4. Wada K, Kimura K, Minematsu K, Yasaka M, Uchino M, Yamaguchi T. Combined carotid and transcranial color-coded sonography in acute ischemic stroke. *Eur J Ultrasound.* 2002;15:101–108.
5. Yasaka M, Omae T, Tsuchiya T, Yamaguchi T. Ultrasonic evaluation of the site of carotid axis occlusion in patients with acute cardioembolic stroke. *Stroke.* 1992;23:420–422.
6. Wolverson MK, Heiberg E, Sundaram M, Tantanasirviongse S, Shields JB. Carotid atherosclerosis: high-resolution real-time sonography correlated with angiography. *AJR Am J Roentgenol.* 1983;140:355–361.
7. Trattnig S, Hubsch P, Schuster H, Polzleitner D. Color-coded Doppler imaging of normal vertebral arteries. *Stroke.* 1990;21:1222–1225.
8. Trattnig S, Schwaighofer B, Hubsch P, Schwarz M, Kainberger F. Color-coded Doppler sonography of vertebral arteries. *J Ultrasound Med.* 1991;10:221–226.
9. Bendick PJ, Jackson VP. Evaluation of the vertebral arteries with duplex sonography. *J Vasc Surg.* 1986;3:523–530.
10. Bartels E, Fuchs HH, Flugel KA. Duplex ultrasonography of vertebral arteries: examination, technique, normal values, and clinical applications. *Angiology.* 1992;43:169–180.
11. Davis PC, Nilsen B, Braun IF, Hoffman JC Jr. A prospective comparison of duplex sonography vs angiography of the vertebral arteries. *AJNR Am J Neuroradiol.* 1986;7:1059–1064.
12. Kimura K, Yamaguchi T, Yasaka M, Tsuchiya T. [Hemodynamics of the vertebral artery in subclavian steal syndrome and subclavian steal phenomenon]. *Rinsho Shinkeigaku.* 1991;31:970–973.
13. Nicolau C, Gilabert R, Chamorro A, Vazquez F, Bargallo N, Bru C. Doppler sonography of the intertransverse segment of the vertebral artery. *J Ultrasound Med.* 2000;19:47–53.
14. Kimura K, Yasaka M, Moriyasu H, Tsuchiya T, Yamaguchi T. Ultrasonographic evaluation of vertebral artery to detect vertebrobasilar axis occlusion. *Stroke.* 1994;25:1006–1009.
15. Hennerici M, Klemm C, Rautenberg W. The subclavian steal phenomenon: a common vascular disorder with rare neurologic deficits. *Neurology.* 1988;38:669–673.
16. Yip PK, Liu HM, Hwang BS, Chen RC. Subclavian steal phenomenon: a correlation between duplex sonographic and angiographic findings. *Neuroradiology.* 1992;34:279–282.
17. Kliewer MA, Hertzberg BS, Kim DH, Bowie JD, Courneya DL, Carroll BA. Vertebral artery Doppler waveform changes indicating subclavian steal physiology. *AJR Am J Roentgenol.* 2000;174:815–819.
18. Touboul PJ, Mas JL, Bousser MG, Laplane D. Duplex scanning in extracranial vertebral artery dissection. *Stroke.* 1988;19:116–121.
19. Bartels E, Flugel KA. Evaluation of extracranial vertebral artery dissection with duplex color-flow imaging. *Stroke.* 1996;27:290–295.
20. Hoffmann M, Sacco RL, Chan S, Mohr JP. Noninvasive detection of vertebral artery dissection. *Stroke.* 1993;24:815–819.
21. Sturzenegger M, Mattle HP, Rivoir A, Rihs F, Schmid C. Ultrasound findings in spontaneous extracranial vertebral artery dissection. *Stroke.* 1993;24:1910–1921.
22. Osborn AG. The vertebrobasilar system. In: Osborn AG, ed. *Cerebral angiography.* 2nd ed. Philadelphia: Lippincott Williams & Wilkins; 1999: 173–194.
23. Delcker A, Diener HC. [Various ultrasound methods for studying the vertebral artery—a comparative evaluation]. *Ultraschall Med.* 1992;13: 213–220.
24. Tegeler CH, Babikian VL, Gomez CR. Vertebral sonography. *Neurosonology.* 1996;91–92.
25. Fisher CM, Gore I, Okabe N, White PD. Atherosclerosis of the carotid and vertebral arteries—extracranial and intracranial. *J Neuropathol Exp Neurol.* 1965;24:455–476.
26. de Bray JM, Pasco A, Tranquart F, Papon X, Alecu C, Giraudeau B, Dubas F, Emile J. Accuracy of color-Doppler in the quantification of proximal vertebral artery stenoses. *Cerebrovasc Dis.* 2001;11:335–340.

Neuronal cyclooxygenase-2 expression during spreading depression and focal brain ischemia

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Abstract

In order to clarify the pathophysiology of ischemic stroke, we examined a primate model eliciting SD, a primate thromboembolic model, and a rat model of focal brain ischemia. Immediately after the first SD, focal cortical hyperemia was demonstrated without being followed by spreading or persistent hypoperfusion. Cyclooxygenase-2 (COX-2) induction was detected in SD monkeys by microarray analysis. Immunoreactive neurons were observed in SD animals. In the thromboembolic model, upregulation of COX-2 mRNA expression was observed after 2 h of ischemia, but disappeared by 24 h in the ischemic core. In peri-infarct areas, where flow-metabolism uncoupling was observed, COX-2 expression persisted even after 24 h of ischemia. In focal ischemic rats, diffuse, neuronal COX-2 staining was found in peri-infarct areas as well as in discrete, immunoreactive neurons in the ischemic core. Robust increases in prostaglandin E_2 levels in the peri-infarct areas were demonstrated following 24 h of ischemia. In conclusion, neuronal COX-2 induction was observed in SD animals as well as within potentially viable hypoperfused brain areas. COX-2 expression and prostaglandin production in ischemic tissues depended on the degree and duration of the reduction in cerebral blood flow.

Key words: spreading depression, focal brain ischemia, cyclooxygenase 2, cerebral blood flow

1. Introduction

Cortical spreading depression (SD)[1] has been suggested to play a significant role in the development of ischemic injury under conditions of focal brain ischemia in rat models[2,3]. As proposed by the Stroke Therapy Academic Industry Roundtable[4], nonhuman primate studies are required to clarify the pathophysiology of ischemic stroke, and to verify the safety and efficacy of newly developed drugs that show promising results in rodents. In order to investigate the pathophysiology of acute ischemic stroke, we have developed a primate model eliciting SD and a primate thromboembolic stroke model.

Cyclooxygenase-2 (COX-2), a rate-limiting en-

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zyme in prostaglandin synthesis, was induced associated with either eliciting SD or focal brain ischemia in the cortex ipsilateral to the SD elicitation or brain ischemia in the nonprimate cortex[5]. Therefore we examined COX-2 expression and its reaction products during SD and focal brain ischemia in primates as well as rats.

All procedures in this study were approved by our Institutional Animal Research Committee and were performed in accordance with the standards published by the National Research Council (Guide for the Care and Use of Laboratory Animals).

2. Material and methods

2. 1. Spreading depression in a primate model

We used nine adult, male cynomolgus monkeys. Animals were anesthetized with pentobarbital (0.1 mg/kg, i.p.). Anesthesia was maintained with a N_2O/O_2 (70% : 30%) gas mixture inhalation under artificial ventilation through an experimental period. They were divided into 2 groups, such as normal control (group C, n = 3) and SD evoked animals (group SD, n = 6).

SD was elicited by applying 3.3 mol/L potassium chloride (KCl) through a burr hole made in the left parietal skull[6]. Two other burr holes were made rostral to the hole for KCl application. DC potentials were monitored with microelectrodes inserted into the cortex to a depth of 1 mm through the burr holes except the hole for KCl application.

Cerebral blood flow (CBF) was measured with PET and the ^{15}O-labeled water bolus injection method. A baseline CBF measurement was done once prior to application of KCl solution. CBF measurements were repeated 5 times, beginning 3 minutes after the first SD at intervals of approximately 15 minutes. After completion of the PET studies (at 120 min after KCl application), the brain tissues in the group SD were quickly removed after exsanguination following perfusion with cold saline. Samples of brain tissues in the group C were also obtained as the same manner as those in the group SD. We investigated the gene expression profile associated with SD by a cDNA array system containing 9,182 human elements, which was confirmed by RNA blot, immunoblot, and immunohistochemical analyses[7].

2. 2. Thromboembolic stroke model in primates

Thromboembolic stroke was produced in male cynomolgus monkeys (n = 4) as described previously[8]. CBF was measured with ^{15}O-labeled water before and 1, 2, 4, 6, and 24 hours after embolization. Cerebral glucose metabolic rate (CMRglc) was measured with [^{18}F] FDG methods 24 hours after embolization[9]. Lesion size and location 24 hours after embolization was determined by the 2, 3, 5-triphenyl-tetrazolium chloride (TTC) staining method.

