to institutions with mRS scores of 0–2 (**a**) and with mRS scores of 3–5 (**b**) at hospital discharge. In both groups, the mortality rate was lower for patients discharged to their own homes ($p < 0.001$).

tutions for both mRS groups (for mRS scores of 0–2: 2.4 ± 0.2% at home vs. 6.5 ± 1.1% in an institution, $p < 0.0001$; for those with mRS scores of 3–5: 10.7 ± 1.5% at home vs. 17.4 ± 0.9% in an institution, $p < 0.0001$). In patients with mRS scores of 0–2, no statistically significant differences were observed in the causes of death between those patients residing in their own home and those in institutions [cerebrovascular diseases: 24.0% (home) vs. 17.1% (institution); heart diseases: 18.4% (home) vs. 11.4% (institution); cancer: 20.8% (home) vs. 31.4% (institution); pneumonia: 13.6% (home) vs. 20.0% (institution); other causes: 23.2% (home) vs. 20.0% (institution)]. On the other hand, in patients with mRS scores of 3–5, pneumonia was less frequently the cause of death in patients residing in their own home than in those patients residing in an institution (13.1 vs. 27.4%, $p = 0.0172$).

Table 4. Multivariate analyses of prognostic variables associated with death after stroke/TIA

Variable	All causes of death		
	HR	95% CI	p value
Age (vs. ≤59 years)			
60–69 years	1.96	1.22–3.16	0.0055
70–79 years	3.20	2.05–5.00	<0.0001
≥80 years	6.37	4.09–9.94	<0.0001
Hypertension	0.86	0.71–1.00	0.051
Hypercholesterolemia	0.80	0.62–1.04	0.937
DM	1.42	1.17–1.71	0.0003
Smoking	1.01	0.78–1.31	0.9278
AF	1.37	1.07–1.76	0.0125
History of stroke	1.28	1.08–1.52	0.0042
Stroke subtype (vs. lacunar)			
Atherothrombotic	1.46	1.16–1.82	0.0011
Cardioembolic	1.49	1.10–2.02	0.0096
Other	2.06	1.43–2.96	0.0001
TIA	1.42	0.89–2.26	0.1355
mRS (vs. score 0–2)			
Score 3–5	2.57	2.00–3.29	<0.0001
Institution (vs. own home)	2.18	1.73–2.75	<0.0001

HR = Hazard ratio; CI = confidence interval.

Discussion

We prospectively conducted the first large hospital-based registration study in Japan and examined the 1-year cumulative mortality rate and cause of death, to identify the factors predictive for death after hospital discharge following stroke or TIA. The data demonstrated a 1-year cumulative mortality rate of 7.0% in 10,234 ischemic stroke patients and 3.5% in 747 TIA patients. The present results were based on hospital statistics, and the 1-year cumulative mortality rate found in this study was lower than previously reported by community-based studies [2–9]. The frequency of patients who were functionally independent (mRS scores of 0–2) at the time of hospital discharge was 67.6%, which was higher than that found in western countries. As well, the proportion of lacunar strokes (39.5%) was higher than that reported in western countries [16–19]. These lacunar stroke patients had the lowest mortality and a significantly better functional outcome as compared to patients with other stroke subtypes, confirming what has been previously reported [4, 20]. Therefore, the low mortality rate in this study can be explained by the higher proportion of lacunar strokes in Japan as compared to western countries.

In our study, pneumonia was the second overall leading cause of death, while in functionally dependent patients (mRS scores 3–5) at discharge, pneumonia was the leading cause. Salive et al. [21] have reported that disability and cognitive impairment were strong risk factors for pneumonia-related mortality in older adults. Thus, strategies to prevent pneumonia among these patients are of great importance in reducing death following stroke.

Multivariate analysis showed that male gender, increasing age, DM, AF, a history of stroke, nonlacunar stroke, a lower functional activity level and institutional residence after hospital discharge were associated with an increased risk of death after stroke. Our results are comparable to previous reports, which demonstrated that older age, degree of functional disability, DM, AF, history of stroke and the particular stroke subtype were significant independent predictors of death among stroke survivors [4, 5, 7, 10, 11]. Other predictors of death were reported to be residence in a nursing home prior to stroke, brainstem involvement, electrocardiographic abnormalities and lesion size [22]. In the present study, neither hypertension nor smoking was found to be an important predictor of death following stroke. One could hypothesize that, based on these results, most hypertensive stroke patients may have had appropriate treatment for their hypertension during the follow-up period. It is also likely that the smokers who quit smoking did so because of poor health, while smokers in good health were more likely to have continued smoking. As well, since the follow-up period was relatively short, the adverse effects of hypertension and smoking did not have the time to become manifest.

Interestingly, patients returning home after hospital discharge were more likely to have a good outcome. Primarily, this association reflects the fact that patients who had risk factors for death, such as cancer or severe cardiac, pulmonary or renal diseases, were likely to be transferred to an institution or other hospital after discharge. Thus, we can expect that the mortality of these patients would be higher than that of those of patients who had returned to their own homes. Secondly, among patients who were functionally dependent, pneumonia was less frequently the cause of death in those receiving care at home than in those sent to institutions. Thus, it seems that the patients who returned home, irrespective of their functional disability, may have benefited from the attentive care of their spouse, children and other family members. Therefore, as long as financial and familial circumstances permit, we should emphasize the benefits of sending patients back to their own home after hospitalization.

Some limitations are present in this study. Firstly, this study was a hospital-based and not a population-based study. Therefore, our study was not representative of all patients with a stroke or TIA in Japan. Secondly, we included TIA patients in the present study because the number of TIA patients who died was very small. Prognostic factors for TIA patients may be different than those for stroke. Thirdly, some selection bias may have existed in the study. Nonhospitalized stroke and TIA patients were not evaluated, although the number of such patients is very small because of the well-organized health insurance system (universal medical care system) in Japan. Fourthly, 28% of the patients were not enrolled into the present study. There were some differences in clinical characteristics between the enrolled and the nonenrolled patients. In particular, the NIHSS score at admission and the mRS at discharge were higher in the nonenrolled patients than in the enrolled patients, which would indicate that nonenrolled patients had severer strokes. Therefore, the overall mortality rate may be in fact higher than the present results would indicate.

In conclusion, to improve survival after hospital discharge, in addition to the appropriate management of vascular risk factors following a stroke, it is important to take measures to prevent pneumonia, and, where possible, discharge patients back to their own home.

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□ ORIGINAL ARTICLE □

Is Stroke a Paradoxical Embolism in Patients with Patent Foramen Ovale?

Masahiro YASAKA, Ryoichi OTSUBO, Hiroshi OE and Kazuo MINEMATSU

Abstract

Objective **Purpose was to assess the stroke mechanism in patients with patent foramen ovale (PFO).**

Methods **We reviewed the medical records of 111 stroke patients with PFO and sinus rhythm (PFO-S group), 25 with PFO and atrial fibrillation (AF) (PFO-AF group) and 67 with AF but not PFO (AF group), who had received contrast transesophageal echocardiography. The clinical and neuroradiological findings were then compared among the three groups. Deep vein thrombosis was investigated in 93 patients with PFO. We determined the number of patients with definite paradoxical embolism who met three criteria: deep vein thrombosis, neuroradiological features indicating embolic stroke, and the absence of other sources of emboli. We also evaluated those with probable paradoxical embolism who met two of the three criteria.**

Results **The PFO-S group more frequently exhibited hypercholesterolemia ($p<0.0001$) and lesions limited to the posterior circulation ($p<0.0004$), and less frequently exhibited large or cortical lesions in the anterior circulation ($p=0.0008$, $p<0.0001$, respectively), than the PFO-AF and AF groups. In the PFO-S and PFO-AF groups, other sources of emboli such as a cardiac source of emboli, cerebral artery stenosis ≥50%, or complicated atheroma in the aortic arch were identified in 72 cases (52.9%). In the 93 patients with examination for deep vein thrombosis, the definite and probable criteria of paradoxical embolism were fulfilled only in three (3.2%) and 33 cases (35.5%), respectively.**

Conclusion **In stroke patients with PFO, not only paradoxical brain embolism through the PFO but also other causes of stroke may contribute to the development of stroke.**

(Internal Medicine 44: 434–438, 2005)

Key words: stroke, patent foramen ovale, atrial fibrillation, paradoxical brain embolism

Introduction

Patent foramen ovale (PFO) is found in about 30% of autopsies and may be associated with paradoxical brain embolism (1). The prevalence of PFO in patients with stroke is higher than in control subjects and PFO is more frequently detected in cryptogenic stroke than in stroke of known etiology (2–4). Contrast transesophageal echocardiography (TEE) enables the detection of PFO with a higher degree of sensitivity, which has contributed to the diagnosis of paradoxical brain embolism (5).

