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脳血管疾患の再発に対する高脂血症治療薬のHMGCoA阻害剤の予防効果に関する研究

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Relationships between Angiographic Findings and National Institutes of Health Stroke Scale Score in Cases of Hyperacute Carotid Ischemic Stroke

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BACKGROUND AND PURPOSE: **Stroke severity in cases of hyperacute carotid ischemic stroke may be related to site of arterial occlusion. We evaluated the relationships between National Institutes of Health Stroke Scale (NIHSS) scores and findings on intra-arterial digital subtraction angiograms (IA-DSA) of patients with ischemic stroke within 6 hr of stroke onset.**

METHODS: **A total of 43 consecutive patients (38 men and five women; mean age, 69.4 ± 8.7 years) with ischemic stroke in the carotid territory underwent IA-DSA within 6 hr of stroke onset. Baseline NIHSS score was assessed immediately before IA-DSA. Patients were divided into four groups according to site of arterial occlusion: 1) the internal carotid artery (ICA group, n = 10); 2) stem of the middle cerebral artery or stem of the anterior cerebral artery (Stem group, n = 14); 3) branches of middle cerebral artery or anterior cerebral artery (Branch group, n = 11); and 4) no arterial occlusion (Normal group, n = 8).**

RESULTS: **Mean (±SD) NIHSS score was 14.7 ± 7.4. The interval from stroke onset to IA-DSA study was 205 ± 76 min. NIHSS score was higher in the ICA group (median, 23; range, 6–32) than in the Branch (median, 17; range, 11–25; $P = .02$) or Normal (median, 15; range, 2–17; $P < .001$) groups but was not higher than in the Stem group (median, 6; range, 1–11; $P = .73$). Sensitivity-specificity curve analysis suggested an NIHSS score ≥10 as indicative of arterial occlusion of the carotid system. A total of 96.9% of patients with NIHSS scores ≥10 displayed arterial occlusion, and 63.6% of patients with NIHSS scores <10 displayed no arterial occlusion.**

CONCLUSION: **NIHSS score is related to site of arterial occlusion in cases of hyperacute carotid ischemic stroke. An NIHSS score of 10 seems to represent the cut-off for discriminating between patients with arterial occlusion and patients without.**

The National Institutes of Health Stroke Scale (NIHSS) is a widely used and well-validated neurologic impairment scale, measuring speech and language, cognition, visual field deficits, motor and sensory impairments, and ataxia (1). NIHSS score assessed during the hyperacute phase of stroke strongly predicts the likelihood of patient recovery after stroke and has been used to include or exclude patients from trials of acute stroke therapy, including thrombolysis (2–7). The NIHSS therefore represents a standard part of clinical assessment for patients with acute stroke in many stroke centers.

Fink et al (8) reported a significant correlation between diffusion-weighted MR imaging lesion volume and NIHSS score. Several studies examined relationships between initial NIHSS score and vascular imaging techniques such as ultrasonography (9, 10), CT angiography (11), and MR angiography (12), reporting that a higher NIHSS score was associated with more severe vascular lesions in patients with acute stroke. However, vascular imaging methods have limitations in clearly displaying occlusion or stenosis of the main stem and branches of the middle cerebral artery (MCA) and anterior cerebral artery (ACA). Relationships between NIHSS score and site of arterial occlusion during the hyperacute phase of stroke, therefore, have yet to be accurately determined.

Intra-arterial digital subtraction angiography (IA-

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DSA) is superior to other methods for detecting the site of arterial occlusion and is considered to represent the gold standard for vascular imaging. During the hyperacute phase of stroke, detailed knowledge of arterial occlusion can be clinically important, particularly regarding thromobolysis (13, 14). The aim of the present study was to evaluate relationships between NIHSS score and IA-DSA findings in patients with ischemic stroke within 6 hr of stroke onset.

Methods

Patients and Techniques

Of the 112 patients admitted to our division within 6 hr of ischemic stroke onset between April 1999 and June 2002, IA-DSA was performed in 43 patients with carotid acute ischemic stroke (38 men and five women; mean age, 69.4 ± 8.7 years). We excluded the patients with posterior circulation strokes because anterior circulation and posterior circulation strokes were thought to be separate entities, with different underlying pathogenesis and natural histories. These 43 patients were enrolled into this study. All patients were assessed by using the NIHSS immediately before IA-DSA. If the patient was aware of symptoms on waking from sleep, time of onset was defined as the last time they were free from symptoms. A modified Rankin Scale (15) score ≥2 before stroke onset was used as an exclusion criterion.

Sex, age, history of stroke or transient ischemic attack, and modified Rankin Scale score were examined, along with vascular risk factors including hypertension, diabetes mellitus, hyperlipidemia, smoking, and potential embolic sources of emboli (atrial fibrillation, patent foramen ovale, left ventricular aneurysm, prosthetic heart valves, infective endocarditis, sick sinus syndrome, dilated cardiomyopathy, and complicated lesions in aortic arch).

Vascular risk factors were identified as follows: 1) use of antihypertensive agents for hypertension, with systolic blood pressure ≥160 mmHg or diastolic blood pressure ≥95 mmHg at admission for hypertension; 2) use of oral hypoglycemic agents, insulin, or glycosylated hemoglobin >6.4% for diabetes mellitus; 3) use of antihyperlipidemic agents or serum cholesterol level >220 mg/dL for hypercholesterolemia; or 4) history of smoking. To detect potential embolic sources of emboli, all patients underwent 12-lead ECG, 24-hr ECG monitoring, and transthoracic or transesophageal echocardiography.

Informed consent for performance of IA-DSA was obtained from patients and/or their family members. Selective IA-DSA was performed by using a biplane, high resolution angiography system (Angio Rex Super-G and DFP-2000A, Toshiba) with a matrix of 1024 × 1024 pixels. A catheter was inserted into the right brachial artery or femoral artery in accordance with the Seldinger method and then guided to the cerebral arteries for diagnostic four-vessel angiography.

Patients were divided into four groups according to the site of arterial occlusion: 1) occlusion of the internal carotid artery (ICA group); 2) occlusion of the main stem of the MCA or A1 segment of the ACA (Stem group); 3) occlusion of the MCA or ACA branch, including occlusion of M2 or A2 or more distal sites (Branch group); or 4) no arterial occlusion (Normal group). If a patient displayed two or more occluded arteries, the patient was placed in the largest artery group (eg, if occlusions of both the right main trunk of the MCA and the ipsilateral A2 portion were present, the patient was placed in the Stem group).

CT of the brain was performed immediately at admission and 3 days after stroke onset to evaluate ischemic lesions. Within 7 days of stroke onset, MR imaging was performed by using a 1.5-T system (Magnetom Vision, Siemens) equipped with single shot echo-planar imaging to obtain rapid diffusion images. MR imaging studies included axial T1-weighted, axial T2-weighted, and diffusion-weighted sequences (approximately 30 min of imaging time). Imaging parameters were as follows: 4000/103 (TR/TE); matrix, 128 × 128; field of view, 230 mm; section thickness, 4 mm; section gap, 2 mm. Two b values were used (0 and 1000 s/mm^2). Diffusion gradients were applied in successive images in each of the x, y, and z directions, and diffusion-weighted images were formed from the mean of these values. Criteria for diagnosis of acute infarcts on diffusion-weighted images included focal hyperintensity judged not to be due to normal anisotropic diffusion or magnetic susceptibility artifact. These lesions were also categorized as cortical, subcortical, or lacunar infarcts according to location.