For biochemical analyses for brain tissues in thromboembolic stroke model, we used 9 adult male cynomolgus monkeys ; 3 monkeys were served as normal control and the remaining 6 were as ischemic animals[10]. Two hours after a single autologous blood clot injection in 3 monkeys or after the completion of the PET studies in the other monkeys with 24 h-ischemia, brain tissues were perfused with cold saline and the animals were sacrificed. Three normal controls were also sacrificed as the same manner. Expression ratios of COX-2 mRNA were calculated as ratios of COX-2 mRNA against those of normal brains. Cell injury was evaluated by incorporation of digoxigenin deoxy-uridine-5'-triphosphate (dUTP) with the use of DNA polymerase I.

2. 3. Focal brain ischemia in rats

Male Sprague-Dawley rats (300-350 g, n = 40) were used. Focal brain ischemia was produced by the intraluminad occlusion of the ostium of the right middle cerebral artery with nylon monofila-

ments, as previously described[11]. Rats were sacrificed at time 0 and at different times points after arterial occlusion (1, 2, 3, 4, 6, 8, 12, and 24 h, n = 4-5/time point) and their brains immediately immersed in ice-cold saline. Several blocks were frozen in isopentane-dry ice and stored at −80℃ until use, whereas others were embedded in paraffin for immunohistochemistry. Analysis of COX-2 expression (mRNA, protein), and measurement of the concentrations of PGE_2 and the prostacyclin metabolite, 6-keto-$PGF_{1\alpha}$ in the peri-infarct areas and the ischemic core were performed. In some animals, N-isoproryl-*p*-[^{125}I]-iodoamphetamine ([^{125}I]IMP) (2.22 MBq/kg body weight) was injected into the femoral vein 5 min before sacrifice and *ex-vivo* autoradiography was performed to measure cerebral blood flow (CBF) as described previously[12].

3. Results

3. 1. SD in primates

SD waves were recorded in eight of the 9 monkeys. Single episode in three monkeys, twice in two, and six episodes in one were recorded in the rostral sites. In two of three animals with the caudal hole, one had eight episodes and another did once in the caudal sites for chemical stimulation while they did no SD waves in the rostral sites. The remaining one had twice episodes in the rostral and six episodes in the caudal sites. Focal hyperemia was demonstrated adjacent to the site of KCl application immediately after the first SD. Average cortical CBF in the ipsilateral hemisphere increased significantly immediately after the chemical stimulation ($p < 0.05$ by paired t-test), and the significant increase in CBF persisted throughout the experimental period of 2 hours. In the contralateral hemisphere, no significant changes in CBF were observed.

As a result of microarray analysis, increases in normalized signals of gene expression above 1.5-fold was cyclooxygenase-2 (COX-2) gene (1.6-fold), and signal levels in 265 genes were different by at least 1.3-fold between the 2 groups. COX-2 induction was confirmed by RNA blot, immunoblot, and immunohistochemical analyses. Intense immunoreactive neurons were induced in the animals with SDs.

3. 2. Focal brain ischemia in primates

CBF in the temporal cortex and the basal ganglia decreased to < 40% of the contralateral values 1 hour after embolization, following further decline in CBF as well as CMRglc at 24 hour of ischemia. These regions were consistently unstained with TTC, being indicated that both temporal cortex and basal ganglia ipsilateral to the arterial embolization were regarded as the ischemic core. While CBF was > 40% of the contralateral values 1 hour after the embolization and recovered gradually with time in the parietal cortex ipsilateral to the embolization. No obvious TTC-unstained lesions were demonstrated in these regions, implicated that the parietal cortex ipsilateral to the embolization was regarded as the ischemic penumbra. Increased in CMRglc at 24 hours of ischemia compared with those in the contralateral regions, an uncoupling of CBF and CMRglc, were demonstrated in these regions.

The upregulation of COX-2 mRNA expression was observed at 2 h (expression ratio was 7.4), but disappeared by 24 h in the ischemic temporal cortex, where cell injury was apparent by incorporation of dUTP. In the ischemic parietal cortex, where flow-metabolism uncoupling was observed, COX-2 mRNA was persistently induced even at 24 h after ischemia (expression ratio was 4.7), and few damaged cells could be detected by incorporation of dUTP as well as in each region from the hemisphere contralateral to the clot injection. Intense COX-2 immunoreactivity was found in discrete neurons in the ischemic parietal

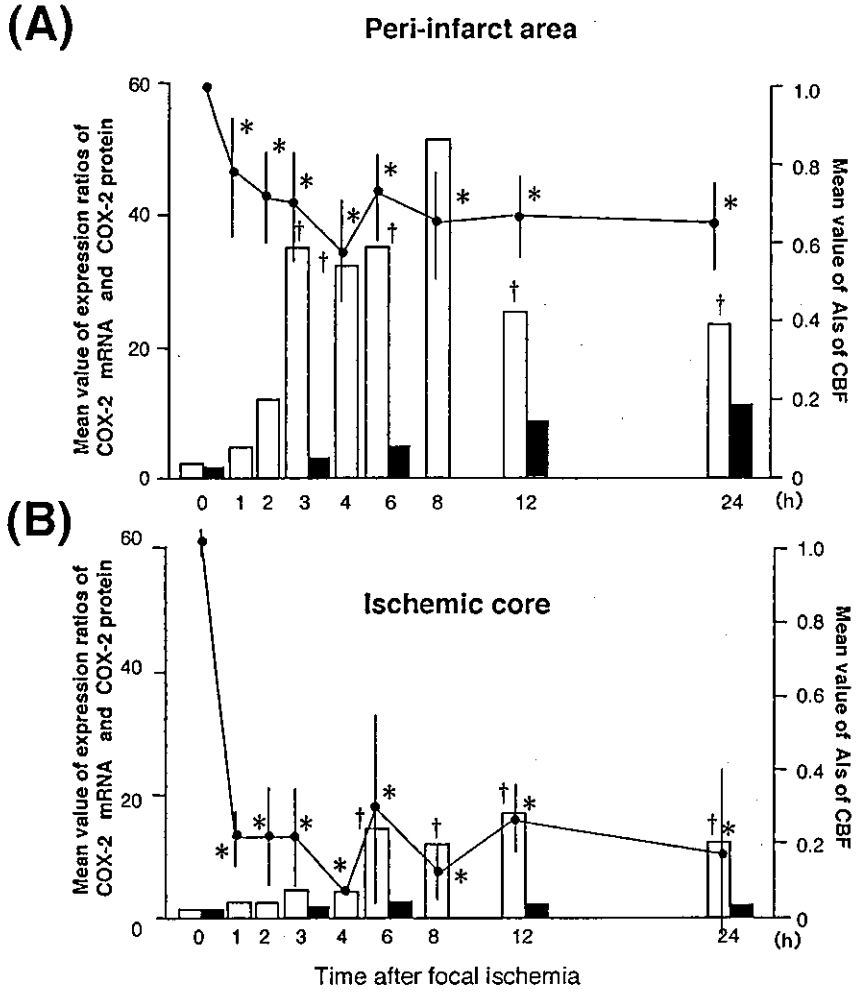

Fig. 1. Temporal profile of COX-2 expression associated with changes in CBF during 24 h of ischemia

Lines indicate the mean asymmetry index (AI) values of CBF. The open and solid columns correspond to the mean expression ratios of COX-2 mRNA and COX-2 protein, respectively. Figures A and B show the time course of COX-2 expression in the peri-infarct areas and ischemic core, respectively. A one-way ANOVA and post-hoc Fisher's tests were used to assess the differences in AIs and expression ratios of COX-2 mRNA between the different ischemic time points. CBF in the peri-infarct areas and ischemic core were significantly reduced compared to controls immediately after arterial occlusion (*: $p<0.05$). The mean CBFs in the ischemic core and peri-infarct areas were 0.19 ± 0.07 (mean ± SD) and 0.67 ± 0.06, respectively. The time course of COX-2 expression in the peri-infarct areas was different from that in the ischemic core. Thus, the expression ratios of COX-2 mRNA increased significantly after 3 h of ischemia (†: $p<0.05$), with COX-2 protein increasing with time in the peri-infarct areas. On the other hand, significant increases in COX-2 mRNA were found 6 h after ischemia (†: $p<0.05$), and increases in COX-2 protein were not observed during the ischemic period in the ischemic core.

cortex, although no significant increases in COX-2 protein level were shown either in the ischemic temporal or parietal cortices.

3. 3 Focal brain ischemia in rats

Table 1. Prostaglandin production (pg/mg total protein) in the ischemic hemisphere

Prostaglandin	Duration of ischemia	Peri-infarct area	Ischemic core
PG E_2	0 hours	60.8 ± 16.6	21.4 ± 11.4
	3 hours	156.6 ± 70.1	54.4 ± 22.3
	24 hours	2,609.0 ± 2,522.0 *†	414.6 ± 226.3 *†
Prostacyclin	0 hours	122.3 ± 47.6	47.6 ± 23.0
metabolite	3 hours	200.8 ± 59.7	93.4 ± 43.5
(6-keto-PG $F_{1\alpha}$)	24 hours	1,143.0 ± 623.7 *†	341.6 ± 84.5 *†

* $p < 0.05$ vs. 0 h (control) ; † : $p < 0.05$ vs. 3 h ischemia by ANOVA
The values were the mean ± SD.