Typical patients with paradoxical brain embolism through PFO demonstrate a venous thrombus as the direct source of emboli and neuroradiological features of cerebral embolism. However, numerous stroke patients with PFO do not have a venous thrombus or neuroradiological findings of brain embolism. Therefore, the contribution of paradoxical embolism through PFO to the development of stroke may be smaller than previously thought. Although clarifying the causes of stroke in patients with PFO is important, the clinical characteristics of stroke patients with PFO have not been fully elucidated. Thus, we retrospectively reviewed the medical records of stroke patients having PFO with or without atrial fibrillation (AF) and those of stroke patients with AF but not PFO, and compared their clinical and neuroradiological findings. In addition, we proposed definite and probable criteria for paradoxical brain embolism and determined how many stroke patients with PFO met the criteria.

For editorial comment, see p 401.

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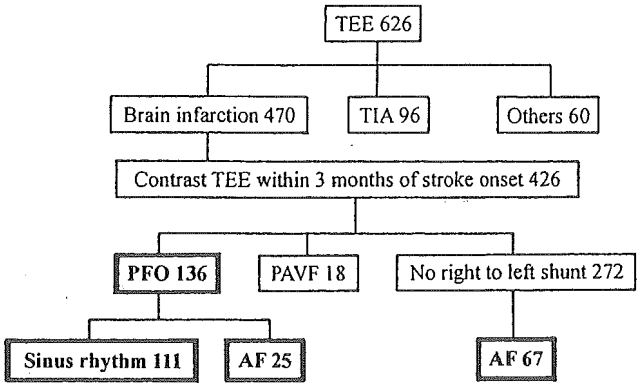

Figure 1. Diagram of the study subjects. TEE: transesophageal echocardiography, TIA: transient ischemic attack, PFO: patent foramen ovale, PAVF: pulmonary arteriovenous fistula, AF: atrial fibrillation. Arabic numerals indicate number of patients.

Methods

From January 2000 to December 2001, we performed TEE in 626 patients (Fig. 1); 470 with brain infarction, 96 with TIA, and the remaining 60 with other neurological disorders. In 426 of the 470 patients with brain infarction, contrast TEE was performed within 3 months of onset. PFO was demonstrated in 136 patients and pulmonary arteriovenous fistula was suspected in 18. We divided the 136 patients with PFO into 111 patients with sinus rhythm (PFO-S group) and 25 patients with AF (PFO-AF group). No right to left shunt was observed in the other 272 patients, and of these patients, AF was noted in 67 patients (AF group) at the time of TEE. We compared the clinical background, atherosclerotic risk factors, vascular territory of the brain infarction, site and size of the infarct, and cerebral angiographic findings among the PFO-S, PFO-AF and AF groups.

We performed contrast TEE using a commercially available real-time two-dimensional echocardiography system (model SSD-2200, Aloka, Tokyo) equipped with a 5.0 MHz phased array omniplane transesophageal transducer. Without any contrast medium, we inspected the left atrium for debris appearing inside the left atrium during the Valsalva maneuver and after release of the maneuver. Next, the contrast medium, a mixture of 9 ml saline and 1 ml air, was infused into the right antecubital vein during the Valsalva maneuver. When the right atrium was opacified by the contrast medium as seen on the monitor, we asked the patient to release the Valsalva maneuver. When contrast medium different from the debris was found in the left atrium within three cardiac cycles after the release of the Valsalva maneuver, we diagnosed the patient with PFO, and when the medium was observed after three cardiac cycles, we suspected the presence of pulmonary atriovenous fistula (5–8).

Previously diagnosed hypertension, diabetes mellitus and hypercholesterolemia were considered atherosclerotic risk factors. Patients taking antihypertensive medicine and with a systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg were considered hypertensive, while diabetic patients were defined as those taking insulin or oral antidiabetic agents, and exhibiting a fasting plasma glucose level of ≥126 mg/dl or plasma glucose at any time of ≥200 mg/dl. Patients taking antihypercholesterolemic medicine, or with a plasma total cholesterol of ≥220 mg/dl were defined as having hypercholesterolemia.

We investigated the circulation territory, either anterior or posterior circulation, responsible for the infarction, whether the lesions involved cortical areas, and whether the lesions were larger than 3.0 cm in diameter in patients with infarctions of the anterior circulation (9–11).

We also compared the incidence of cervical or cerebral artery stenosis ≥50% demonstrated by carotid ultrasonography, MRA, or cerebral angiography, in the major artery proximal to the responsible infarction, intraluminal filling defect indicating an embolus on angiogram, reopening of the previously occluded artery confirmed by MRA, and atherosclerotic lesions thicker than 4.0 mm at the aortic arch among the three groups. We reviewed medical records for other sources of emboli, such as arterial dissection, ulcerative plaque at the carotid artery, and cardiac and cerebral catheter manipulation.

In the 136 patients having PFO with or without AF, we investigated underlying heart diseases by electrocardiography, TEE and transthoracic echocardiography. Thrombus in lower leg veins was investigated by ultrasonography in 86 patients, by RI scintigraphy in 46 patients, and by either procedure in 93 patients. Using the diagnostic criteria given in Table 1, we ascertained the number of patients who met the definite or probable criteria for paradoxical embolism.

Continuous data were expressed as mean ± SD. We used the Chi squared test for analysis of discrete variables and analysis of variance with the multiple comparison test with Scheffe's test for analysis of continuous variables.

Results

Patients in the PFO-S, PFO-AF, and AF groups were 63.5±11.7 years old, 68.0±11.7 years old, and 70.7±7.8 years old, respectively (ANOVA, $p<0.0001$). Patients in the PFO-S were significantly younger than those in the AF group (multi comparison test with Scheffe, $p<0.0001$, Table 2). Hypercholesterolemia was noted in 54.1%, 20.0%, and 38.8% of the PFO-S, PFO-AF, and AF groups, respectively ($p<0.0001$) and was significantly more frequent in the PFO-S group than the PFO-AF and AF groups ($p<0.0001$).

Infarction occurred in the anterior circulation in 68.5%, 84.0%, and 79.1%, in the posterior circulation in 31.5%, 8.0%, and 11.9%, and in both in 0%, 8.0% and 9.0% of the PFO-S, PFO-AF and AF groups, respectively (Table 2). Patients in the PFO-S group had a significantly higher incidence of lesions restricted to the posterior circulation than did the PFO-AF and AF groups ($p=0.0004$).