Statistical analysis was performed by using StatView 5.0 for Windows (SAS Institute, 1998). The χ^2 test or Kruskal-Wallis U test was used to compare baseline characteristics among the four groups. Relationships between baseline NIHSS score and site of arterial occlusion were tested by using the Kruskal-Wallis U test, and the differences between NIHSS scores for each group were tested by post hoc analysis under Scheffe's method. To obtain the NIHSS score as the cut-off point for discriminating between patients with arterial occlusion and those without, a sensitivity and specificity curve was drawn. The study protocol followed all principles outlined in the Declaration of Helsinki.

Results

Of the 43 patients enrolled in this study, 20 underwent IA-DSA within 3 hr of stroke onset. Intervals from stroke onset to arrival at hospital and to IA-DSA study were 88 ± 58 min and 205 ± 76 min, respectively.

The Stem group was the largest (n = 14), with the other groups in descending order being the Branch (n = 11), ICA (n = 10), and Normal (n = 8) groups. Demographic data and clinical features of each group are shown in Table 1. Atrial fibrillation was observed most frequently in the Branch group ($P < .014$). No other significant differences in baseline characteristics were observed.

In the ICA group, seven patients displayed ICA occlusion. For one, occlusion of both the ICA and ipsilateral ACA A2 portion was show, and for the remaining two, both ICA occlusion and ipsilateral MCA stem occlusion was shown, despite good collateral flow from the contralateral ACA via the anterior communicating artery. In the Stem group, 11 patients displayed MCA stem occlusion, one had bilateral MCA stem occlusion, one had both MCA stem occlusion and ipsilateral A2 occlusion, and the remaining had both MCA stem occlusion and occlusion of the distal site of ipsilateral ACA. In the Branch group, five patients had MCA M2 branch occlusion, two had M3 branch occlusion, two had both M2 and A2 portion occlusion, one had M2 and A4 portion occlusions, and the other one displayed M4 and A3 portion occlusions.

The median NIHSS score was 16 (range, 1–32). No significant differences were observed between NIHSS scores of patients with left- and right-sided stroke ($P = .874$, Mann-Whitney U test). NIHSS score was higher in the ICA group (median, 23; range, 6–32) than in the Branch (median, 15; range, 2–17; $P = .02$)

Clinical characteristics of all patients

Group	ICA	Stem	Branch	Normal	P
Number of patients	10	14	11	8	
Age, median (range) (yr)	71 (48–78)	68 (56–82)	74 (57–86)	64 (55–84)	0.671
Sex, male	10 (100%)	12 (86%)	9 (82%)	7 (88%)	0.598†
Hypertension	8 (80%)	9 (64%)	5 (45%)	8 (100%)	0.065
Diabetes mellitus	2 (20%)	5 (36%)	4 (36%)	1 (13%)	0.405
Hyperlipidemia	5 (50%)	4 (29%)	4 (36%)	6 (75%)	0.181
Smoking	6 (60%)	8 (57%)	2 (18%)	3 (38%)	0.163
Atrial fibrillation	5 (50%)	10 (71%)	9 (82%)	1 (13%)	0.014
Potential emboligenic diseases	2 (20%)	3 (21%)	3 (27%)	4 (50%)	0.467
Patent foramen ovale	0	2	2	2	
Left ventricular aneurysm	0	1	0	0	
Complicated lesion in aorta	2	0	1	2	
History of stroke/transient ischemic attack	1 (10%)	1 (7%)	2 (18%)	1 (13%)	0.858
Interval from stroke onset to angiography mean ± SD (min)	173 ± 75 (150)	211 ± 86 (194)	223 ± 67 (185)	223 ± 76 (199)	0.272†
Affected side (right/left)	6/4	8/6	3/8	3/5	0.353

* Analyzed by using the χ^2 test.
† Analyzed by using the Kruskal-Wallis U test.

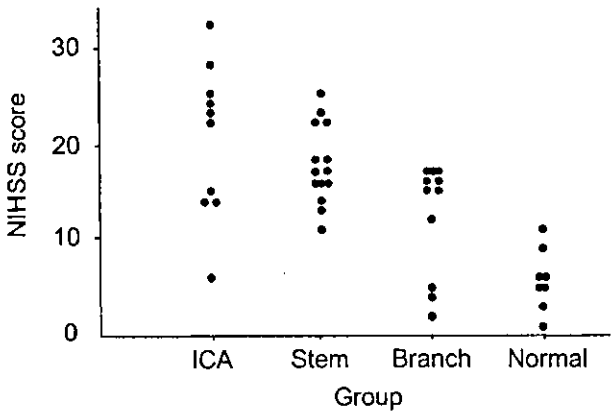

FIG 1. Distribution of the NIHSS score of four groups.

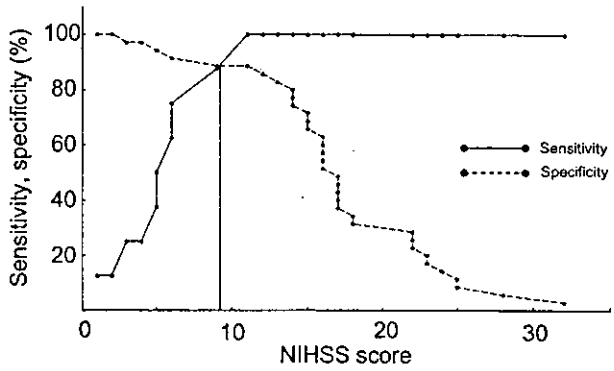

FIG 2. Sensitivity-specificity curve analysis for predicting arterial occlusion. The optimal threshold value of the NIHSS score was 10.

or Normal (median, 6; range, 1–11; $P < .001$) groups but not higher than in the Stem group (median, 17; range, 11–25; $P = .73$). Patients in the Stem group displayed higher NIHSS scores than those in the Normal group ($P < .001$). In sensitivity-specificity curve analysis for predicting arterial occlusion, the optimal threshold value of the NIHSS score was 10 (Figs 1 and 2). Using an NIHSS score of 10 as the cut-off, sensitivity, specificity, positive predictive value, and negative predictive value for any arterial occlusion were 88.6%, 87.5%, 63.6%, and 96.9%, respectively.

MR imaging revealed 33 cortical infarcts, six subcortical infarcts, one lacunar infarct, and no ischemic lesions in three patients. Cortical and subcortical infarcts were present in seven and three of the 10 ICA group patients, respectively, 14 and 0 of the 14 MCA group patients, respectively, 10 and 0 of the 11 Branch group patients, respectively, and two and three of the eight Normal group patients, respectively. Lacunar infarct was observed in only one patient from the Normal group. One patient in the Branch group and three in the Normal group displayed no fresh lesions.

Discussion

Our study showed that an NIHSS score ≥10 represented the optimal value for predicting arterial occlusion in patients within 6 hr of stroke onset; 31 (97.0%) of 32 patients with NIHSS score ≥10 displayed arterial occlusion. Lewandowski et al (4) studied patients with stroke within 3 hr of stroke onset in the Emergency Management of Stroke bridging trial by using angiography. In that study, 17 (77.3%) of 22 patients with NIHSS scores ≥10 displayed occluded arteries in the carotid system. This minor discrepancy may be attributable to the differing interval between stroke onset and vascular imaging and to the methods to evaluate occlusive lesions.