Significant reductions in CBF in the peri-infarct areas and ischemic core were demonstrated in animals at each ischemic time point compared to the controls (Fig. 1). The expression ratios of COX-2 mRNA increased significantly between 3 and 24 h of ischemia in the peri-infarct areas compared to the controls. In the ischemic core, significant increases in COX-2 mRNA were seen following 6 h of ischemia, which remained through 24 h. The peak value of the expression ratio of COX-2 protein in the peri-infarct area was 10.7 at 24 h of ischemia, while the peak expression ratio in the ischemic core was 2.0 at 6 h of ischemia. COX-2 immunoreactive neurons were found predominantly in the peri-infarct area. Elevations in the immunohistochemical staining of discrete neuronal populations were also observed in the ischemic core. Although no significant increases in PGE_2 and prostacyclin levels were observed in the peri-infarct and ischemic core areas following 3 h of ischemia, significant increases in prostaglandin levels were found in the ischemic hemisphere following 24 h of ischemia. In particular, PGE_2 levels in the peri-infarct area increased significantly (Table 1).

4. Discussion

The CBF pattern obtained in the SD model of primates differed from those obtained in other studies using rat- and cat-SD models[13,14]. The focal hyperemia was not followed by prolonged hypoperfusion. The changes in CBF during SD phenomenon in primates also differed from those in patients with migraine[15]. In biochemical analysis for brain tissues, COX-2 was induced in the cortices where SD was recorded, being in accord with prvious observations in rodent models[5].

We observed COX-2 expression during focal brain ischemia in a primate thromboembolic stroke model. In the ischemic core, in which a significnat decrease in CBF were accompanied by reduced CMRglc, upregurated COX-2 mRNA at 2 h-ischemia but decreased by 24 h. Disappearance of COX-2 at 24 h-ischemia was parallel to a housekeeping GAPDH-mRNA reduction, indicating that ischemic injury was already apparent at 24-ischemia in the temporal cortex and the basal ganglia. In the peri-infarct area, on the contrary, induced expression of COX-2 mRNA was still found at 24-h ischemia in the parietal cortex with a mild CBF reduction and maintained CMRglc.

In the focal ischemia in rats, the time course of COX-2 expression in the ischemic core was different from that seen in the peri-infarct area. The upregulation of COX-2 mRNA in the peri-infarct area persisted for at least 24 h after ischemia, as did the production of COX-2 protein, which lead

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A Case-Control Analysis of Intra-Arterial Urokinase Thrombolysis in Acute Cardioembolic Stroke

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Key Words
Intra-arterial urokinase thrombolysis · Cardioembolic stroke · Acute ischemic stroke · National Institute of Health Stroke Scale score · Modified Rankin scale score

Abstract
Background: Intra-arterial urokinase (IA-UK) thrombolysis is frequently given in Japan to selected patients with acute cerebral artery occlusion. However, it is not clear whether or not IA-UK thrombolysis has an efficacy for acute stroke patients. The purpose of this study was to assess the effects of IA-UK thrombolysis in acute cardioembolic stroke patients, by performing a case-control analysis using data from Japan's Multicenter Stroke Investigator's Collaboration (J-MUSIC). ***Methods:*** 16,922 acute ischemic stroke patients were enrolled into J-MUSIC. From these patients, we selected 91 patients (UK group) who met the following criteria: treatment with IA-UK; 20–75 years of age; cardioembolic stroke; presenting with a carotid stroke; admission within 4.5 h of symptom onset, and a National Institutes of Health Stroke Scale (NIHSS) score of 5–22 points on admission. A control group of 182 patients without IA-UK treatment and matched to the NIHSS score, gender, and age was chosen. We compared the modified Rankin scale (mRS) score at discharge and the mortality between the 2 groups. ***Results:*** In both groups, the mean age was 65 ± 8 years, and the median NIHSS score was 14. The mean interval between symptom onset and UK administration was 3.4 ± 1.3 h, and the IA-UK dose was 392,000 ± 200,000 units. The mRS score at discharge was lower in the UK group than in the control group (mean, SD, median; 2.8, 2.9, 2 in UK group vs. 3.3, 1.8, 4, in the control, respectively $p = 0.031$). A favorable outcome (mRS of 0–2) was more frequently observed in the UK group (50.5%) than in the control group (34.1%, $p = 0.0124$). No difference in the mortality rate was seen between the UK group (11.0%) and the control group (13.3%). As well, there was no difference in the length of hospital stay between the UK group (46 ± 41 days, mean ± SD) and the control group (42 ± 42 days, mean ± SD). ***Conclusions:*** IA-UK thrombolytic therapy may improve the outcome in hyperacute cardioembolic stroke patients.

Intravenous (IV) thrombolytic therapy using recombinant tissue plasminogen activator (rt-PA) has been shown to be an effective treatment for ischemic stroke if used within 3 h of stroke onset [1, 2]. Recently, prolyse in acute cerebral thromboembolism (PROACT) I and II reported that local and intra-arterial (IA) thrombolytic therapy with pro-urokinase (proUK) could improve the outcome

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for ischemic stroke patients if used within 6 h of symptom onset [3, 4]. In Japan, the use of rt-PA for acute ischemic stroke has not been approved by the government. Therefore, IA thrombolytic therapy with urokinase (IA-UK) is mainly performed as a replacement of IV-rt-PA thrombolysis for acute cerebral artery occlusion, and in particular, for embolic occlusion of the middle cerebral artery. Several investigators have reported that IA-UK therapy was safe and effective for acute ischemic stroke [5–15]. However, their sample sizes were small, and not all their studies were randomized controlled trials.. Therefore, it remains unclear whether IA-UK therapy is effective for acute stroke patients. The aim of this study was to assess the efficacy of IA-UK thrombolysis for acute stroke patients by a case-control analysis using data from J-MUSIC [16, 17].

Subjects and Methods

We conducted a multicenter, prospective, hospital-based registration study (J-MUSIC) from May 1999 to April 2000 in which 156 hospitals from all over Japan participated [16, 17]. A total of 16,922 consecutive patients with acute ischemic stroke and transient ischemic attack within 7 days of onset were registered in this study.

The following data were assessed in all the patients, using common data-sheets prepared by the protocol committee: (1) age and gender; (2) time from onset to hospital arrival; (3) a history of stroke; (4) National Institutes of Health Stroke Scale (NIHSS) score on admission; (5) site of acute lesions on CT or MRI; (6) stroke subtype (clinical category); (7) thrombolytic therapy (IV and IA rt-PA, IA UK) within 12 h of onset; and (8) outcome at discharge.

Clinical categories were defined by using clinical and radiographic diagnosis rubrics according to the classification of cerebrovascular diseases III developed by National Institute of Neurological Disorders and Stroke [18]. The main subtypes included: lacunar, atherothrombotic, cardioembolic, and other stroke. The modified Rankin Scale (mRS) [19] score and mortality were used to assess clinical outcome at hospital discharge.

We selected patients treated with IA-UK (UK group) and patients who had been treated without thrombolytic therapy (control group) from 16,922 patients. The UK group was identified as the patients treated with IA-UK who met the following criteria: aged 20–75 years; presence of a cardioembolic stroke or a carotid stroke; admission within 4.5 h of symptom onset, and an NIHSS score of 5–22 points on admission. We randomly selected control patients who had no thrombolytic therapy, such as IA-UK, IA-rt-PA, and IV-rt-PA and were matched to the UK group patients with respect to age, gender, and NIHSS score. The number of control group patients was set to be twice the number of the UK group patients.

Statistical Analysis

Analyses were made with a commercially available software package (Stat-View, version 4.5; ASA Institute, Cary, N.C.). We compared the mRS score, mortality, and length of hospital stay between the two groups. The statistical significance for differences between the two groups was assessed by the Wilcoxon signed-rank test for the mRS score, the χ^2 test for favorable outcome (mRS score 0–2) and mortality, and the paired t-test for length of hospital stay. A p value <0.05 was considered statistically significant.