Table 1. Diagnostic Criteria for Paradoxical Brain Embolism

1) Brain infarction demonstrated by CT or MRI.
2) Patent foramen ovale diagnosed by TEE.
3) Intravenous thrombus demonstrated by ultrasonography or RI venography.
4) Neuroradiological features of brain embolism,
   such as cortical infarction demonstrated by CT or MRI and angiographic findings of intraluminal filling defect (embolus shadow) or reopening of previously occluded arteries.
5) Absence of other sources of embolism, such as
   heart disease (atrial fibrillation, prosthetic valves, rheumatic heart disease, dilated cardiomyopathy, sick sinus syndrome, acute myocardial infarction, ventricular aneurysm), atherosclerotic plaque at the aortic arch thicker than 4.0 mm, and arterial stenotic lesion (≥50%) proximal to the lesion.

Diagnosis of paradoxical brain embolism.

Definite	1)+2)+3)+4)+5)
Probable	1)+2)+3)+4)
	1)+2)+3)+5)
	1)+2)+4)+5)

Table 2. Demographics

Number	PFO-S group 111	PFO-AF group 25	AF group 67	p
Age (years)	63.5±11.7	68.0±11.7	70.7±7.8*	<0.001
Gender, male	84 (75.7)	22 (88.0)	47 (70.1)	0.21
Hypertension	71 (64.0)	11 (44.0)	45 (67.2)	0.11
Diabetes Mellitus	42 (37.8)	6 (24.0)	24 (35.8)	0.52
Hypercholesterolemia	60 (54.1)	5 (20.0)	14 (20.9)	<0.0001 (<0.0001)+
Territory of the infarction				
Anterior circulation	76 (68.5)	21 (84.0)	53 (79.1)	0.24
Posterior circulation	35 (31.5)	2 (8.0)	8 (11.9)	0.0018** (0.0004)+
Both	0 (0)	2 (8.0)	6 (9.0)	
Stenotic lesion***	31 (22.8)	2 (8.0)	5 (7.5)	0.0011 (0.0002)+
Aortic arch atheroma	10 (9.0)	10 (40.0)	20 (30.0)	<0.0001 (<0.0001)+
Number of patients with infarction located in the anterior circulation.				
	76	23	55	
Infarction >3.0 cm diameter	21 (27.6)	13 (56.5)	34 (61.8)	0.0002 (0.0008)+
Cortical infarction	35 (46.0)	22 (95.7)	46 (83.6)	<0.0001 (<0.0001)+
Number of patients with cerebral angiography.				
	28	13	19	
Embolic shadow	2 (8.8)	2 (15.4)	2 (10.5)	0.71
Intracranial artery occlusion	14 (50.0)	11 (84.6)	16 (84.2)	0.017 (0.0046)+
Follow-up MRA	7	9	7	
Reopening on MRA	0	8 (88.9)	7 (100)	<0.0001 (<0.0001)+

(%), *multi comparison test with Scheffe p<0.0001 vs. PFO-S group, **vs. anterior circulation, ***at the artery proximal to the infarction. +PFO-S group vs. PFO-AF and AF groups.

In patients with infarction in the anterior circulation, lesions larger than 3.0 cm in diameter were seen in 27.6%, 56.5%, and 61.8% (p=0.0002), and cortical lesions were observed in 46.0%, 95.7%, and 83.6% (p<0.0001) of the PFO-S, PFO-AF and AF groups, respectively. Large infarcts and cortical lesions were significantly less frequent in the PFO-S group than in the PFO-AF and AF groups (p=0.0008, p<0.0001, respectively, Table 2).

We performed carotid ultrasonography in all patients, MR angiography (MRA) in 77 (69.4%) of the PFO-S group, in 20 (80.0%) of the PFO-AF group, and in 50 (74.6%) of the AF group. Cerebral angiography was carried out in 28 (25.2%), 13 (52.0%), and 19 (28.4%) of the PFO-S, PFO-AF, and AF groups, respectively. The incidence of arterial stenotic lesion proximal to the infarction was 22.8%, 8.0%, and 7.5% of the PFO-S, PFO-AF, and AF groups, respectively (p=0.0011). The stenotic lesions were significantly more commonly complicated in the PFO-S group than in the PFO-S and AF groups (p=0.0002, Table 2).

The incidence of intracranial arterial occlusion demonstrated by cerebral angiography was 50.0%, 84.6%, and 84.2% (p=0.017), and reopening of a previously occluded artery detected by follow-up MRA was demonstrated in 0%, 88.9%, and 100% (p<0.0001) of the PFO-S, PFO-AF and AF groups, respectively. The incidence of intracranial arterial occlusion and reopening phenomenon was significantly less frequent in the PFO-S group than in the PFO-AF and AF groups (p<0.0043 and p<0.0001, respectively, Table 2). The incidence of embolic shadow was low in all three groups (Table 2). All patients with findings of intraluminal filling defect or reopening phenomenon had cortical infarction.

TEE revealed complicated atheroma at the aortic arch in 9.0%, 40.0%, and 30.0% of the PFO-S, PFO-AF, and AF groups, respectively (p<0.0001). In the 136 patients with PFO, an underlying heart disease was demonstrated in 26 patients (19.1%); non-valvular atrial fibrillation in 25 and sick sinus syndrome in one. Other sources of emboli in the PFO group were ulcerative carotid plaque (n=1), arterial dissection (n=2), and cardiac catheter manipulation (n=1).

In total, sources of emboli including a cardiac source of emboli (n=26), cerebral artery stenosis ≥50% (n=33), complicated aortic atheroma (n=20), and other sources mentioned above (n=4) except for PFO and deep vein thrombosis were demonstrated in 72 (52.9%) of the 136 patients with PFO (eight had both AF and aortic atheroma, one had both AF and stenotic lesion, and one had AF, stenotic lesion and aortic atheroma). Deep vein thrombosis was found in 25 of the 93 patients (26.9%) who were examined by ultrasonic examination or RI scintigraphy. Of these 93 patients, the definite and probable criteria for paradoxical brain embolism were fulfilled in only 3 (3.2%) and 33 cases (35.5%), respectively.

Discussion

Several studies have revealed that paradoxical embolism through PFO is an important stroke mechanism (2–4). However, in the present study, we found that 3.2% and 35.5% of stroke patients with PFO fitted the criteria for definite and probable paradoxical brain embolism, respectively. We also found that a considerable number of stroke patients with PFO had other sources of emboli (52.9%) and risk factors of atherosclerosis. Neuroradiological features of embolic stroke such as large or cortical infarction, or reopening of a previously occluded artery were less common in the PFO-S group than in the PFO-AF and AF groups. On the other hand, the clinical and neuroradiological features in the PFO-AF group were similar to those in the AF group. These distinguishing characteristics of the PFO-S and PFO-AF groups suggest that a considerable number of patients developed stroke not only by paradoxical embolism through PFO but also by other embolic mechanisms from a cardiac source, proximal arterial stenosis, atherosclerotic lesions in the aortic arch, or thrombotic or hemodynamic mechanisms in the large or small arteries. Therefore, the risk of stroke and other sources of emboli in stroke patients with PFO must be investigated to determine if they meet the criteria for paradoxical embolism, which requires anticoagulant therapy against recurrent attacks. The present study was retrospective, and thus prospective studies examining consecutive stroke patients are required to obtain an accurate prevalence rate for paradoxical embolism in stroke.

PFO is an important mechanism by which stroke develops in the young (2, 3), whereas in the elderly, non-valvular atrial fibrillation (NVAF) is the most frequent embolic source of brain infarction (12). Recent population-based surveys have revealed that 10% of people over 80 have AF (13). Thus, the differences in several features of stroke patients among the PFO-S, PFO-AF, and AF groups may be reflected by a difference in age.

Infarction in the posterior circulation was common in the PFO-S group. Small emboli passing through the PFO may enter the vertebral arteries more easily than the common carotid arteries. Otsubo et al reported that aortogenic infarction tends to occur at the posterior circulation (14). Therefore, emboli from atherosclerotic lesions in the aortic arch may play an important role in developing stroke in the PFO-S group, although aortic atherosclerotic lesions were also reported to play an important role in the development of stroke in patients with NVAF (15).