Some studies have reported no significant differences in NIHSS scores between patients with MCA trunk occlusion and ICA/MCA tandem occlusion, a result that is compatible with the present results (9, 16). When the ICA is occluded, the severity of neurologic deficit is contingent on collateral blood flow through the anterior communicating or leptomeningial arteries from the ACA or posterior cerebral artery.

Our study included a small number of small artery diseases presenting as lacunar syndrome. We do not

frequently perform IA-DSA but MRA to evaluate occlusive lesions in patients with lacunar stroke. A previous study reported that 205 (67.0%) of 306 patients with small artery disease had NIHSS scores of 0 to 6 (2). Patients with small artery disease may therefore be likely to achieve NIHSS scores <10.

In the present study, only one (3.0%) of 33 patients with NIHSS scores ≥10 displayed no arterial occlusion. In this case, neurologic symptoms improved immediately after angiography; this was attributed to spontaneous reopening of the occluded artery immediately before IA-DSA. Conversely, four (36.4%) of 11 patients with NIHSS scores <10 displayed arterial occlusions. Naylor et al (17) reported that patients with hyperacute stroke with MCA or ICA occlusions may occasionally display mild stroke severity or mimic lacunar events. Of the four patients, angiography in one case revealed occlusion of the right ICA origin and ipsilateral ACA and good collateral blood flow through the anterior communicating artery from the contralateral ICA system to the ipsilateral MCA. MR imaging of the brain revealed only a small infarct in ACA territory. In the remaining three patients with MCA branch occlusion, good leptomeningeal collateral blood supply from the ACA or posterior cerebral artery was present, and MR imaging revealed small infarcts. The mild neurologic deficits in these cases may therefore be due to good collateral flow.

The present study displayed some limitations. We did not perform IA-DSA for all patients with stroke within 6 hr of onset. In particular, IA-DSA was infrequently performed for patients older than 80 years and patients with lacunar stroke. This represents a source of selection bias in the study.

In conclusion, NIHSS score is associated with site of arterial occlusion in patients with hyperacute carotid ischemic stroke. An NIHSS score ≥10 is predictive of arterial occlusion in hyperacute ischemic stroke within 6 hr of onset.

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Original Articles

Hospital-based Prospective Registration of Acute Ischemic Stroke and Transient Ischemic Attack in Japan

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The purpose of this study was to obtain fundamental information on patients with acute ischemic stroke and transient ischemic attack (TIA) in Japan. We prospectively registered consecutive stroke and TIA patients who visited 156 participating hospitals within 7 days of onset between May 1, 1999 and April 30, 2000. A total of 16,922 patients with 70.6 ± 11.5 years old (median 71, range 18-107) were enrolled in the study. TIA was seen in 7% of registered patients, lacunar stroke in 36%, atherothrombotic in 31%, cardioembolic stroke in 20%, and other in 6%. Hypertension was present in 61%, diabetes mellitus in 24%, atrial fibrillation (AF) in 21%, smoking in 18%, and hypercholesterolemia in 17%. Overall, 37% of patients arrived at hospital within 3 hours of symptom onset, and 50% within 6 hours. Among those who visited the hospital within 6 hours, 64% used an ambulance service. Mean NIHSS score was 8.0 ± 7.9 (median, 5). Only 3% were treated with thrombolytic agents in acute phase of stroke. Only 19% of all patients were treated in stroke care unit or intensive care unit. The modified Rankin Scale score of 0 to 2 at discharge was observed in 61% of the patients, 3 to 5 in 32%, and the mortality rate was 7%. More than half of the acute stroke patients arrived at the hospital after 6 hours of onset, and the stroke care unit was used only in one fifth of all patients. Establishment of ideal emergency system and arrangement of stroke units are also awaited for better management and improvement of patients' outcome. **Key Words:** Brain infarction—prospective study—statistics—stroke—acute—stroke management—transient ischemic attack.

Although stroke mortality has gradually but remarkably decreased during the recent three decades in Japan,[1] about 140,000 people died because of stroke in 1999. The proportion of deaths from stroke was about 14% of the total national deaths, making stroke the third leading cause of death, following total neoplasms and heart diseases. Morikawa et al[2] studied secular changes in stroke incidence between 1977 and 1991 in Japanese rural areas, and reported that the proportion of brain hemorrhage decreased from 24% to 16%, but brain infarction increased from 64% to 74%. Kodama et al[3] also reported that the incidence of brain hemorrhage has decreased, but brain infarction has not. Therefore, the number of patients with disability caused by brain infarctions may have increased in recent decades.

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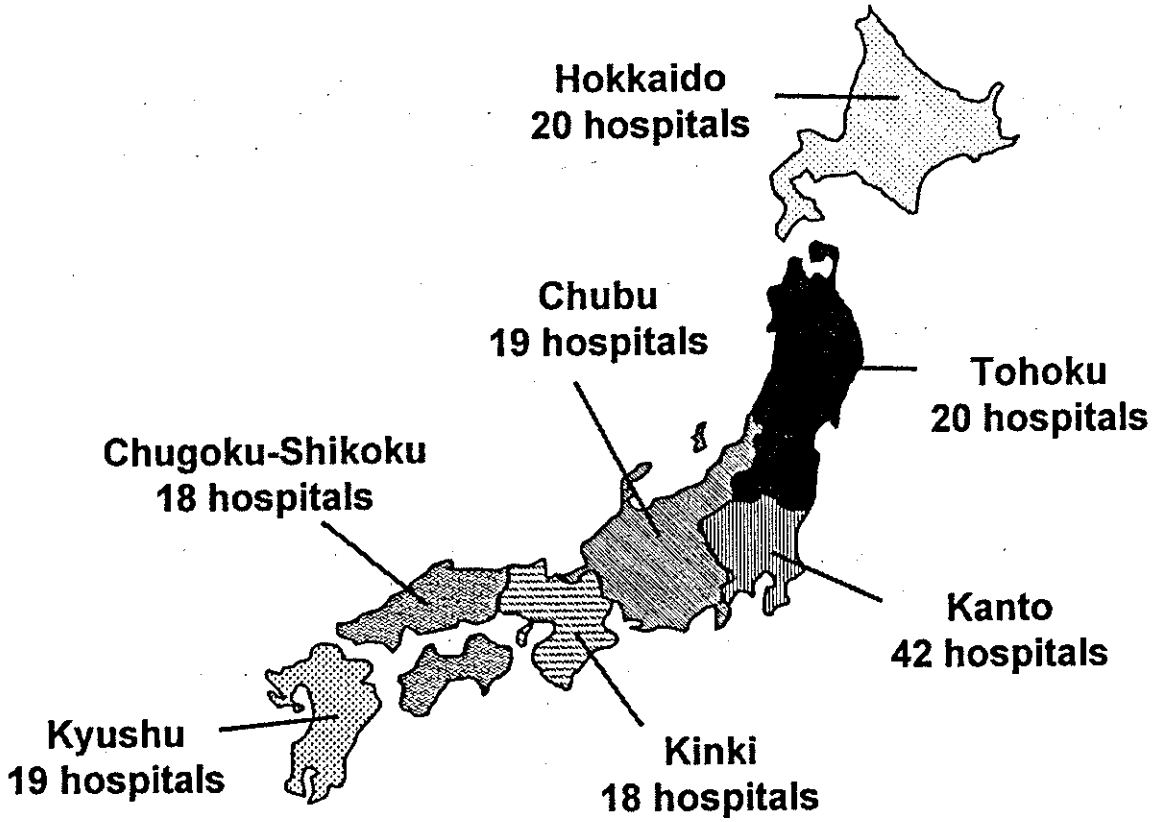

Figure 1. *The seven Japanese districts involved in the study, as defined by the Ministry of Health, Labour and Welfare, Japan, and the number of participating hospitals in each district.*

Total medical expense in Japan has been increasing every year, and reached about 24 trillion yen (US $200 billion) in 1999.[4] Stroke is the second most costly disease among all diseases, and it is the top in elderly people (≥65 years). The medical expenses for stroke will probably continue to increase, leading to major social problems, including burdening the health insurance system. To improve these circumstances, we need to know the present status of stroke medicine, and analyze the data to utilize for reconstruction of socio-medical systems.