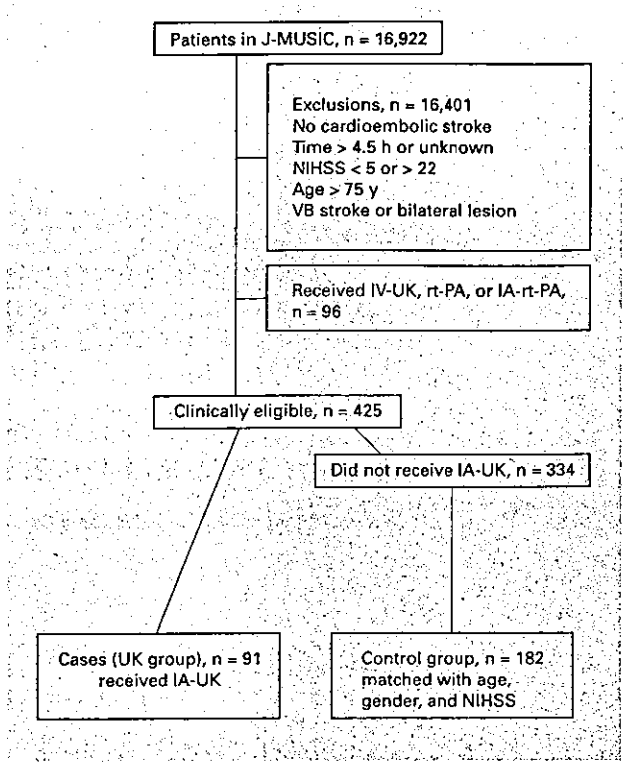

Fig. 1. Flow chart showing process of patient selection.

Results

Ninety-one patients met the criteria for inclusion into the UK group and 182 patients were selected for the control group (fig. 1). Table 1 shows the baseline characteristics of the two groups. In each group, the average age was 65 ± 8 years. The median NIHSS score for the two groups was 14. In the UK group, the mean interval between the onset of symptoms and UK administration was 3.4 ± 1.3 h, and the IA-UK dose was 392,000 ± 200,000 units. The mRS score at discharge was lower in the UK group than in the control group (mean, SD, median; 2.8, 2.9, 2 vs. 3.3, 1.8, 4, respectively; p = 0.031). Patients with

Table 1. Characteristics of the two groups

	UK group	Control group	p
Patients	91	182	
Gender, F/M	24/67	48/134	
Age, years (mean ± SD)	65±8	65±8	
NIHSS score on admission	14	14	
Interval time from stroke onset to hospital, h (mean ± SD)	1.1±0.2	1.1±0.3	
Interval time from stroke onset to treatment, h (mean ± SD)	3.4±1.3	–	
Range	1–6		
Mega units of UK administered (mean ± SD)	0.39±0.20	–	
Mean, SD, median of mRS score at discharge	2.8, 2.9, 2	3.3, 1.8, 4	0.031
mRS ≤2, %	50.5	34.1	0.012
Mortality, %	11.0	13.2	0.745
Length of hospital stay, days (mean ± SD)	46±41	42±42	0.347

favorable outcome were more frequently found in the UK group than in the control group (50.5 vs. 34.1%, p = 0.0124). However, no difference between the two groups was observed in the mortality rate (11.0 vs. 13.2%) or the length of hospital stay (46 ± 41 vs. 42 ± 42 days, mean ± SD).

We analyzed the relationship between time interval from stroke onset to IA-UK thrombolytic therapy and patients' outcome. The percentage of favorable outcome was higher in patients treated within 2 h of stroke onset than in those between 2–4 h and over 4 h [63% (17/27), 45% (21/47), and 47% (8/17)]. However, no significant differences among them were observed (p = 0.30).

Discussion

This case-control study based on the data from J-MUSIC demonstrates the effectiveness of IA-UK thrombolysis in acute stroke patients. Patients with IA-UK thrombolysis had an increased frequency of good outcomes, approximately 1.5 times greater than patients without IA-UK thrombolysis. However, no difference in mortality rate was observed between patients with and without IA-UK thrombolysis.

The PROACT II study [4] demonstrated a significant benefit from treatment with IA proUK in patients with a middle cerebral artery occlusion treated within 6 h of stroke onset. Their proUK group had a higher recanalization rate (66 vs. 18%) with a greater number of patients with good outcomes (mRS score 0–2) after 3 months of stroke onset (40 vs. 25%). However, the incidence of symptomatic intracranial hemorrhage was 10% in the proUK group, but only 2% in the placebo group.

In 1988, del Zoppo et al. [5] studied 20 patients and showed that local IA fibrinolytic therapy using UK or streptokinase might lead to cerebral arterial recanalization in patients with an acute carotid territory thrombotic stroke. Mori et al. [6] also assessed 22 patients and reported on the safety and efficacy of UK thrombolytic therapy for acute thromboembolic occlusion of the middle cerebral artery. Recently, Gonner et al. [8] performed IA-UK thrombolytic therapy in 43 ischemic stroke patients within 6 h of symptom onset, and reported that therapy was effective except in patients with a carotid artery occlusion. Arnold et al. [20] analyzed the clinical and radiological findings, and assessed the functional outcome 3 months after IA-UK thrombolysis for 100 consecutive patients. They concluded that IA-UK thrombolytic therapy was safe and could be efficacious. The results of the present study also lead us to conclude that local IA thrombolytic therapy using UK could be effective for acute ischemic stroke.

The therapeutic time window of IV thrombolytic therapy with rt-PA is within 3 h [1, 2]. However, in the PROACT II study proUK could be administered within 6 h of stroke onset [4]. Therefore, IA thrombolytic therapy may allow the extension of the therapeutic time window for treating acute stroke from 3 to 6 h. In the future, thrombolysis using proUK as well as UK may provide an alternative to IV thrombolysis with rt-PA in selected patients with acute ischemic stroke.

Our study has some limitations. Firstly, the aim of the J-MUSIC [15] study was to determine the present state of stroke managements in Japan, and not to investigate the effectiveness of thrombolytic therapy. Secondly, we did not require to describe the presence and frequency of symptomatic cerebral hemorrhage after thrombolytic therapy in J-MUSIC. There was a higher rate of symptomatic intracranial hemorrhage with IA proUK in PROACT II (10.2%) [4] compared to IV-rt-PA in NINDS (6.4%) [2]. However, there is no evidence that the rate of symptomatic brain hemorrhage is lower with IV thrombolysis than with IA thrombolysis. Thirdly, this was not a randomized study. Therefore, there may be some selection bias against choosing stroke patients with complications, such as heart diseases and infection. Patients with

such complications were not likely to be treated with thrombolytic therapy, and outcomes of such patients were not as good as those in patients without such complications. Furthermore, control patients did not always undergo angiography. The catheter placement itself might be benefit for destruction of the clot. Moreover, physicians who assessed patients' outcome were not blinded to treatment. Therefore, it is possible that efficacy of IA-UK thrombolysis is overestimated.

In conclusion, IA thrombolysis using UK could potentially be effective for acute ischemic stroke patients, and would allow the possible extension of the 3-h therapeutic window. This would lead to an increased number of patients being eligible for thrombolytic therapy.

References

1 Hacke W, Kaste M, Fieschi C, Toni D, Lesaffre E, von Kummer R, Boysen G, Bluhmki E, Hoxter G, Mahagne MH, et al: Intravenous thrombolysis with recombinant tissue plasminogen activator for acute hemispheric stroke. The European Cooperative Acute Stroke Study (ECASS). JAMA 1995;274:1017–1025.

2 Tissue plasminogen activator for acute ischemic stroke: The National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group. N Engl J Med 1995;333:1581–1587.

3 del Zoppo GJ, Higashida RT, Furlan AJ, Pessin MS, Rowley HA, Gent M: PROACT: A phase II randomized trial of recombinant prourokinase by direct arterial delivery in acute middle cerebral artery stroke. PROACT Investigators. Prolyse in Acute Cerebral Thromboembolism. Stroke 1998;29:4–11.

4 Furlan A, Higashida R, Wechsler L, Gent M, Rowley H, Kase C, Pessin M, Ahuja A, Callahan F, Clark WM, Silver F, Rivera F: Intra-arterial prourokinase for acute ischemic stroke. The PROACT II study: A randomized controlled trial. Prolyse in Acute Cerebral Thromboembolism. JAMA 1999;282:2003–2011.

5 del Zoppo GJ, Ferbert A, Otis S, Bruckmann H, Hacke W, Zyroff J, Harker LA, Zeumer H: Local intra-arterial fibrinolytic therapy in acute carotid territory stroke. A pilot study. Stroke 1988;19:307–313.

6 Mori E, Tabuchi M, Yoshida T, Yamadori A: Intracarotid urokinase with thromboembolic occlusion of the middle cerebral artery. Stroke 1988;19:802–812.

7 Hacke W, Zeumer H, Ferbert A, Bruckmann H, del Zoppo GJ: Intra-arterial thrombolytic therapy improves outcome in patients with acute vertebrobasilar occlusive disease. Stroke 1988;19:1216–1222.

8 Gonner F, Remonda L, Mattle H, Sturzenegger M, Ozdoba C, Lovblad KO, Baumgartner R, Bassetti C, Schroth G: Local intra-arterial thrombolysis in acute ischemic stroke. Stroke 1998;29:1894–1900.

9 Barnwell SL, Clark WM, Nguyen TT, O'Neill OR, Wynn ML, Coull BM: Safety and efficacy of delayed intra-arterial urokinase therapy with mechanical clot disruption for thromboembolic stroke. AJNR Am J Neuroradiol 1994;15:1817–1822.