Exploration for deep vein thrombus is essential for proper diagnosis of paradoxical embolism. The detection rate of thrombus was 26.9% among the cases investigated in the present study. Recently, echo examination was applied to small veins for detecting thrombi. The more widely the echo examination is applied, the higher the detection rate of venous thrombi in stroke patients with PFO. If thrombi are detected, anticoagulant therapy should be applied and if not, antiplatelet treatment may achieve prevention to the same extent as the anticoagulant therapy (16).

In conclusion, the clinical features of patients having PFO with sinus rhythm appear to differ from those of patients

with AF. Other causes of stroke should be considered in stroke patients with PFO because not only paradoxical brain embolism through the PFO but also other causes of stroke may contribute to development of the stroke.

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Bilateral induction of the S-100A9 gene in response to spreading depression is modulated by the cyclooxygenase-2 activity

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Abstract

Cyclooxygenase-2 (COX-2) was reported to be induced in the infarcted human brain. Spreading depression (SD) is thought to play a role in this induction. In this study, we correlated the expression of SD-associated genes with COX-2 production in brains after SD. Rats were divided into 3 groups: rats that did not undergo SD (group I saline controls, $n=7$), rats that underwent unilateral SD as a result of KCl application (group II, $n=9$), and rats that were pretreated with the selective COX-2 inhibitor, JTE-522 3 h before the induction of SD (group III, $n=7$). The expression of the SD-associated genes, S-100A9, and mitogen-activated proteinkinase phosphatase (cpg21) was analyzed 2 h later using a cDNA array. In group II, COX-2 and cpg21 mRNA expression, as determined by RT-PCR, were significantly upregulated in the hemisphere undergoing SD. While the expression of S-100A9 mRNA was bilaterally upregulated in these animals, this expression was significantly reduced in group III, and was accompanied by reduced bilateral production of PGE_2. Thus, the bilateral induction of expression of the S-100A9 gene in response to SD was associated with COX-2 activation.

Keywords: Spreading depression; S-100A9; cpg21; Cyclooxygenase-2; JTE-522; Rat

1. Introduction

Spreading depression (SD), characterized by reversible depression of cortical electrical activity in the brain that spreads like a wave, can be induced by a variety of electrical, chemical, and mechanical stimuli in the normal brain [1]. While preconditioning by the repeated induction of SD was shown to induce tolerance to subsequent ischemia [2], the induction of repetitive SDs in the ischemic cortex within a few hours after ischemia was found to contribute to the expansion of the infarcted areas in rat and cat models [3–6]. Thus, SD appears to have dual effects on the brain.

The activity of cyclooxygenase-2 (COX-2), the rate-limiting enzyme in prostaglandin synthesis, was upregulated by SD [7] and focal brain ischemia [8] in the cortex of primates and nonprimates [9,10]. Koistinaho and Chan [11] reported that SD directly induced COX-2 expression in focal brain ischemia by stimulating the *N*-methyl-D-aspartate (NMDA) receptor and activating phospholipase A2. Nogawa et al. [12] suggested that COX-2, induced at the infarct border, might be involved in delaying neuronal death, though they did not assess COX-2 expression in the contralateral hemisphere. On the other hand, COX-2 expression was shown to be globally induced in the infarcted human brain, and delayed COX-2 induction in the hemisphere contralateral to the ischemia was speculated to play a role in promoting the remodeling of neural networks [13]. SD is thought to be involved in the induction of COX-2 in brain areas great distances from the ischemic

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regions [14], however, the mechanisms of COX-2 expression in remote brain areas from affected site have remained to be clarified.

To help clarify these mechanisms, we examined the alteration of gene expression in the whole brain under eliciting SDs after administration of a selective COX-2 inhibitor.

2. Materials and methods

2.1. SD model and brain preparation

All animal procedures were approved by our Institutional Animal Research Committee and were performed in accordance with the standards published by the National Research Council.

Male, Sprague–Dawley rats (240–350 g, $n=23$) were randomly divided into 3 groups. Rats in group I (control, $n=7$) were not subjected to SD, while rats in groups II ($n=9$) and III ($n=7$) were subjected to SD, with the rats in group III also receiving a selective COX-2 inhibitor JTE-522 (4-[4-cyclohexyl-2-methyloxazol-5-yl]-2-fluorobenzenesulfonamide; Central Pharmaceutical Research Institute of Japan Tobacco Inc., Osaka, Japan) 3 h prior to SD elicitation. JTE-522, which was suspended in a 0.5% carboxy methylcellulose solution and administered orally at a dose of 10 ml/kg, was reported to selectively inhibit COX-2 without affecting COX-1 [15]. All animals were anesthetized with chloral hydrate (400 mg/kg body weight i.p.) prior to the induction of SD.

SD was evoked by applying 3.3 mol/L KCl to the cortex. The anesthetized animals were mounted on a stereotaxic instrument in the prone position, with their head restrained using teeth and ear-bars. After the frontoparietal cranium was exposed by a midsagittal incision, two small burr holes were made in the right parietal skull bone and the dura carefully excised; the rostral burr hole was used to apply potassium chloride (KCl) while the more caudal hole was used to take direct current (DC) potential recordings. These two burr holes were made 7-mm apart (Fig. 1A). The DC potential was monitored with an amplifier (Iso-DAM8, World Precision Instruments, Sarasota, FL, USA) that was connected to microelectrodes (TM33B10, World Precision Instruments, Sarasota, FL, USA) that were inserted into the cortex to a depth of 1 mm. In the rats in group I, physiological saline, instead of KCl, was applied to the cortex through the rostral burr hole. Rectal temperature of all treated rats was monitored and their body temperature maintained around 37 °C with the aid of heating pads. An arterial catheter placed into the right femoral artery was used to continuously monitor heart rate and arterial pressure.

Two hours after KCl or saline application, brain tissues were perfused with cold saline and the animals were sacrificed by exsanguination under chloral hydrate anesthesia. The brains were then cut into 3 coronal sections as shown in Fig. 1. The section between slices 1 and 2 was frozen in isopentane-dry ice and stored at −80 °C for biochemical analyses.

2.2. Gene analyses

cDNA array analyses were used to search for SD-associated genes that were modulated by the administration of JTE-522. These analyses were conducted using Motorola CodeLink Bioarrays (Motorola Life Science, IL, USA), each of which contained 10,060 elements. Poly $(A)^+$ RNA extracted from the right cortices of 3 animals in each of the 3 groups (animal no 1~no 9) was pooled together (the total

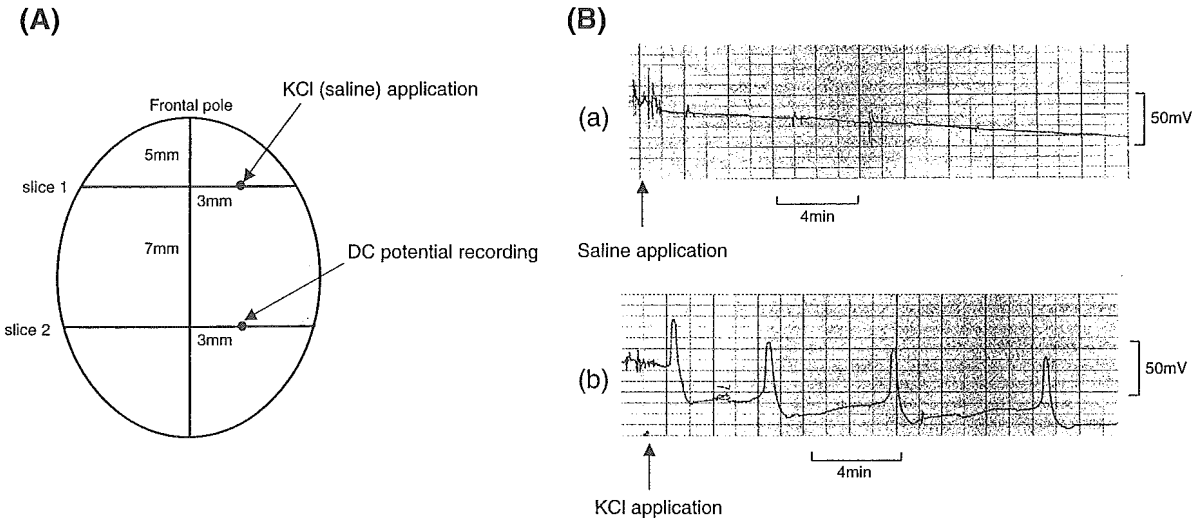

Fig. 1. Stereotaxic parameters and DC potential recordings from rats in groups I and II. (A) The brain was stereotaxically divided, on ice, into 3 coronal sections. (B) DC potentials were not detected after saline application to rats in group I (a). In the rats in group II, a total of four DC potentials were detected after KCl application (b). Tracings represent the data from a single rat in each group.