We conducted a large prospective hospital-based registration study to create a fundamental database on acute ischemic stroke (AIS) or transient ischemic attack (TIA) in 156 hospitals throughout Japan. The data were recorded using the common protocol and data sheets in all participating hospitals.

Materials and Methods

All the consecutive patients (age ≥16 years old) with AIS and TIA admitted within 7 days of onset to the 156 participating hospitals were registered to the central office, using the common protocol and data sheets, for a 12-month period between May 1, 1999 and April 30, 2000. We divided Japan into 7 districts according to juridical regions defined by the Ministry of Health, Labour and Welfare, Japan, and selected the hospitals in which more than 50 acute ischemic stroke patients were treated between April 1997 and March 1998 (Fig 1). Of the 156 participating hospitals, 16 (10%) and 70 (45%) equipped specialized stroke care unit (SCU) and intensive care unit (ICU) services, respectively.

All data were registered with the central office. The doctor in charge of each participating hospital reported the number of AIS and TIA admissions to the central office by fax at the end of every month. Documented data sheets were mailed to the central office within 1 month after patient discharge. If the central office judged the data as "incomplete" because of an insufficient description, the data sheets were mailed back to and revised by the doctor in charge, then re-mailed to the central office.

Diagnosis of acute brain infarction or TIA (≥7 days of onset) was made by a neurologist or neurosurgeon, and confirmed by computed tomography (CT) and/or magnetic resonance imaging (MRI) in all registered patients. The following data were assessed in all of the patients using common data-sheets prepared by the protocol committee: (1) age and gender; (2) patient's activity at onset (resting, working, sleeping, and unknown), time and place of onset (home, office, outdoor, hospital, and others); (3) time from onset to hospital arrival; (4) method of transportation to hospital (ambulance, walking by him-/

herself, assisted by family, in hospital, or others); (5) clinical symptoms at onset; (6) a history of stroke; (7) ward (SCU or ICU, general ward mainly for stroke patients, or general ward); (8) NIH stroke scale (NIHSS) score on admission; (9) site of acute lesions on CT or MRI (side, area supplied by the carotid artery, or vertebrobasilar and posterior cerebral arteries); (10) stroke subtype (clinical categories of ischemic stroke); (11) stroke risk factors; (12) therapy within 7 days of onset; (13) length of hospital stay; and (14) outcome at discharge.

Clinical categories (stroke subtypes) were defined using clinical and radiographic diagnosis rubrics according to the "classification of cerebrovascular diseases III" by National Institute of Neurological Disorders and Stroke (NINDS)[5]: lacunar, atherothrombotic, cardioembolic stroke and other.

Clinical symptoms assessed at stroke onset were level of consciousness, convulsion, speech disturbance, headache, nausea/vomiting, vertigo/dizziness, visual, motor, sensory, and gait disturbances.

We investigated the application of thrombolytic agents (intravenous or intra-arterial urokinase [UK] and recombinant tissue plasminogen activator [rt-PA] within 12 hours of stroke onset), and medical (ozagrel sodium, argatroban, heparin, aspirin, ticlopidine, and warfarin,) and surgical treatment (decompression craniotomy, carotid endarterectomy [CEA], stenting, and percutaneous transluminal angioplasty [PTA]) within 7 days of stroke onset.

Risk factors were defined as follows: hypertension; use of antihypertensive agents, or a systolic blood pressure (SBP) reading $\geq$ 160 mmHg or diastolic blood pressure (DBP) reading $\geq$ 95 mmHg before onset, diabetes mellitus (DM); use of oral hypoglycemic agents or insulin, or a glycosylated hemoglobin (HbA1C) level $\geq$ 6.4%, hypercholesterolemia; use of antihyperlipidemic agents, or a serum cholesterol level $\geq$ 220 mg/dl, current cigarette smoking, and potential cardiac sources of emboli; nonvalvular atrial fibrillation (AF), acute myocardial infarction, old myocardial infarction with intraventricular thrombus, mitral valve disease, prosthetic cardiac valve, pacemaker, and dilated cardiomyopathy.

Outcome at hospital discharge was evaluated by the mRs[6] and death.

Statistical analyses were performed using a commercially available software package (Stat-View, version 4.5; ASA Institute, Cary, NC). The Mann-Whitney *U* test or Kruskal-Wallis test was applied to detect difference in age and NIHSS score among subgroups. All other findings were assessed by the Chi square test for stroke subtypes and TIA. Differences were assumed to be significant at $P < .05$.

Results

During the study period, 17,728 AIS or TIA patients were registered. We excluded 806 patients because of protocol violation such as double registration (n=446), no documentation of onset day (n=237), non-stroke patients (n=2), visit later than day 7 from stroke onset (n=16), patient's age < 16 (n=8), and stroke onset after the study period (n=97). Thus, 16,922 patients (men 10,370 [61%], women 6,552 [39%]) were enrolled in this study.

TIA was seen in 7% of registered patients, lacunar stroke in 36%, atherothrombotic in 31%, cardioembolic stroke in 20%, and other in 6% (Table 1).

Age

Patients' age was 70.6 (standard deviation [SD], 11.5) years (median 71, range 18-107). Women (73.6 [SD 11.7], median 75, range 18-100) were older than men (68.7 [SD 11.0], median 69, range 18-107; ($P < .0001$) (Table 2). Only 2% of the enrolled patients were younger than 45 years old. Overall, 71% were older than 65 years old, and 10% over 85 years. In 11,321 patients with first-ever stroke or TIA, mean age was 69.6 [SD 12.1] years (median 70, range 18-107). Women (n=4,492; mean age, 72.8 [SD 12.2]; median 74; range, 29-100) were again significantly older than men (n=6,829; mean age, 67.4 [SD 11.5]; median 67; range, 18-102; $P < .0001$).

Time from Onset to Admission

Cumulative frequency was 50% within 6 hours, 60% within 12 hours, 73% within 24 hours, 84% within 48 hours, and within 72 hours 91% of patients were admitted to the hospital (Table 3). Among 15,831 stroke patients, 35% were admitted within 3 hours, and 48% within 6 hours. The frequency of early hospital admission < 3 hours of onset by stroke subtype was the highest in cardioembolic stroke ($P < .0001$).

Use of Ambulance and Ward Admitted

Overall, 43% of patients were transferred to hospital by an ambulance, 17% of patients came to the hospital by themselves using public transportation or private car, and 37% visited with their family's assistance. Excluding the 354 patients who developed stroke or TIA during hospital stay, the ambulance was used in 64% of patients who arrived within 6 hours of onset, but in only 28% of patients who visited the hospital after 6 hours of onset ($P < .0001$). SCU or ICU admission accounted for only 19% of patients, and 58% were admitted to general ward mainly for stroke, and 27% to mixed general ward.