10 Barr JD, Mathis JM, Wildenhain SL, Wechsler L, Jungreis CA, Horton JA: Acute stroke intervention with intra-arterial urokinase infusion. J Vasc Interv Radiol 1994;5:705–713.

11 Berg-Dammer E, Mobius E, Nahser HC, Kuhne D: Local thrombolytic therapy for thromboembolic occlusion of the middle cerebral artery. Neurol Res 1992;14:164–166.

12 Bourekas EC, Slivka AP, Shah R, Sunshine J, Suarez JI: Intra-arterial thrombolytic therapy within 3 hours of the onset of stroke. Neurosurgery 2004;54:39–44.

13 Lee BI, Lee BC, Park SC, Shon YH, Kim DI, Jung TS, Suh JH: Intra-carotid thrombolytic therapy in acute ischemic stroke of carotid arterial territory. Yonsei Med J 1994;35:49–61.

14 Ryu YH, Chung TS, Yoon PH, Kim DI, Lee JD, Lee BI, Suh JH: Evaluation of reperfusion and recovery of brain function before and after intracarotid arterial urokinase therapy in acute cerebral infarction with brain SPECT. Clin Nucl Med 1999;24:566–571.

15 Urbach H, Ries F, Ostertun B, Solymosi L: Local intra-arterial fibrinolysis in thromboembolic 'T' occlusions of the internal carotid artery. Neuroradiology 1997;39:105–110.

16 Kimura K, Kazui S, Minematsu K, Yamaguchi T, for the Japan Multicenter Stroke Investigators' Collaboration (J-MUSIC): Hospital-based prospective registration of acute ischemic stroke and transient ischemic attack in Japan. J Stroke Cerebrovasc Dis 2004;13:1–11.

17 Kimura K, Kazui S, Minematsu K, Yamaguchi T, for the Japan Multicenter Stroke Investigator's Collaboration (J-MUSIC): Analysis of 16,922 patients with acute ischemic stroke and TIA in Japan hospital-based prospective registration study. Cerebrovasc Dis 2004;18:47–56.

18 Special report from the National Institute of Neurological Disorders and Stroke. Classification of cerebrovascular diseases III. Stroke 1990;21:637–676.

19 van Swieten JC, Koudstaal PJ, Visser MC, Schouten HJ, van Gijn J: Interobserver agreement for the assessment of handicap in stroke patients. Stroke 1988;19:604–607.

20 Arnold M, Schroth G, Nedeltchev K, Loher T, Remonda L, Stepper F, Sturzenegger M, Mattle HP: Intra-arterial thrombolysis in 100 patients with acute stroke due to middle cerebral artery occlusion. Stroke 2002;33:1828–1833.

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Mortality and Cause of Death after Hospital Discharge in 10,981 Patients with Ischemic Stroke and Transient Ischemic Attack

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Key Words
Brain infarction · Transient ischemic attack · Stroke, acute · Stroke management

Abstract
Background: The aim of this study was to examine the 1-year cumulative mortality rate and cause of death, and to identify the predictive factors for death after hospital discharge following ischemic stroke and transient ischemic attack (TIA) using data from the Japan Multicenter Stroke Investigators' Collaboration study. ***Methods:*** We prospectively registered 16,922 consecutive patients with acute ischemic stroke or TIA from May 1999 to April 2000 in 156 Japanese hospitals. We mailed a questionnaire to the 15,322 patients who were alive at hospital discharge. ***Results:*** 10,981 patients (6,945 men, 4,036 women, age 70 ± 11 years, median 71, range 19–100 years) were enrolled in the follow-up study. The mean follow-up period was 271 ± 110 days (median 272 days; range 1–487 days). The 1-year cumulative mortality was 6.8% (7.0% for 10,234 stroke patients and 3.5% for 747 TIA patients). The causes of death were: cerebrovascular disease, 24.1%; pneumonia, 22.6%; heart disease, 18.1%; cancer, 11.0%, and miscellaneous causes, 24.1%. Multivariate analysis suggested that male gender, age, diabetes mellitus, atrial fibrillation, history of stroke, nonlacunar stroke, functional disability and transfer to another hospital or nursing home on discharge were significant independent predictors of death during the follow-up period. ***Conclusions:*** The major causes of death after hospital discharge were found to be cerebrovascular diseases, pneumonia and heart diseases. Thus, in order to improve survival after hospital discharge, in addition to appropriate management of vascular risk factors following stroke, it appears to be important to take measures to prevent pneumonia and to discharge patients to their own home, if possible.

Stroke is the third leading cause of death and a major cause of long-term disability in Japan. Stroke mortality has gradually but markedly decreased during the past 3 decades [1]. In western countries, the 1-year survival rate after ischemic stroke has been reported to be 50–70% [2–9]. Many investigators have shown that older age, a greater degree of functional disability, a nonlacunar stroke, the presence of heart diseases, diabetes mellitus (DM), atrial fibrillation (AF) and a history of stroke or transient isch-

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emic attack (TIA) may increase the risk of death after a stroke [4, 5, 7, 10, 11]. Unfortunately, in Japan, there is a paucity of reliable follow-up information about mortality and the cause of death following an acute ischemic stroke.

With the cooperation of 156 hospitals we conducted a large prospective hospital-based registration study to develop an acute ischemic stroke/TIA database [12, 13]. Several studies have demonstrated that the risk of mortality after stroke is highest during the first year, and that, thereafter, the risk remains almost constant [2, 3, 5, 7–9, 11]. Therefore, we examined the 1-year mortality rate and cause of death, and identified the factors that could be used as predictors of death occurring after an ischemic stroke or TIA.

Subjects and Methods

Of 16,922 patients with an acute ischemic stroke or TIA admitted within 7 days of onset, who were consecutively registered in 156 hospitals during the period from May 1999 to April 2000, 1,177 patients died during admission [12, 13]. Twelve of the 156 initially collaborating hospitals declined to participate in the follow-up study, and thus the 423 patients from these hospitals were excluded from the follow-up study. Therefore, 15,322 patients were enrolled in the study. To identify the factors that could predict death within 1 year after stroke, we used data recorded during hospitalization [12, 13]. The data included: (1) age and gender; (2) history of stroke; (3) NIH stroke scale (NIHSS) score on admission; (4) stroke subtype (clinical categories of ischemic stroke); (5) vascular risk factors, including hypertension, DM, hypercholesterolemia, current cigarette smoking and AF; (6) functional outcome at hospital discharge, and (7) residence after hospital discharge.

Clinical categories (stroke subtypes) were defined using clinical and radiographic diagnostic rubrics according to the 'Classification of Cerebrovascular Diseases III' developed by the National Institute of Neurological Disorders and Stroke [14]. Stroke subtypes included lacunar, atherothrombotic, cardioembolic and other strokes. The functional outcome at the time of hospital discharge was evaluated using the modified Rankin scale (mRS) [15]. Residence after hospital discharge was categorized into 2 groups, either own home or an institution (including nursing home and another hospital for chronic rehabilitation and medical management).

The following risk factors were studied: hypertension (defined as the use of antihypertensive agents, a systolic blood pressure reading ≥160 mm Hg or a diastolic blood pressure reading ≥95 mm Hg before stroke onset or 2 weeks after stroke onset); DM (defined as the use of oral hypoglycemic agents or insulin, or a glycosylated hemoglobin level ≥6.4%); hypercholesterolemia (defined as the use of antihyperlipidemic agents or a serum cholesterol level ≥220 mg/dl); current cigarette smoking, and potential cardiac sources of emboli (including nonvalvular AF, acute myocardial infarction, old myocardial infarction with intraventricular thrombus, mitral valve disease, prosthetic cardiac valve, pacemaker and dilated cardiomyopathy).

During the study, the central office reported the names and registration numbers of patients enrolled in the follow-up study to the doctor in charge of each participating hospital. To obtain patients' follow-up information as of September 1, 2000, the doctors in charge mailed a questionnaire to all participating patients. Responses from the patients or their families were collected by the doctor in charge of each hospital. They deleted the patient's name and address from the data sheet to protect patient privacy and sent completed forms to the central office by September 30, 2000. The follow-up information that was collected included: (1) whether the patient was alive or dead and, if dead, the date and cause of death; (2) the patient's level of activities of daily living. The cause of death was classified into five groups: cerebrovascular diseases; cancer; heart disease; pneumonia, and miscellaneous causes. The level of disability was evaluated by the patients and their families using the mRS.

Statistical analyses were performed with a commercially available software package (StatView, version 4.5; SAS Institute, Cary, N.C., USA). The Kaplan-Meier method was applied to estimate the survival rate and to determine the 1-year cumulative mortality rate for all patients and subgroups. We examined a variety of factors associated with death by using a univariate analysis. A multivariate analysis using the Cox proportional hazard model was also performed to identify the independent risk factors for death and to calculate the hazard ratios. The risk factors included in the multivariate analysis were gender, age, hypertension, DM, hypercholesterolemia, current cigarette smoking, history of stroke, mRS score at hospital discharge, stroke subtype and residence after hospital discharge. These factors were used as covariates in the analyses. The Mann-Whitney U test or the Kruskal-Wallis test was applied to detect differences in age among the subgroups. The frequency of the stroke subtypes and an association between causes of death and the mRS score or residence after hospital discharge was assessed by the χ^2 test. A p value of <0.05 was considered statistically significant.