Table 1
Oligonucleotide primers used in RT-PCR

Gene	Sequences (Forward primer) (Reverse primer)	Cycling number (*N*) PCR product size
COX-2	CCCAGCACTTCACTCATCAGTTTTTCAAGA (F563)	32
	TTCCACCAGCAGGGCGGGATACAGTTCCAT (R1459)	926 bp
cpg21	GAGTATATCAAGCAGAGGAGGAGCGTGGTC (1045F)	32
	TTCCCTGAAGTGACAGAGGACAGAGACAGA (1761R)	746 bp
S-100A9	AGCGCAGCATAAGCACCATCATCAATGTTT (60F)	32
	ATTATTTCCCAGCCCCAGAACCAAGGTCAT (431R)	401 bp
GAPDH	ACCACAGTCCATGCCATCAC (586F)	23
	TCCACCACCCTGTTGCTGTA (1018R)	439 bp

COX-2, cyclooxygenase 2; cpg21, mitogen-activated protein kinase; GAPDH, glyceraldehydes-3-phosphate dehydrogenase.

amounted to about 30 μg) and was used to synthesize complementary DNA. The specific protocol involved the use of a Motorola CodeLink™ microarray and is described in detail elsewhere [16,17].

2.3. Reverse transcriptase-polymerase chain reaction (RT-PCR)

RT-PCR was used to examine the expression of COX-2 and SD-associated genes. Twenty samples were obtained from bilateral cortices in 10 animals (animal no10~no19). Primers for selected genes were obtained from Proligo (Kyoto, Japan). RT-PCR analysis was performed using KOD DNA polymerase (Toyobo, Osaka) as previously described [18]. The sequence of the primer pairs for each of the target genes and their cycling number (*N*) are described in Table 1. The basic cycling parameters were as follows: 3 min at 96 °C, followed by N cycles at 94 °C, 15 s; 55 °C, 2 s; 68 °C, 1 min. Cycling numbers were determined empirically using semi-quantitative PCR amplification. The amplification products were visualized by electrophoresis using a 2% ethidium bromide-stained agarose gel, and digitized using a DC290 with Kodak™ 1D 3.5.3 software. The digitized values of each gene were normalized with those of glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

2.4. Measurement of prostaglandin concentration by radioimmunoassay

Tissue concentrations of prostaglandin E_2 (PGE_2) in both hemispheres of all animals ($n=23$) were determined using radioimmunoassay kits (PerkinElmer Life Sciences, Inc., MA, USA). Values were normalized for protein content.

2.5. Data analysis

A one-way analysis of variance (ANOVA) followed by a Fisher's post-hoc test was used to assess the differences in hemispheric gene and PGE_2 expression between the 3 groups. A Student's *t* test was used to analyze differences between the hemispheres in each group. A two-tailed *p*-value of <0.05 was considered to be significant. Data are expressed as the mean±standard deviation.

3. Results

The physiological parameters of all test animals did not change significantly during the experimental period (Table 2). No episodes of SD were observed in the control group (I; Fig. 1). The mean number of SDs evoked by KCl application in group II (4.7±2.4) was not significantly different from that seen in group III (3.6±1.6).

3.1. cDNA array analysis

The expression signals for S-100A9 and mitogen-activated protein kinase phosphatase (cpg21) in group II increased more than 2.5-fold compared to their expression in the rats in groups I and III (2.8-fold vs. group I, 2.8-fold vs. group III for S-100A9; 6.0-fold vs. group I and 2.7-fold vs. group III for cpg21). In group II, 35 elements were found to be at least 2.5-fold different than in the rats in group I,

Table 2
Physiological measurements

Group	*N*	Weight (mg)	SD	MAP (mm Hg)	Temp. (°C)	pH	Po_2 (mm Hg)	Pco_2 (mm Hg)
I	7	302±30	0	66.4±6.5	37.2±0.6	7.30±0.02	108±15	43±4
				66.1±6.4	37.2±0.5	7.26±0.02	108±15	42±4
II	9	301±33	4.7±2.4	65.3±10.7	37.4±0.6	7.30±0.04	97±19	44±5
				68.1±12.1	37.0±0.9	7.29±0.05	108±20	42±5
III	7	305±25	3.6±1.6	74.0±9.9	37.2±0.6	7.31±0.02	104±12	44±2
				68.1±15.4	37.2±0.4	7.30±0.04	109±13	43±5

Values are the mean±S.D.
Upper rows of each column indicate baseline measurements, while the lower rows indicate values obtained 2 h after KCl or saline application.