Tables 4 and 5 show the patients' activity and place of onset, and symptoms at onset of events, respectively.

NIHSS on Admission

The mean and median of NIHSS score were 8.0 (SD 7.9) and 5, respectively (Fig 2). Overall, 59% of patients had an NIHSS score of "0-6," 24% ranged from "7-15," and

Table 1. *Age, NIHSS score, risk factors, and length of hopital stay by each stroke subtype*

	Total (n = 16,922)	Lacunar (n = 6,146)	Atherothrombotic (n = 5,267)	Cardioembolic (n = 3,451)	Other (n = 967)	TIA (n = 1,091)
Mean (Median) age, years	70.6 (71)	69.6 (70)	70.8 (71)	73.5 (74)	66.0 (68)	69.5 (70)
History of stroke*	5,160/16,481	1,870/6,004 (31%)	1,625/5,121 (32%)	1,062/3,337 (32%)	258/948 (27%)	345/1,017 (32%)
Mean (Median) NIHSS score	8.0 (5)	4.6 (4)	8.7 (6)	14.7 (14)	8.2 (5)	—
Hypertension	10,302 (61%)	4,143 (68%)	3,469 (66%)	1,558 (45%)	519 (54%)	613 (56%)
Diabetes mellitus	4,113 (24%)	1,601 (26%)	1584 (30%)	556 (16%)	168 (17%)	204 (19%)
Hypercholesterolemia	2,836 (17%)	1,185 (19%)	980 (19%)	313 (9%)	149 (15%)	209 (19%)
Atrial fibrillation	3,521 (21%)	245 (4%)	385 (7%)	2614 (76%)	91 (9%)	186 (17%)
Smoking	2,964 (18%)	1231 (20%)	1019 (19%)	346 (10%)	149 (15%)	219 (20%)
Mean (median) length hospital stay, days	35.0 (23)	29.0 (20)	40.1 (29)	40.5 (29)	31.4 (22)	14.4 (11)

*441 patients with unknown about a history of stroke were excluded from 16,922 patients, and 16,481 patients were analyzed.

Table 2. *Age of patients*

	Men (n = 10,370)	Women (n = 6,522)
≤45	204 (2%)	123 (2%)
46-55	957 (9%)	342 (5%)
56-65	2,411 (23%)	885 (14%)
66-75	3,768 (36%)	1,859 (28%)
76-85	2,392 (23%)	2,335 (36%)
86≤	638 (6%)	1,008 (15%)

16% were greater than "15." In 11,356 patients admitted within 24 hours of onset, the mean and median NIHSS scores were 9.5 (SD 8.4) and 6, respectively. An NIHSS score less than "6" was observed in 51% of patients, "7-15" in 27% did, and more than "15" in 22%. The NIHSS score for each stroke subtype is presented in Table 1.

Therapy Within 12 Hours of Stroke Onset

One thousand twenty-seven (6%) patients received intravenous UK (n=750) or intra-arterial UK (n=277). The mean dose of intra-arterial UK was 359, 000 IU (SD, 217,000 IU). One hundred thirty-eight (1%) patients were treated with intravenous rt-PA; 50 with intra-venous and 88 with intra-arterial application. When intravenous UK with more than 200, 000 IU and intravenous rt-PA, intra-arterial UK and rt-PA were considered as thromboliytic therapy, 477 (3%) patients were treated with thrombolytic agents in acute phase of stroke.

Therapy Within 7 Days of Stroke Onset

Ozagrel sodium was most frequently used in 49% of patients, followed by argatroban in 21%. Heparin, ticlopidine, and aspirin were given in 16%, 14%, and 10%, respectively. Surgical treatment was performed in 262 (2%) patients; decompression craniotomy in 106 patients, PTA in 54, CEA in 41, carotid stenting in 13, and miscellaneous surgical produces in 69.

Risk Factors

In all, 61% of the patients had hypertension, 24% and 17% had diabetes mellitus and hypercholesterolemia, respectively. Cigarette smoker was observed in 18%. AF was the potential cardiac sources for brain embolism in 21% of patients, and the remaining 13% had miscellaneous risk factors for stroke. The frequencies of risk factors for each stroke subtype are presented in Table 1. Patients with a history of stroke had AF more frequently than those without (24% *v* 19%; $P < .001$).

Site of Lesion

CT or MRI detected acute relevant lesions in 93% (n=15,794) of patients. Among them, 73% of the lesions

Table 3. *Stroke subtypes and arrival time*

	All patients (n = 16,922)	Lacunar (n = 6,146)	Atherothrombotic (n = 5,267)	Cardioembolic (n = 3,451)	Other (n = 967)	TIA (n = 1,091)
<3h	6,211 (37%)	1,361 (22%)	1,737 (33%)	2,122 (61%)	387 (40%)	614 (56%)
3-6 h	2,147 (13%)	763 (12%)	707 (13%)	417 (12%)	113 (12%)	147 (13%)
6-12 h	1,725 (10%)	745 (12%)	554 (11%)	252 (7%)	92 (10%)	82 (8%)
12-24 h	2,199 (13%)	1046 (17%)	714 (14%)	245 (7%)	99 (10%)	95 (9%)
24-48 h	1,948 (12%)	964 (16%)	628 (12%)	188 (5%)	96 (10%)	72 (7%)
48-72 h	1,205 (7%)	604 (10%)	393 (7%)	110 (3%)	70 (7%)	28 (3%)
72-96 h	680 (4%)	343 (2%)	240 (1%)	37 (0%)	42 (0%)	18 (0%)
96-120 h	334 (2%)	136 (0%)	130 (1%)	22 (0%)	31 (0%)	14 (0%)
120-144 h	231 (1%)	99 (1%)	77 (1%)	27 (0%)	18 (0%)	10 (0%)
144-168 h	227 (1%)	83 (1%)	85 (1%)	28 (0%)	19 (0%)	11 (0%)
Unknown	5 (0%)	2 (0%)	0 (0%)	3 (0%)	0 (0%)	0 (0%)

were located on either side of the carotid territories (46% on the right and 54% on the left) and 2% was on carotid territories on both sides, and 24% were on the vertebrobasilar and posterior cerebral arteries, and 1% on both carotid and vertebrobasilar arteries. The proportion of lesions in carotid and vertebrobasilar (posterior cerebral artery inclusive) territories was 75% and 23% in lacunar, 69% and 28% in atherothrombotic, 82% and 17% in cardioembolic stroke, and 59% and 36% in other, respectively. The proportion of lesion in the carotid artery territory was largest in cardioembolic stroke among stroke subtypes ($P < .0001$).

Length of Hospital Stay

The mean length of hospital stay was 35.0 ± 33.5 days (median 23, range 0-429). Mean length of hospital stay by baseline NIHSS score was 26.3 ± 24.1 days (median 20, range 0-337) for a score of 0-6, 42.2 ± 37.6 days (median 31, range 0-429) for a score of 7-10, 46.4 ± 38.5 days (median 36, range 0-332) for a score of 11-15, and 47.4 ± 45.4 days (median 35, range 0-325) for a score of ≥ 16. The length of hospital stay for each stroke subtype is presented in Table 1.