Results

Of the 15,322 patients contacted for the follow-up study, we received replies from 11,266 patients (73.5%). We excluded 285 incomplete patients' replies. Thus, the data from 10,981 patients (71.7%) – 6,945 men (63.2%), 4,036 women (36.8%) – were used for the analysis. Table 1 shows the baseline clinical characteristics of the enrolled and nonenrolled patients. The enrolled patients' age (mean ± standard deviation) was 70.4 ± 11.1 years (median 71 years; range 19–100 years). Women (73.2 ± 11.4 years; median 75 years; range 20–100 years) were older than men (68.8 ± 10.6 years; median 70 years; range 19–100 years; $p < 0.0001$). The follow-up period for all patients was 271 ± 110 days (median 272 days; range 1–487 days).

Cardiovascular risk factors in the enrolled subjects included: hypertension in 60.0%; DM in 24.0%; hypercholesterolemia in 18.1%; AF in 18.3%, and current smoking in 18.0%. As well, 29.7% of the patients had a

Table 1. Baseline characteristics of enrolled and nonenrolled patients

Characteristics	Nonenrolled patients (n = 4,764)	Enrolled patients (n = 10,981)	p value
Mean age, years	69.9 (12.4)	70.3 (11.1)	0.321
Male gender, %	59.6	63.2	<0.0001
History of stroke, %	30.9	29.7	0.149
Risk factors, %			
Hypertension	60.4	62.0	0.487
DM	25.7	24.0	0.022
Hyperlipidemia	15.8	18.1	0.001
AF	20.2	18.3	0.007
Smoking	18.4	17.9	0.490
NIHSS score at admission			<0.0001
Median	7	5	
Mean	7.8 (7.5)	6.8 (6.6)	
Stroke subtype, %			0.001
Lacunar stroke	36.6	39.5	
Atherothrombotic	31.0	31.3	
Cardioembolic	19.5	17.1	
Other	5.9	5.3	
TIA	7.1	6.8	
mRS score at discharge, %			<0.0001
mRS 0	18.7	20.4	
mRS 1	28.2	32.2	
mRS 2	13.2	14.8	
mRS 3	9.5	8.7	
mRS 4	17.4	14.6	
mRS 5	12.9	9.1	
Institution after hospital discharge	45.7	31.0	<0.0001

1,177 patients who died during admission were excluded. Figures in parentheses indicate SD.

history of previous stroke. Lacunar stroke (39.5%) was the most frequent stroke subtype, followed by atherothrombotic (31.3%), cardioembolic (17.1%) and other strokes (5.3%). TIA was diagnosed in 6.8% of the patients.

The distribution of the mRS scores at hospital discharge was as follows: 20.4% of patients scored 0; 32.2% scored 1; 14.8% scored 2; 8.7% scored 3; 14.6% scored 4, and 9.1% scored 5. When functional disability was classified into 2 categories, namely mRS of 0–2 indicating independence and 3–5 indicating dependence, 7,410 patients (67.6%) were independent, while 3,553 (32.4%) were classified as dependent. Figure 1 shows the relationship between the NIHSS score on admission and the mRS score at hospital discharge.

Sixty-nine percent of patients were discharged home, and 31% were sent to an institution. Of the patients with mRS scores 0–2, 90.6% returned to their own home, while only 24.5% of patients with mRS scores 3–5 returned home. Among patients with mRS scores 0–2, the patients who returned to their own home were slightly younger (68.3 ± 10.7 years; median 69 years; range 19–100 years) than those who were discharged to an institution (69.5 ± 10.7 years; median 70 years; range 30–100 years; p = 0.0105). However, among patients with mRS scores 3–5, no difference in age was observed between those discharged to their own home (74.9 ± 10.4 years; median 76 years; range 21–98 years) and those sent to an institution (74.6 ± 10.7 years; median 76 years; range 21–100 years; p = 0.6429).

A total of 604 patients died during the follow-up period. Overall, the 1-year cumulative mortality rate after discharge was 6.8 ± 0.3% (mean ± SEM), i.e. 7.0 ± 0.3% for the 10,234 stroke patients and 3.5 ± 0.8% for the 747 TIA patients. Table 2 shows the 1-year cumulative mortality rate by gender and age groups. The mortality rate increased progressively with age, reaching a maximum at

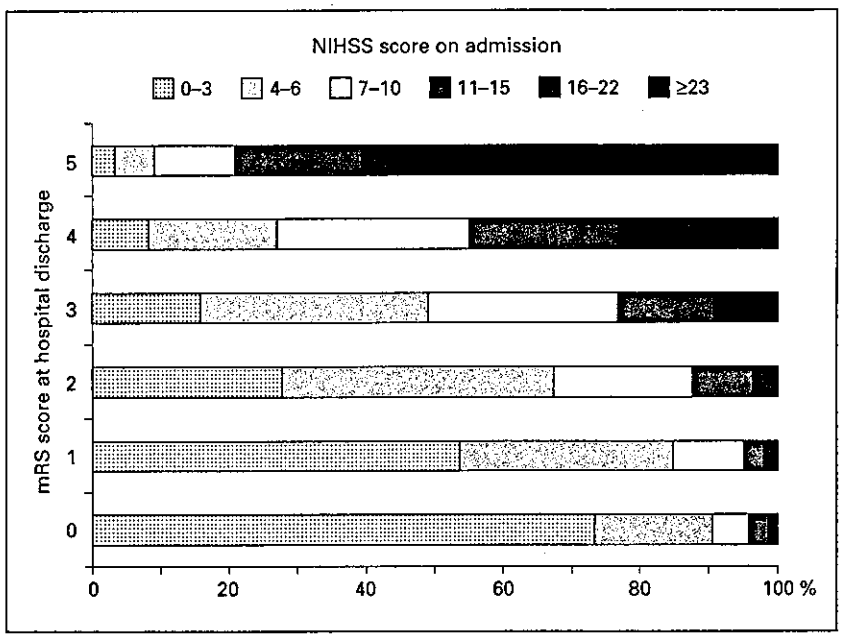

Fig. 1. NIHSS score on admission and mRS at hospital discharge.

Table 2. One-year cumulative mortality rates risk (±SD) by gender and age

Age	Male	Female	p value
≤59 years	1.7±0.4	1.0±0.5	0.3143
60–69 years	3.8±0.5	2.4±0.7	0.0562
70–79 years	6.9±0.6	5.7±0.7	0.4964
≥80 years	18.0±1.5	14.4±1.3	0.0331

80 years and older in both men and women; it was higher in men than in women (p = 0.0331) in patients aged 80 years and older.

The most frequent causes of death were cerebrovascular diseases (24.1%) followed by pneumonia (22.6%), heart diseases (18.1%), cancer (11.0%) and miscellaneous causes (24.1%).

We compared the mortality rate among stroke subtypes; the 1-year cumulative mortality was highest in cardioembolic stroke patients (12.5%) as compared to those with the remaining subtypes (4.0% in lacunar stroke, 7.8% in atherothrombotic stroke, 8.1% in other and 3.0% in TIA, $p < 0.001$).

Patients with an mRS score of 5 at discharge showed a markedly higher mortality (25.3%) compared to those with other scores (1.6% in those who scored 0; 1.9% in those who scored 1; 3.4% in those who scored 2; 4.5% in those who scored 3; 9.2% in those who scored 4; $p < 0.0001$). The cumulative survival rate was higher in patients with mRS scores of 0–2 than in those with mRS scores of 3–5.

At the time of follow-up, 15.5% of patients had an mRS score of 0; 28.9% of patients had a score of 1; 14.1% had a score of 2; 12.4% had a score of 3; 12.6% had a score of 4, and 11.0% had a score of 5. Comparing the mRS scores at follow-up with those at discharge, the number of patients who were independent (mRS 0–2) decreased from 67.6 to 58.5%, and those who were dependent (mRS 3–5) increased from 32.4 to 36.0%. Follow-up mRS scores improved in 10.8% of patients, remained unchanged in 63.6%, and deteriorated in 25.6% of the patients as compared to the mRS scores at discharge (death was assigned an mRS score of 6).

There was a significant difference in the cause of death between patients with mRS scores of 0–2 and those with 3–5 at hospital discharge ($p < 0.0001$; fig. 2). For patients with mRS scores of 0–2, the most frequent cause of death was cancer (23.1%), while for those with mRS scores of 3–5, it was pneumonia (25.5%).