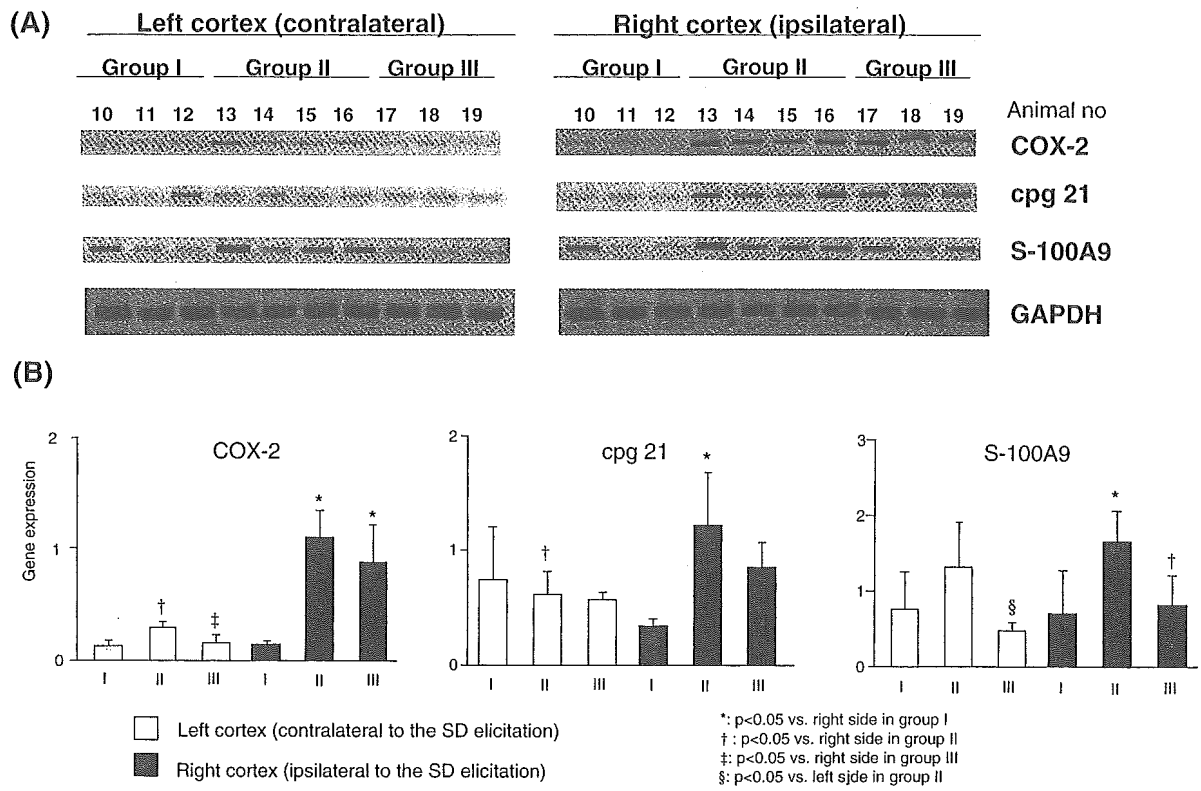

Fig. 2. RT-PCR analysis of COX-2, cpg21, S-100A9, and GAPDH mRNA in each group. (A) Autoradiograms of COX-2, cpg21, S-100A9, and GAPDH mRNA in each group. (B) Densitometric analysis showing the expression of the genes of interest normalized to GAPDH mRNA. Note that S-100A9 expression, which was upregulated in both cortices of rats in group II, was downregulated by the administration of JTE-522 (group III). The expression of COX-2 and cpg21 was prominent in the cortices undergoing SD in the rats in group II. Expression of GAPDH mRNA was equivalent between groups.

and 8 elements were found to be at least 2.5-fold different than in the rats in group III.

3.2. SD-associated genes that were modulated by JTE-522

There were no significant differences in the expression of cpg21, S-100A9, and COX-2 in the right vs. left cortex of rats in group I. However, mRNA expression of all of these genes was significantly upregulated in the right cortex of rats in group II that had undergone SD, compared to controls (Fig. 2). The expression of both COX-2 and cpg21 mRNA in the right hemisphere in group II rats was also significantly upregulated compared to their left hemisphere. On the other hand, the expression of S-100A9 mRNA in the left cortex of these rats was similar to that seen in their contralateral hemisphere. The expression of S-100A9 mRNA in both hemispheres of the rats in group III was significantly reduced compared to that seen in corresponding sides of the brain in group II rats.

Table 3
Concentration of PGE_2 in brain samples

Group	Right cortex (ipsilateral)	Left cortex (contralateral)
I	22.8±12.3	36.3±16.9
II	17.8±12.6	29.5±13.8
III	8.0±4.9***	18.4±6.9***

pg/TP (total protein) mg.
* $p<0.05$ vs. right cortex in group I.
** $p<0.05$ vs. left cortex in group III.
*** $p<0.05$ vs. left cortex in group I.

3.3. PGE_2 synthesis

Mean tissue levels of PGE_2 in both hemispheres of group III rats were significantly lower than those seen in corresponding regions of the brains of control, group I rats (Table 3). The PGE_2 concentration in the right cortex of group III rats was significantly reduced compared to levels in their contralateral hemisphere.

4. Discussion

In this study, we examined the effects of the selective COX-2 inhibitor, JTE-522, on gene expression in brains that underwent SD. We confirmed the findings of Choudhuri et al. [19] that COX-2 was only upregulated in the hemisphere that underwent SD. On the other hand, PGE_2 production levels in the rats of group II were similar to those seen in group I; levels in the right cortex of the rats in group III were significantly lower than those seen in the contralateral cortex. These findings were likely

attributable to either suppression of protein synthesis during the repetitive SDs [20], or the rapid kinetics of the COX enzyme [21,22].

cpg21, which shows 92% homology with dual specificity phosphatase 5 in humans, dephosphorylates and inactivates phosphorylated extracellular signal regulated kinase 1 (ERK1). Transient phosphorylation of ERK1/2 in a MAP kinase/ERK kinases (MEK)-dependent manner was shown to occur following SD, with phosphorylated ERK levels returning to control levels 45 min later [23]. Because JTE-522 did not suppress the expression of the cpg21 gene as shown by RT-PCR in the present study, upregulation of the cpg21 gene probably occurs concomitantly with phosphorylation of ERK1/2 after SD, and is not associated with COX-2 activation.

S-100A9 belongs to the S-100 family of calcium-binding proteins. The S-100 protein, which was first isolated from the brain by Moore in 1965 [24], exists in 3 dimeric forms [25] i.e., an alpha–alpha form known as S-100A(0), an alpha–beta form known as S-100A, and a beta–beta form known as S-100B [26]. Enhanced synthesis of S-100B by reactive astrocytes within the peri-infarct area was shown to participate in the inflammatory response that delayed infarct expansion after permanent focal ischemia in rats [27]. S-100A8 and S-100A9, which are produced by activated neutrophils and monocytes, are translocated to the cell membrane where they form a heterodimer that co-localizes with cytoskeletal proteins [28,29]. Postler et al. [30] demonstrated that microglial cells in the peri-infarct area expressed S-100A8 and S-100A9 in the early phase of human cerebral ischemia, though the role that they played in this process is not fully understood.

In the present study, we found that the expression of the S-100A9 gene was upregulated not only in the cortex that underwent SD but also in the contralateral cortex. The S-100A8/A9 complex was reported to specifically bind to polyunsaturated fatty acids in a calcium-dependent manner [31,32], and it has been implicated in the modulation of the activity of arachidonic acid-metabolizing enzymes [33]. Neuronal activity during SD that is mediated by the NMDA receptor could help propagate astrocytic calcium waves into the brain areas contralateral to the affected side [34–36]. Since S-100A9 gene expression and PGE_2 production were bilaterally downregulated in the hemispheres of rats administered JTE-522, the expression of the S-100A9 gene is likely affected not only by calcium mobilization but also by increased COX-2 activity during SD. Thus, expression of the COX-2 gene in the cortices undergoing SD could be modulated by the S100A9 gene, though it remains unclear whether the S100A9 gene could modulate contralateral COX-2 gene expression.

In conclusion, the bilateral induction of expression of the S-100A9 gene in response to SD may be modulated by prostaglandin synthesis, even though there was no upregulation of the COX-2 gene in the cortex contralateral to that which underwent SD.

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Regular Article

Optimal dose of prothrombin complex concentrate for acute reversal of oral anticoagulation

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Abstract We investigated optimal dose of prothrombin complex concentrate (PCC) for acute reversal of oral anticoagulation in patients with major hemorrhagic complications or who required invasive procedures. We also checked how rapidly international normalized ratio (INR) was reversed after PCC administration.

INR was measured before and 10–60 min after administration of PCC with or without vitamin K in 42 patients (men 28, women 14, median age of 70 years old) who had received warfarin but required rapid reversal of INR because of a hemorrhagic complication or medical procedure. The amount of PCC administered was 200 IU in six patients, 500 IU in 30, 1000 IU in 3, and 1500 IU in the other 3. Additional administration of PCC was performed when the correction of INR was inadequate. In 10 of the 42 cases, INR was measured serially, before, 10 and 60 min and 12–24 h after the administration of PCC and vitamin K.

In the six patients who received PCC of 200 IU, INR values of 3.34 median (range 2.06 to 5.08) decreased to 1.85 (range 1.23 to 2.43) significantly (Wilcoxon's rank sum test, $p=0.028$), but in three patients (50%), INR values were still above 2.0 after the administration. In 30 patients treated with PCC of 500 IU, values decreased from 2.49 median (range 1.54 to 10.00) to 1.19 (range 0.87 to 1.55) significantly ($p<0.0001$). The corrected INR values were below 1.5 in 25 of 26 patients (96%) who had initial INR values from 2.0 to 4.9. In four patients with initial INR of 5.0 or more, the reversed INR was below 1.5 in one (25%), between 1.5 and 2.0 in two (50%), and above 2.0 in one (25%) who had additional administration of 500 IU PCC lowering INR from 2.01 to 1.48. Values of INR in the six patients receiving 1000 IU or 1500 IU, INR decreased from 2.33 median (range 1.96 to 4.00) to 0.96 (range 0.87 to 1.24, $p=0.028$).