Outcome at Hospital Discharge

The distribution of the mRs score at discharge was as follows: 19% scoring 0, 29% scoring 1, 13% scoring 2, 8% scoring 3, 14% scoring 4, and 10% scoring 5, and 7% were dead. When mRs scores of 0, 1, and 2 were considered as good outcome, 61% showed good outcome. Fig 3 demonstrates outcomes expressed in mRs score at discharge by each stroke subtype. The frequency of good outcome was highest in lacunar stroke (76%), followed by atherothrombotic (52%), and cardioembolic stroke (37%; $P < .0001$). The proportion of patients with good outcomes and death at discharge were 84% and 1% of patients with baseline NIHSS score of 0-6, in 46% and 7% with score 7-10, and 26% and 18% with score 11-15, respectively. In patients with baseline NIHSS score greater than 15, good outcome was seen only in 9%, and 37% were dead.

Table 4. *Patients' activity and place of onset*

	Stroke n = 15,831	TIA n = 1,091
Patients' activity of onset		
Moving	43%	57%
Rest	34%	32%
Sleep	13%	8%
Unknown	10%	3%
Place of onset		
Home	80%	71%
Office	4%	6%
Outside	9%	15%
Hospital	4%	6%
Others	3%	2%

Discussion

Median of patients' age in this study was above 70 years, being slightly higher than those previously re-

Table 5. *Symptoms at onset of events*

Symptom	Number of patients (%)
Weakness	11,955 (71%)
Speech disturbance	7,757 (49%)
Gait disturbance	6,209 (37%)
Loss of consciousness	4,277 (25%)
Sensory disturbance	2,593 (15%)
Vertigo/dizziness	1,449 (9%)
Nausea/vomiting	1,175 (7%)
Visual disturbance	745 (4%)
Headache	590 (4%)
Convulsion	118 (1%)

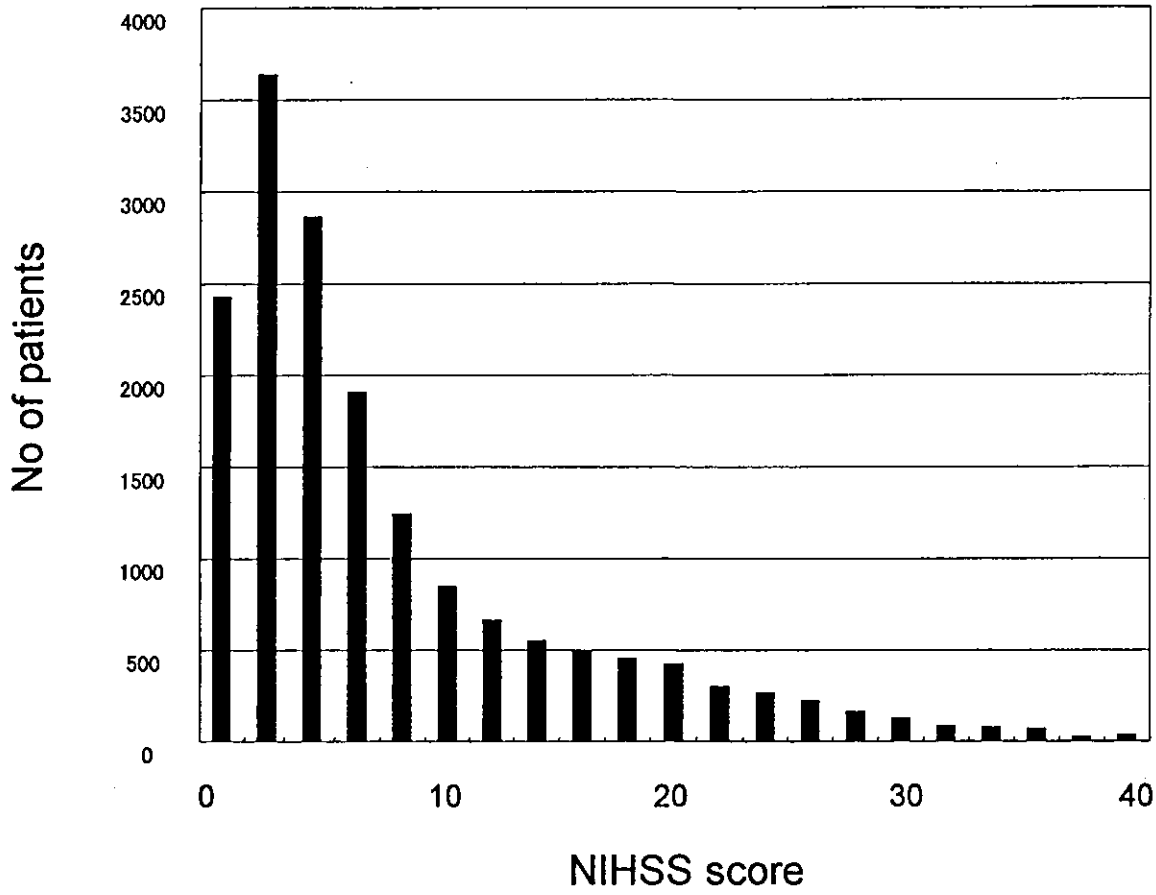

Figure 2. *Distribution of NIHSS scores at admission.*

ported from Western and Asian countries.[7-15] The proportion of patients under the age of 45 years was only 2%, which was much smaller than that in previous reports.[9-11,14] The Japanese life expectancy is the longest in the world, which may explain the results that the proportion of elderly people is higher than those in other countries.

In this study, lacunar stroke was the most frequent stroke subtype, followed by atherothrombosis, and cardioembolism. In an epidemiological study in Hisayama, Japan, they followed stroke-free subjects (n=1,621) for 32 years from 1961, and identified 298 ischemic stroke patients, in which lacunar stroke was 56%, atherthromotic stroke 21%, and cardioembolic stroke 19%.[16] The incidence of lacunar stroke in Japan was higher than in Western countries.[10,17-19] Japanese are considered to be at higher risk for arteriosclerosis of intracranial small arteries compared with whites. However, the proportion of lacunar stroke in the present study was smaller, and those of atherothrombotic stroke was larger compared with those in Hisayama study,[16] which may indicate that lacunar stroke has been decreasing, and in contrast, atherothrombotic stroke has been increasing. We assume that lifestyle including dietary habits has been changed or westernized in recent years, which may reduce the incidence of lacunar stroke in Japan.

Weakness of extremities was a prominent clinical feature, followed by gait disturbance and speech disturbance, loss of consciousness, sensory disturbance, and vertigo/dizziness. The majority of strokes or TIAs occurred while the patients were at their homes. It is important to conduct public education, particularly to family members, on the early symptoms of stroke or TIA as mentioned above.

About half of the stroke patients arrived at hospitals within 6 hours of stroke onset. Of stroke or TIA patients who arrived at hospital within 6 hours, 64% used an ambulance. However, only 28% of patients arrived later than 6 hours did so. Public education on the use of the ambulance service could result in a significant reduction of delayed hospital arrival after stroke onset.[20] The frequency of early arrival was higher for cardioembolic stroke compared with other stroke subtypes. This can be explained by the fact that cardioembolic stroke more frequently develops abruptly with severe neurological deficits than the other stroke subtypes.