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-

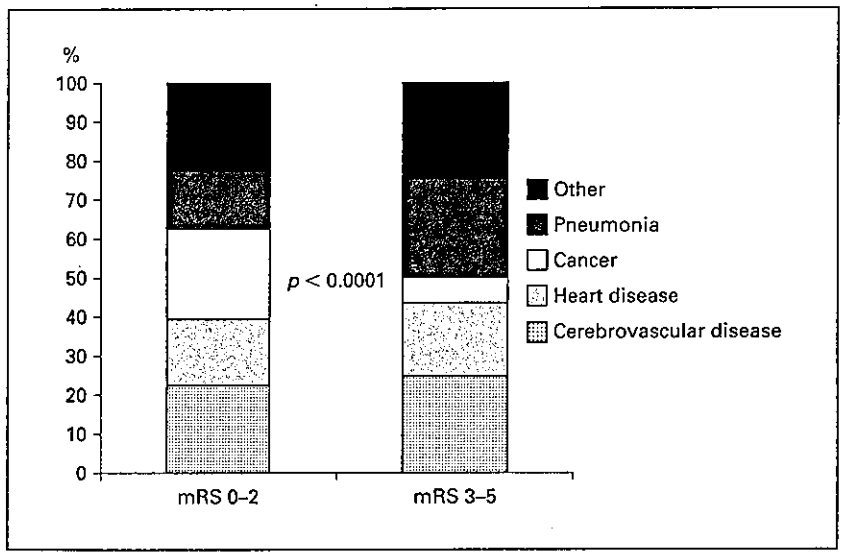

Fig. 2. The cause of death during follow-up for patients with mRS scores at hospital discharge of 0–2 and 3–5 ($p < 0.0001$). The most frequent cause of death was cancer (23.1%) in patients with mRS scores of 0–2, and pneumonia (25.5%) in those with mRS scores of 3–5.

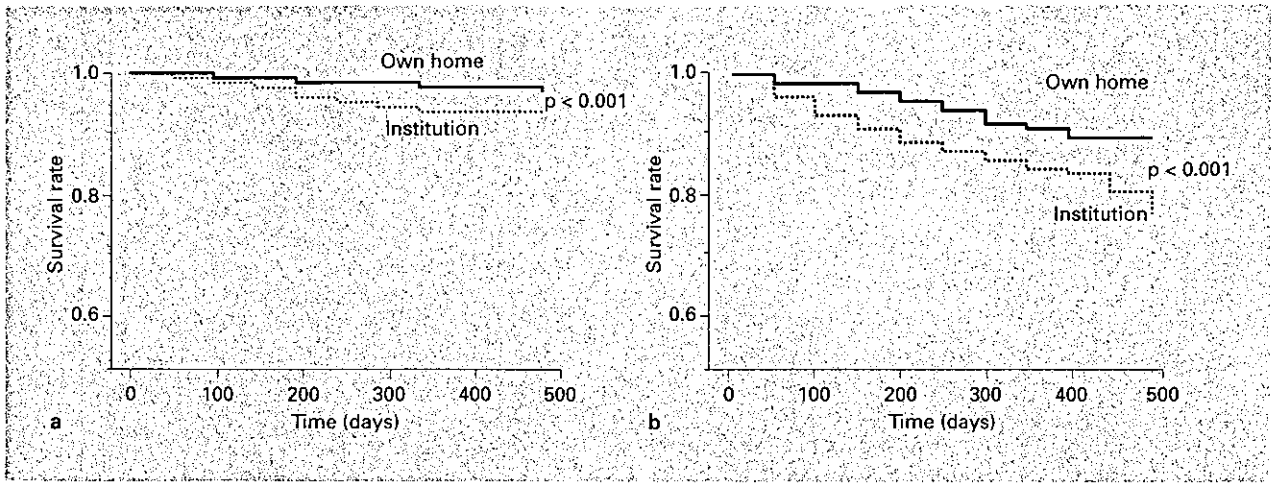

Fig. 3. The cumulative survival rates of patients discharged to their own homes and to institutions with mRS scores of 0–2 (**a**) and with mRS scores of 3–5 (**b**) at hospital discharge. In both groups, the mortality rate was lower for patients discharged to their own homes ($p < 0.001$).

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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References

1 Sarti C, Rastenyte DR, Cepaitis Z, Tuomilehto J: International trends in mortality from stroke, 1968–1994. Stroke 2000;31:1588–1601.
2 Schmidt EV, Smirnov VE, Ryabova VS: Results of the seven-year prospective study of stroke patients. Stroke 1988;19:942–949.
3 von Arbin M, Britton M, de Faire.U: Mortality and recurrences during eight years following stroke. Intern Med 1992;231:43–48.
4 Sacco RL, Shi T, Zamanillo MC, Kargman DE: Predictors of mortality and recurrence after hospitalized cerebral infarction in an urban community: The Northern Manhattan Stroke Study. Neurology 1994;44:626–634.
5 Elneihoum AM, Goransson M, Falke P, Janzon L: Three-year survival and recurrence after stroke in Malmö, Sweden: An analysis of stroke registry data. Stroke 1998;29:2114–2117.
6 Kolominsky-Rabas PL, Sarti C, Heuschmann PU, Graf C, Siemonsen S, Neundoerfer B, Katalinic A, Lang E, Gassmann KG, von Stockert TR: A prospective community-based study of stroke in Germany – The Erlangen Stroke Project (ESPro): Incidence and case fatality at 1, 3, and 12 months. Stroke 1998;29:2501–2506.
7 Hankey GJ, Jamrozik K, Broadhurst RJ, Forbes S, Burvill PW, Anderson CS, Stewart-Wynne EG: Five-year survival after first-ever stroke and related prognostic factors in the Perth Community Stroke Study. Stroke 2000; 31:2080–2086.
8 Westling B, Norrving B, Thorngren M: Survival following stroke: A prospective population-based study of 438 hospitalized cases with prediction according to subtype, severity and age. Acta Neurol Scand 1990;81:457–463.
9 Lai SM, Alter M, Friday G, Sobel E: Prognosis for survival after an initial stroke. Stroke 1995; 26:2011–2015.
10 Broderick JP, Phillips SJ, O'Fallon WM, Frye RL, Whisnant JP: Relationship of cardiac disease to stroke occurrence, recurrence, and mortality. Stroke 1992;23:1250–1256.
11 Bonita R, Ford MA, Stewart AW: Predicting survival after stroke: A three-year follow-up. Stroke 1988;19:669–673.
12 Kimura K, Kazui S, Minematsu K, Yamaguchi T, for the Japan Multicenter Stroke Investigators' Collaboration (J-MUSIC): Analysis of 16,922 patients with acute ischemic stroke and TIA in Japan: A hospital-based prospective registration study. Cerebrovasc Dis 2004;18: 47–56.
13 Kimura K, Kazui S, Minematsu K, Yamaguchi T, for the Japan Multicenter Stroke Investigators' Collaboration (J-MUSIC): Hospital-based prospective registration of acute ischemic stroke and transient ischemic attack in Japan. J Stroke Cerebrovasc Dis 2004;13:1–11.
14 Special report from the National Institute of Neurological Disorders and Stroke: Classification of cerebrovascular diseases III. Stroke 1990;21:637–676.
15 van Swieten JC, Koudstaal PJ, Visser MC, Schouten HJA, van Gijn J: Interobserver agreement for the assessment of handicap in stroke patients. Stroke 1988;19:604–607.
16 Bogousslavsky J, Van Melle GV, Regli F: The Lausanne stroke registry: Analysis of 1,000 consecutive patients with first stroke. Stroke 1988;19:1083–1092.
17 Petty GW, Brown RD Jr, Whisnant JP, Sicks JD, O'Fallon WM, Wiebers DO: Ischemic stroke subtype: A population-based study of incidence and risk factors. Stroke 1999;30: 2513–2516.
18 Kolominsky-Rabas PL, Weber M, Gfeller O, Neundoerfer B, Heuschmann PU: Epidemiology of ischemic stroke subtypes according to TOAST criteria – Incidence, recurrence, and long-term survival in ischemic stroke subtype: A population-based study. Stroke 2001;31: 2735–2740.
19 Friday G, Lai SM, Alter M, Sobel E, LaRue L, Gil-Peralta A, McCoy RL, Levitt LP, Isack T: Stroke in the Lehigh Valley: Racial/ethnic differences. Neurology 1989;39:1165–1168.
20 Salgado AV, Ferro JM, Gouveia-Oliveira A: Long-term prognosis of first-ever lacunar strokes: A hospital-based study. Stroke 1996; 27:661–666.
21 Salive ME, Satterfield S, Ostfeld AM, Wallace RB, Havlik RJ: Disability and cognitive impairment are risk factors for pneumonia-related mortality in older adults. Public Health Rep 1993;108:314–322.
22 Counsell C, Dennis M: Systematic review of prognostic models in patients with acute stroke. Cerebrovasc Dis 2001;12:159–170.