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In the 10 patients with serial measurement, INR changed from 2.67 median (range 2.05 to 10.00) to 1.17 (range 0.99 to 1.60) 10 min after the administration. The INR values remained stable 60 min and 12–24 h after the PCC administration.

The 500 IU of PCC is likely to be optimal dose of PCC for emergent reversal of INR in patients requiring rapid correction of INR below 5.0, but to be inadequate dose in patients with INR of 5.0 or more. PCC administration with vitamin K may finish reversing INR rapidly within 10 min and keep the reversed INR values for 12–24 h.

Introduction

Hemorrhagic complication is a major adverse events in patients with oral anticoagulant therapy [1,2], and often requires reduction in dose or discontinuation of the therapy, administration of vitamin K, fresh frozen plasma (FFP) or prothrombin complex concentrate (PCC). The PCC contains coagulant factors II, VII, IX, and X and can reverse the effect of warfare more rapidly than FFP in warfarin-related coagulopathy [3–7]. We reported earlier that rapid correction of international normalized ratio (INR) prevented enlargement of intracranial hematoma in patients with INR values above 2.0 within 24 h of hemorrhagic stroke onset [8].

However, several questions remain to be resolved in the PCC treatment; how much PCC should be administered initially and how rapidly the PCC treatment can reverse INR. The present study was carried out to solve these questions.

Material and methods

From December 2000 to August 2003, PCC was administered in 42 patients who were given warfarin treatment, but required rapid correction of INR because of a major hemorrhagic complication or invasive procedures, an insertion of drainage tube into the thoracic cavity due to acute pneumothorax in two patients and an operation to remove a part of skull bone due to local infection. The major hemorrhagic complications were cerebral hemorrhage in 27, acute epidural hemorrhage in seven, acute bleeding from the gastrointestinal system in two, acute subdural hemorrhage, massive subcutaneous hemorrhage, and intramuscular hemorrhage in one each.

They were 28 men and 14 women with 24–90 years of age (median 70 years old). The underlying diseases requiring warfarin treatment were atrial fibrillation in 22, prosthetic cardiac valves in 9, deep vein thrombosis in 4, left ventricular assist systems in 2, Buerger disease, basilar stenosis, old myocardial infarction, dilated cardiomyopathy, and aortic arch atherosclerosis in 1 each. Hypertension, brain infarction, hypercholesterolemia, diabetes mellitus and hepatitis was complicated in 32 (76.2%), 24 (58.5%), 14 (34.1%), 8 (19.5), and 2 (4.8%) patients, respectively.

The pharmaceutical council in our hospital discussed the administration of PCC including ethical issue and approved it for emergent INR reversal after obtaining informed consent. Then written informed consent was always obtained from the patients or their family. For each patient, administration and amount of the PCC were decided by physicians in charge according to our previous studies [7,8]. The initial amount of PCC was 200 IU in 6, 500 IU in 30, 1000 IU in 3 and 1.500 IU in the other 3. We administered vitamin K of 10 mg in 20 patients and 20 mg in 11 with PCC. Additional PCC was given if the INR value was still high just after the first PCC administration. We used a commercially available PCC "PPSB-HT Nichiyaku" produced by Nihon Pharmaceutical, Tokyo, Japan, which contained 500 IU of II, VII, IX, X and 380 U of protein C in 25 ml. The PCC was derived from donated plasma, which was negative for HBs antigen, anti HCV antibody, anti HIV-1 antibody, anti HIV-2 antibody, anti-HTLV-I antibody and screened by ALT values. Heat at 65 C for 96 h and nanofiltration were applied to inactivate viruses. PCC was extracted from a bottle through a filter to an injection syringe and infused through a venous line in 5–10 min.

INR values were measured before and 10 to 60 min after administration of PCC with or without vitamin K in 42 patients. In 10 of the 42 cases, they were measured serially, before, 10 and 60 min and 12–24 h after the administration of PCC (500 IU in nine and 1000 in the other one) and vitamin K (10 mg in seven and 20 mg in the other three).

Data were expressed as median and range. We used Wilcoxon's rank sum test for analysis of

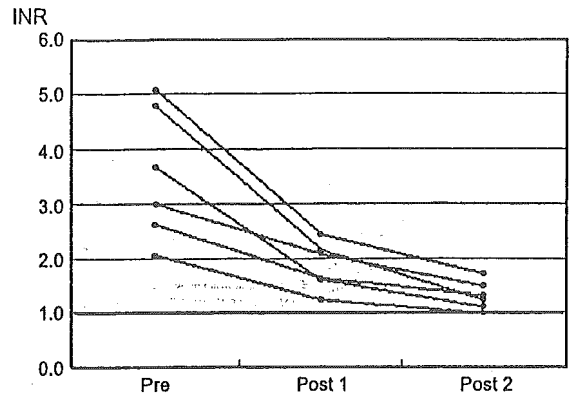

Figure 1 INR values before (Pre) and after PCC administration of 200 IU (Post 1), and those after additional administration of 300 IU (Post 2).

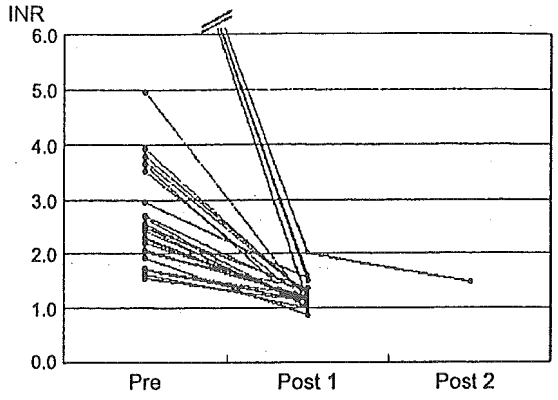

Figure 2 INR values before (Pre) and after PCC administration of 500 IU (Post 1), and those after additional administration of 500 IU (Post 2).

variables. A p-value less than 0.05 was considered significant.

Results

In six patients who received PCC of 200 IU (median 3.3 IU/kg, range 2.6 to 5.3 IU/kg), INR values decreased from 3.34 median (range 2.06 to 5.08) to 1.85 (range 1.23 to 2.43) significantly (p=0.028), but INR values after the PCC administration were still above 2.0 in three patients and between 1.5 and 2.0 in two patients (Fig. 1). Additional 300 IU was administered into the six patients, and INR values decreased to 1.28 (range 0.99 to 1.71).

In 30 patients treated with PCC of 500 IU (median 8.8 IU/kg, range 6.0 to 17.9 IU/kg), INR values decreased from 2.49 median (range 1.54 to 10.00) to 1.19 (range 0.87 to 1.55) significantly (p<0.0001, Fig. 2). INR values after PCC administration were below 1.5 in 25 of 26 patients (96%) who had initial INR values from 2.0 to 4.9 and between 1.5 and 2.0 in two (50%) and above 2.0 in the one (25%) of the four patients that had initial INR of 5.0 or more (Table 1). Only one patient with INR value of 2.01 after 500 IU of PCC administration received additional 500 IU of PCC (1000 IU in total) and his INR decreased to 1.48.

Values of INR in patients receiving 1000 IU (median 18.4 IU/kg, range 18.1 to 18.7 IU/kg) or 1500 IU (median 26.0 IU/kg, range 25.2 to 26.8 IU/kg), INR values decreased from 2.33 median (range 1.96 to 4.00) to 0.96 (range 0.87 to 1.24, p<0.028, Fig. 3).