Only 19% of all patients were treated in SCU/ICU. Several studies have shown better outcome of patients who were treated in stroke units (SU) compared with those in general wards,[21-23] and managements in SU have been strongly recommended in Europe. We need to set

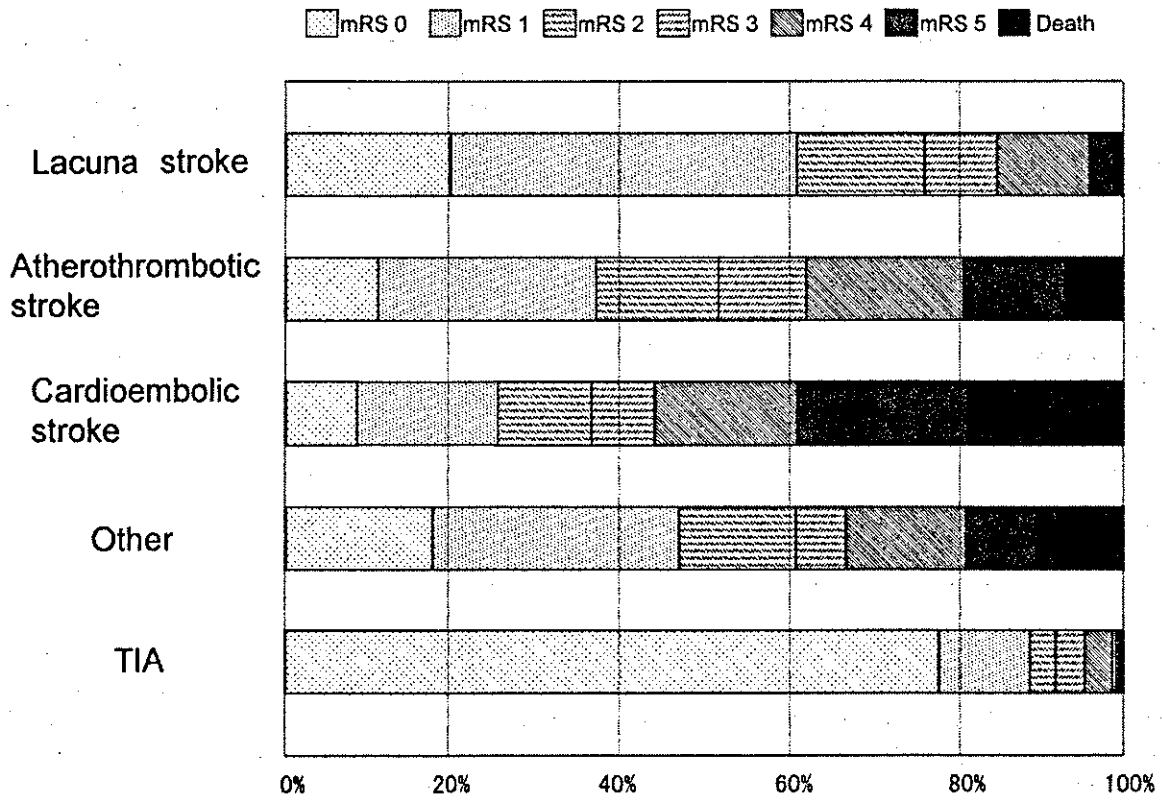

Figure 3. *Stroke subtype and mRs score at discharge. Among stroke subtypes excluding TIA, the frequency of a good outcome (mRs 0-2) was highest in lacunar stroke (76%), followed by other (61%), atherothrombotic stroke (52%), and cardioembolic stroke (37%; $P < .0001$).*

up more SCU/SU supplied with trained stroke team all over Japan for better management and outcome of stroke patients.

Of 11,356 stroke patients admitted within 24 hours of onset, 51% had NIHSS scores of 0-6, 27% scores of 7-15, and 22% scores greater than 16. The distributions of NIHSS score were similar to those reported by Adams,[24] who reported that 46% of 1,281 patients in the TOAST (Trial of Org 10172 in Acute Stroke Treatment) study had a NIHSS scores of 0 to 6, 42% scored 7-15, and 13% scored > 15.24 In the present study, the distribution of NIHSS score of cardioembolic stroke was somewhat different from other subtypes. Cardioembolic stroke patients had a variety of neurological deficits from very mild to extremely severe. In some of them, an embolus suddenly occludes the major cerebral artery without presence of collateral flow, which often results in severe and widespread ischemia compared with other stroke subtypes. On the other hand, an occlusion may spontaneously reopen in certain patients, which results in a case of spectacular shrinking deficits.[25]

The efficacy of thrombolytic therapy in acute ischemic stroke has been proven in recent trials.[26,27] However, rt-PA administration for acute ischemic stroke has not been approved by the Ministry of Health, Labour and Welfare of Japan, and we cannot use legally rt-PA to an acute ischemic stroke patient. Barber et al[28] reported that 27% of 1,168 ischemic stroke patients were admitted within 3 hours of symptom onset, and of these, 27% received rt-PA. Overall, only 7% of ischemic stroke patients in their hospital received rt-PA. Chiu et al[29] also stated that only a small percentage of acute stroke patients received this therapy. The major reason for these results on thrombolytic therapy is short therapeutic time window. Before the official approval of rt-PA, we need to make public education focusing on importance of early treatment and disadvantage of delayed hospital arrival as well as in the United States.

CT or MRI detected acute relevant lesions located in the areas supplied by the carotid arteries in 76%, and in the territories of the vertebrobasilar-posterior cerebral arteries in 24%. Lesions of cardioembolic stroke were more frequently present in the carotid artery territory than those of other stroke subtypes. These findings were similar to previous reports based on stroke registry except for the Lausanne study.[11,13,14] The reason for this difference is unknown.

Table 6 shows risk factors reported in the previous hospital-based or community-based studies. Hypertension was the most prominent risk factor for stroke, and the frequency of hypertension was similar to other previous reports.[10,11,14,24,30-33] Epidemiological studies have confirmed an independent effect of DM with a relative risk for ischemic stroke between 1.8 to 3.0.[34] Hypercho-

Table 6. *Risk factors from selected registry studies*

	No. of patients	Hypertension	Diabetes mellitus	Hypercholesterolemia	Atrial fibrillation	Smoking
Community-based study						
Bogousslavasky, 1988[11]	778	23%	6%	7%	8%	17%
Petty GW, 1999[17]	454	73%	21%	—	—	49%
Feigin VL, 1998[32]	237	85%	7%	—	15%	19%
Kolominsky-Robas PL, 2001[18]	531	57%	25%	—	—	54%
Hospital-based study						
Foulkes MA, 1988[15]	1,253	22%	8%	—	4%	—
Yip PK, 1997[10]	676	64%	31%	14%	16%	33%
Frey JL, 1998[31]	1,290 (White)	66%	17%	19%	—	61%
Frey JL, 1998[31]	242 (Hispanic)	72%	36%	13%	—	46%
Misbach J, 2000[9]	2,065	73%	17%	16%	6%	13%
Samadja D, 2001[33]	463	71%	32%	13%	15%	8%
Lee BI, 2001[14]	1,000	64%	37%	24%	—	35%
Present study, 2002	16,922	61%	24%	17%	21%	18%

lesterolemia has been proven as an important risk factor for coronary heart diseases, but its relation to stroke remains uncertain.[35] AF is the most powerful and treatable cardiac precursor of ischemic stroke. In this study, 21% of patients had AF, which was higher than previous reports from Western and Asian countries.[9-11,14,30] Moreover, patients with a history of stroke had AF more frequently than first-ever stroke or TIA patients. Petty et al[17] demonstrated that the five-years survival rate was poorest in cardioembolic stroke among ischemic stroke subtypes. Therefore, the primary and secondary prevention of embolic events is one of the most important issue for AF patients.