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Transcranial Color-Coded Real-Time Sonographic Criteria for Occlusion of the Middle Cerebral Artery in Acute Ischemic Stroke

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BACKGROUND AND PURPOSE: **Transcranial color-coded real-time sonography (TCCS) is a useful tool to evaluate disease of the middle cerebral artery (MCA). This study was undertaken to identify TCCS criteria for the diagnosis of MCA stem and MCA branch occlusions.**

METHODS: **TCCS and digital subtraction angiography were performed in 55 consecutive patients with acute stroke: 10 with MCA stem occlusion, the MO group; eight with MCA branch occlusion, the MB group; and 37 with nonocclusive lesions, the control group. We measured the end-diastolic velocity (EDV) of the bilateral MCA stems and calculated the end-diastolic ratio by dividing the EDV of the unaffected side by that of the affected side.**

RESULTS: **EDV was highest in the control group, and end-diastolic ratio was highest in the MO group. An EDV of >25 cm/s indicated a nonocclusive lesion in the MCA, with a positive predictive value of 98.4%, a negative predictive value of 81.0%, and an accuracy of 93.9%. An EDV of ≤25 cm/s with an end-diastolic ratio of <2.7 indicated an MCA branch occlusion with a positive predictive value of 85.7%, a negative predictive value of 97.2%, and an accuracy of 95.3%. An EDV of ≤25 cm/s with an end-diastolic ratio of ≥2.7 indicated MCA stem occlusion with a positive predictive value of 100%, a negative predictive value of 100%, and an accuracy of 100%.**

CONCLUSION: **We developed TCCS criteria for the diagnosis of MCA diseases. MCA flow velocity detected by means of TCCS can help identify MCA stem occlusion as well as MCA branch occlusion.**

In the early 1990s, transcranial color-coded real-time sonography (TCCS) was introduced as a new method for imaging the basal cerebral arteries (1–3). TCCS is a noninvasive, easy-to-repeat, diagnostic technique that is widely used for the evaluation of cerebral hemodynamics. As a result of combining the B-mode facility and the color-coded Doppler facility, the brain vessels can be clearly displayed. Furthermore, since the angle of insonation can be measured and corrected for, one can obtain velocity measurements that are closer to true values (4, 5). Thus, TCCS is a useful tool in evaluating vascular diseases of the middle cerebral arteries (MCAs), particularly in patients with ischemic stroke.

However, only a few reports have described the diagnosis of MCA stem occlusion with TCCS (6, 7). In fact, TCCS criteria for MCA occlusion, particularly MCA branch occlusion, have not yet been established. MCA branch occlusion frequently occurs in patients with acute ischemic stroke. Therefore, it is important to be able to evaluate the MCA branch occlusion as well as the MCA stem occlusion when one is deciding on a patient's therapy.

Peripheral resistance, which is found after the point of measurement, is thought to reflect blood flow velocity; the higher peripheral resistance, the lower the blood flow velocity. Therefore, we hypothesized that the blood velocity in the MCA stem of patients with MCA branch occlusion is lower than that in patients without MCA occlusion and that it is higher than that of patients with MCA stem occlusion. The aim of the current study was to establish TCCS criteria for determining the specific sites of MCA occlusion.

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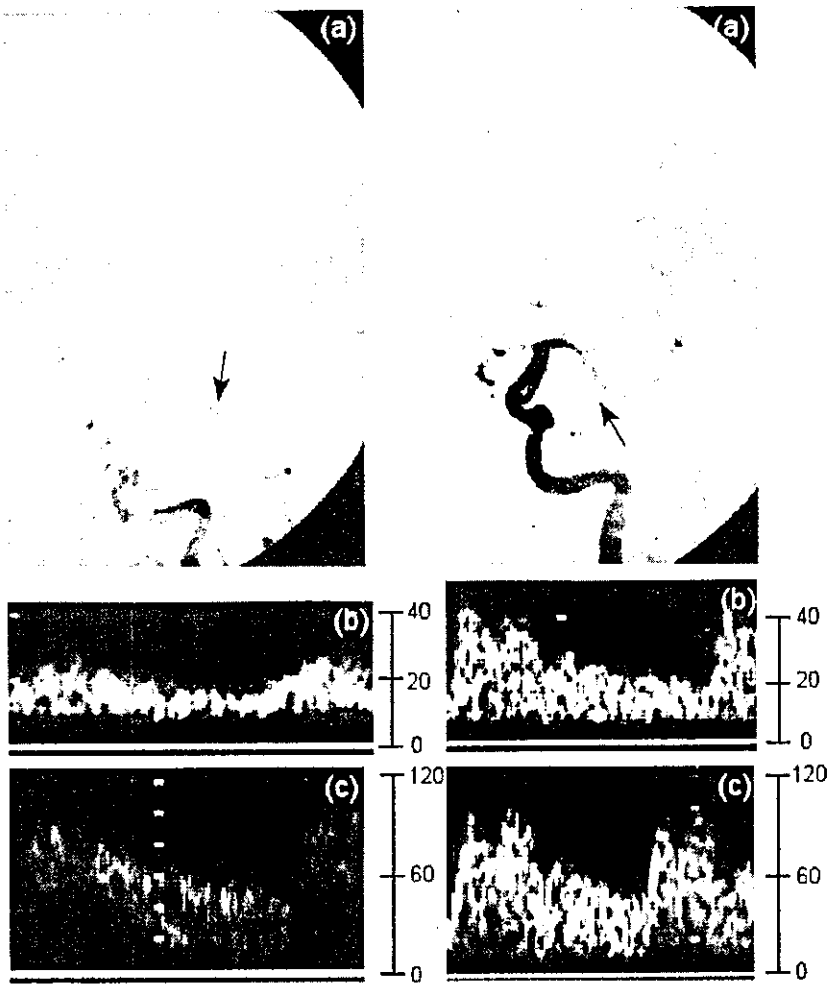

FIG 1. Left anteroposterior carotid angiograms in representative cases evaluated with cerebral angiography and TCCS. Y axis is represented blood flow velocity (cm/s). *Left* image shows occlusion of the horizontal portion of the left MCA (*a*). Occlusion site overlies the external carotid artery branch. Doppler waveforms of the left (*b*) and right (*c*) MCAs show EDVs of 14.5 and 49.9 cm/s, respectively. *Right* image shows occlusion of the branch in the left MCA (*a*). Doppler waveforms of the left (*b*) and right (*c*) MCAs show EDVs of 18.2 and 44.6 cm/s, respectively.

Methods

We prospectively performed TCCS in 66 consecutive patients with acute stroke who underwent intra-arterial digital subtraction angiography (DSA). TCCS examinations were performed 24 hours before and after the intra-arterial DSA study. Eleven patients (three with occlusion or a severe stenosis in internal carotid artery, six with MCA stem stenosis, and two with proximal MCA occlusion) were excluded from this study. Patients with proximal MCA occlusion were excluded because TCCS could not display the flow signal of the MCA, and thus the flow velocity could not be measured. Therefore, 55 patients were enrolled. Their stroke subtypes were as follows: two patients had a lacunar stroke, 10 had atherothrombotic stroke, 20 had cardioembolic stroke, one had a transient ischemic attack, 18 had other types of ischemic stroke, three had hemorrhagic stroke, and one had amourosis fugax.

The study protocol followed all principles outlined in the Declaration of Helsinki. Selective angiography was performed by using biplane DSA (Angio Rex Super-G and DFP-2000A; Toshiba, Tokyo, Japan). All examinations were performed by means of transbrachial or transfemoral catheterization in accordance with the Seldinger method. Standard anteroposterior and lateral images were routinely obtained.

According to the DSA results, we assigned the patients as follows: Patients with an MCA stem occlusion were the MO group, patients with an MCA branch occlusion were the MB group, and patients with no occlusive or stenotic MCA lesions were the control group.

TCCS was performed by using a unit (Sonos 5500; Philips Medical Systems, Japan, Tokyo) with a 1.0–3.0-MHz sector scan. The transtemporal acoustic window was used to visualize the MCA stem in real time by using color signals. We obtained color Doppler flow images and measured flow velocity at the MCAs. Patients were examined first in the left lateral decubitus position and then in the right lateral decubitus position. Particular care was taken to identify an appropriate straight-vessel segment of the MCA by means of tilting, rotating, or shifting the transducer. A 1.9-mm, range-gated, pulsed Doppler sample volume was used to measure the blood flow velocity in the MCA stem. The sample volume was moved slowly from the proximal to the distal position along the horizontal segment of the MCA and displayed as a color flow image on B-mode images. We chose the measured point where the blood flow velocity was the highest. Then we measured the mean end-diastolic velocity (EDV) over five consecutive cardiac cycles. Angle correction was applied when the correction angle did not exceed 60°. Furthermore, the side-to-side ratio of the end-diastolic flow velocity (end-diastolic ratio) was calculated by dividing the velocity of the unaffected side by that of the affected side in the MO and MB groups. We also detected the end-diastolic ratio of patients in the control group by dividing the higher MCA velocity by the lower one.

The age and blood flow velocity data for each group were expressed as the mean ± SD. Statistical comparisons of velocity differences within each group were performed by using one-way factorial analysis of variance and then Scheffé multiple comparison tests. Sensitivity-specificity curve analysis was applied to obtain cutoff values for EDV to distinguish the MO or MB group from the control group and for the end-diastolic ratio to differ-