In 10 patients with serial measurement, INR changed from 2.67 median (range 2.05 to 10.00) to 1.17 (range 0.99 to 1.60) 10 min after the administration (p=0.0051, Fig. 4). The INR values remained stable after 60 min and 12–24 h after the administration.

Symptoms did not deteriorate and hematoma volume did not enlarge in 25 patients of the 27 with cerebral hemorrhage. Deterioration of symptoms with enlargement of hematoma volume was noted in only two, one of whom re-increase of INR from 1.48 to 2.72 half a day after INR reversal by administration of 1000 IU of PCC without vitamin K, and the other one of whom systolic blood pressure after admission remained above 200 mm Hg while INR was kept low. Evacuation of hematoma in six patients with acute epidural hematoma and in a patient with acute subdural hematoma was successfully performed and easy hemostasis during operation was noted by neurosurgeons while a patient with severe epidural hematoma at admission died despite INR reversal. Insertion of drainage

Table 1 Reversed INR according to the initial INR

Amount of PCC administered initially (%)									
	200 IU			500 IU			1000 or 1500 IU		
Reversed INR	≧2.0	≧1.5 & <2.0	<1.5	≧2.0	≧1.5 & <2.0	<1.5	≧2.0	≧1.5 & <2.0	<1.5
Initial INR									
5.0–7.9	0	0	0	1 (25)	2 (50)	1 (25)	0	0	0
3.0–4.9	3 (75)	1 (25)	0 (0)	0	0	6 (100)	0	0	1 (100)
2.0–2.9	0	1 (50)	1 (50)	0	1 (8)	12 (92)	0	0	3 (100)
1.5–1.9	0	0	0	0	0	7 (100)	0	0	2 (100)
									(%)

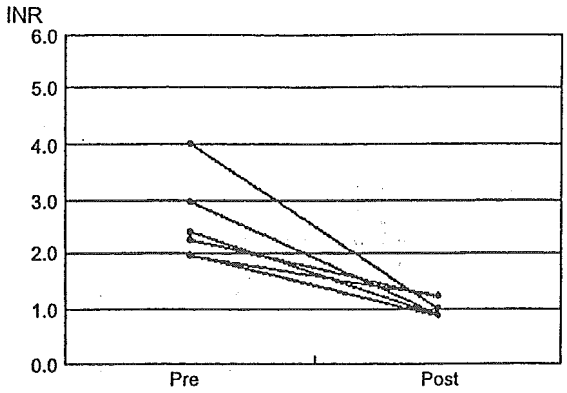

Figure 3 INR values before (Pre) and after PCC administration of 1000 or 1500 IU (Post).

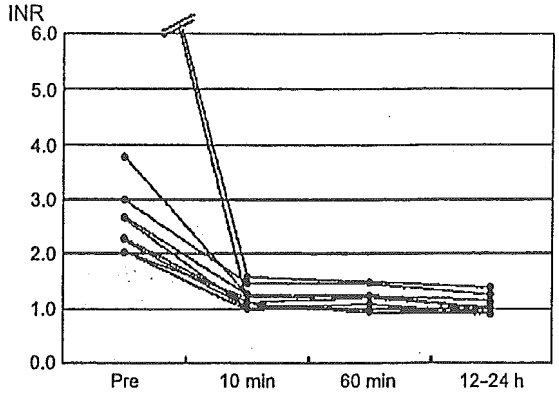

Figure 4 INR values before (Pre) and 10 min, 60 min, 12–24 h after PCC administration of 500 or 1000 IU. min: minutes, h: hours.

tube into the thoracic cavity and an operation to remove a part of skull bone were also successfully done. Subcutaneous, intramuscular and gastrointestinal bleeding stopped after the INR reversal by the PCC administration.

Any adverse effects including shock, allergy, or thrombotic or embolic episodes were not observed in the 42 patients.

Discussion

Previously we reviewed 47 patients on warfarin who developed acute intracerebral hematoma, 10 of whom had PCC treatment within 24 h of onset, and determined relationships among enlargement of the hematoma, INR reversal and clinical data [8]. Multivariate analysis showed an INR value <2.0 at admission or for 24 h after immediate INR reversal with PCC prevented the enlargement of hematoma. Fredriksson et al. retrospectively compared laboratory data and clinical features in 17 patients of anticoagulant-related intracerebral hemorrhage treated with PCC or FFP, and found that clinical progression within 12 h occurred in five of six patients with reversed INR of 1.46 or more [5]. Therefore, it seems that immediate reversal of INR and upkeep of INR values below 2.0 or below 1.5 is necessary to prevent progression of intracerebral hemorrhage.

Effect of 20–50 IU/kg of PCC on reversing INR was reported to be more rapid and effective than FFP [3–6]. Butler et al. [9] recommended administration of 50 IU/kg or 25 IU/kg of PCC with vitamin K in the immediate management of oral anticoagulant-related intracranial hemorrhage when INR values are >=4.5 or <4.5, respectively. The present study demonstrated that 500 IU (median 8.8 IU/kg, range 6.0 to 17.9) of PCC induced a rapid reversal of INR into below 1.5 in 96% of 26 patients who's initial INR values were below 5.0. Because, in the patients with INR above 5.0, INR values remained above 1.5 in 75% after the initial administration of 500 IU PCC and one of them had 1000 IU PCC in total to reverse INR fully, 500 IU PCC may be inadequate and initial administration of 1000 IU or 1500 IU PCC seem required to reverse INR fully in patients with INR above 5.0 (Tables 1 and 2).

Fredriksson et al. [5] reported bilateral renal infarction at autopsy in a case treated with 3000 IU PCC and noted the risk of general thromboembolism triggered by activated prothrombin complex. We did not find any adverse effects in patients treated with smaller amount of PCC (200–1500 IU) than in previous reports [3–6]. Therefore, the smaller amount of PCC might contribute to avoid

Table 2 Amount of PCC and INR change

Investigator	Number	PCC			VK (mg)	INR change	Time
		Amount (IU)	IU/kg	(median)			
Fredriksson et al. [[5]]	5		40–50		10	3.1–1.3	2 h
Makris et al. [[6]]	6		50		10	4.9–1.3	15 min
Boulis et al. [[3]]	10		25.8		10–20	2.8–1.2	4.8 h
Cartmil et al. [[4]]	16		20–50		1–5	5.8–1.3	15 min
Current study	6	200 IU	2.6–5.3	(3.3)	0–20	3.3–1.9	10–60 min
	30	500 IU	6.0–17.9	(8.8)	0–20	2.5–1.2	10–60 min
	3	1000 IU	18.1–18.7	(18.4)	0–10	2.3–1.0	10–60 min
	3	1500 IU	25.2–26.8	(26.0)	0–10	2.4–0.9	10–60 min

thrombotic or embolic adverse effects including disseminated intravascular coagulation. However, the present study showed that initial amount of 200 IU was so inadequate to reverse INR that we had better to administer 500 IU or more initially.

Correction of INR values was reported to be confirmed 15 min, 2 h, or 4.8 h after PCC administration [3–6]. Preston et al. [10] demonstrated rapid reversal of INR by measuring blood samples obtained at 20, 60, and 120 min after treatment. According to the present study, correction of INR seems to be accomplished more quickly, within 10 min after completion of PCC administration than indicated in the previous five reports. Excessive INR values may be counteracted immediately with increases of coagulant factors II, VII, IX, and X by the PCC administration.

Because the present study was not a randomized one, we need prospective randomized research to confirm optimal initial dose of the PCC according to INR.

In conclusion, 500 IU of the PCC is likely to be optimal for rapid correction of INR below 5.0 but to be inadequate in patients with INR of 5.0 or more. PCC administration with vitamin K may finish correcting INR rapidly within 10 min and keep the lower INR values for 12–24 h.

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