Adams et al[24] reported that baseline NIHSS score strongly predicted patient's outcome, which was comparable to our results. The in-hospital mortality was 6.9% in the present study. To the best of our knowledge, this is the lowest rate reported previously in the literatures.[1,36-39] This reason may be explained not only by the high proportion of lacunar stroke, but by a recent improvement of medical management of acute stroke patients.

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Characterisation of [^{123}I]iomazenil distribution in a rat model of focal cerebral ischaemia in relation to histopathological findings

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Abstract. Iodine-123 labelled iomazenil ([^{123}I]IMZ) has been reported to be a useful marker of neuronal viability. The brain distribution of [^{123}I]IMZ, however, has not been correlated with the pathophysiological response in detail after an ischaemic insult. To characterise [^{123}I]IMZ as a marker of neuronal viability, we compared its brain distribution with cyclooxygenase-2 (COX-2) expression, DNA fragmentation and cellular integrity. [^{123}I]IMZ and [^{125}I]IMP were injected into rats with focal cerebral ischaemia for the purpose of dual-tracer autoradiography. COX-2 and microtubule-associated protein-2 (MAP-2, a marker of cellular integrity) were immunostained. In situ DNA polymerase-I-dependent dUTP incorporation into damaged DNA was used as an indicator of DNA fragmentation. Lesion to normal ratios (LNRs) for [^{123}I]IMP and [^{125}I]IMZ were calculated. [^{123}I]IMZ accumulation was preserved in several regions with impaired [^{123}I]IMP accumulation. COX-2 expression was occasionally observed, whereas neither DNA fragmentation nor MAP-2 denaturation was detected in these regions. DNA fragmentation and impaired MAP-2 immunostaining were observed only in the regions with reduced LNRs for both tracers. The LNR for [^{123}I]IMZ was significantly lower in regions with impaired MAP-2 immunostaining (0.120±0.152, P<0.0001), in regions positive for dUTP incorporation (0.488±0.166, P<0.0001) and in regions positive for COX-2 expression (0.626±0.186, P<0.001) than in histologically normal regions (0.784±0.213). Thus, neuronal DNA is still intact and cellular integrity is maintained in the ischaemic regions with preserved [^{123}I]IMZ accumulation. The impairment of [^{123}I]IMZ accumulation precedes DNA fragmentation and denaturation of cellular integrity. These results provide the molecular basis of [^{123}I]IMZ distribution.

Keywords: [^{123}I]iomazenil – Cerebral ischaemia – Neuronal viability – Cyclooxygenase-2 – DNA fragmentation

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Introduction

An ischaemic stroke is one of the most common neuronal disorders, and the number of patients suffering from the disease is increasing. For the clinical evaluation of ischaemic stroke, it is very important to precisely detect the ischaemic penumbra, which is an ischaemically affected but still viable tissue, because the penumbral tissue can be salvaged by pharmacological and/or surgical interventions [1, 2, 3, 4].

Iodine-123 iomazenil ([^{123}I]IMZ) is a probe for central-type benzodiazepine receptor (BZR) for single-photon emission tomography (SPET). Since GABA receptors are abundant in the cortex and sensitive to ischaemic damage, specific radioligands to their subunits, the cerebral BZRs existing in GABA-A receptors, can be used as a marker of neuronal viability [5]. Thus, BZR imaging with [^{123}I]IMZ should be useful for detecting viable neurons, which may help detect the penumbra after an isch-

aemic insult. In this regard, several experimental and clinical investigators have compared the [^{123}I]IMZ distribution with the cerebral blood flow, oxygen metabolism and/or glucose metabolism, and shown the potential of [^{123}I]IMZ for evaluation of neuronal viability after an ischaemic stroke [3, 6, 7, 8, 9, 10, 11, 12]. A few authors [6, 10, 11] have also correlated [^{123}I]IMZ distribution with histological findings obtained using the haematoxylin-eosin stain.

To the best of our knowledge, however, the brain distribution of [^{123}I]IMZ has not been correlated with the molecular response after an ischaemic insult in detail. The pathophysiological significance of findings that are actually imaged by [^{123}I]IMZ also remains to be elucidated. Accordingly, we compared the brain distribution of [^{123}I]IMZ with (1) cerebral blood flow, (2) the expression of cyclooxygenase-2 (COX-2), (3) fragmentation of DNA and (4) cellular integrity, in order to characterise [^{123}I]IMZ as a marker of neuronal viability. COX-2, a prostanoid synthesising enzyme, is expressed early after an ischaemic insult and contributes to the progression of ischaemic damage [13, 14, 15, 16, 17]. Thus, we examined COX-2 expression to evaluate the neuronal response early after an ischaemic insult. In situ DNA polymerase-I-dependent dUTP incorporation into damaged DNA was used as an indicator of DNA fragmentation. Techniques for visual detection and localisation of DNA injury/repair in situ include: TdT-dependent dUTP labelling of free 3'-OH ends of double-stranded DNA (TUNEL); Klenow fragment of DNA polymerase-I-dependent labelling of staggered 3'-OH ends and gaps; and DNA polymerase-I incorporation in nicks, gaps and staggered 3'-OH ends [18]. Of these, only DNA polymerase-I has 5'–>3' exonucleolytic activity, which allows nick translation and visualisation of randomly occurring single-strand scission of double-stranded DNA. MAP-2, a cellular structural protein existing on the surface of neurodendrites, is also immunostained as a marker of cellular integrity.

Materials and methods

Animal preparation. The experimental protocol was fully approved by the Laboratory Animal Care and Use Committee of Hokkaido University. Male Sprague-Dawley rats weighing 300–350 g were used. The rats had free access to water and laboratory chow. The animals were initially anaesthetised with 400 mg/kg body weight IP chloral hydrate. The body temperatures were monitored with rectal probes and maintained at 37°C with heating pads during the operation. The rats were subjected to permanent unilateral major artery occlusion. The right middle cerebral artery (MCA) of each rat was occluded intraluminally according to a method described in detail previously [19, 20, 21]. The rats were allowed to recover from anaesthesia and any induced neurological deficits were confirmed. The animals not showing any neurological deficits were excluded from this experiment.

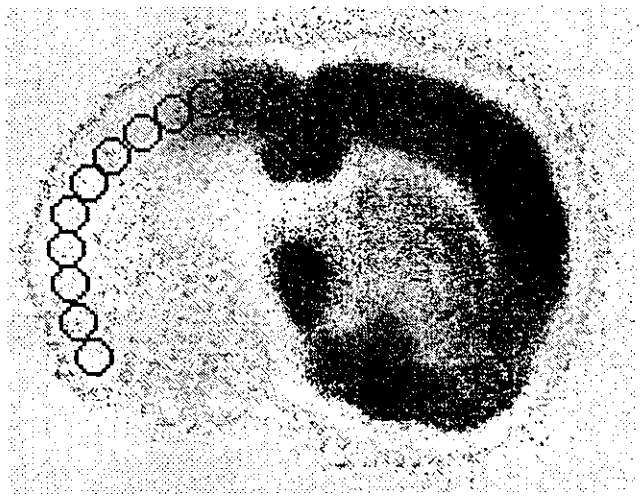
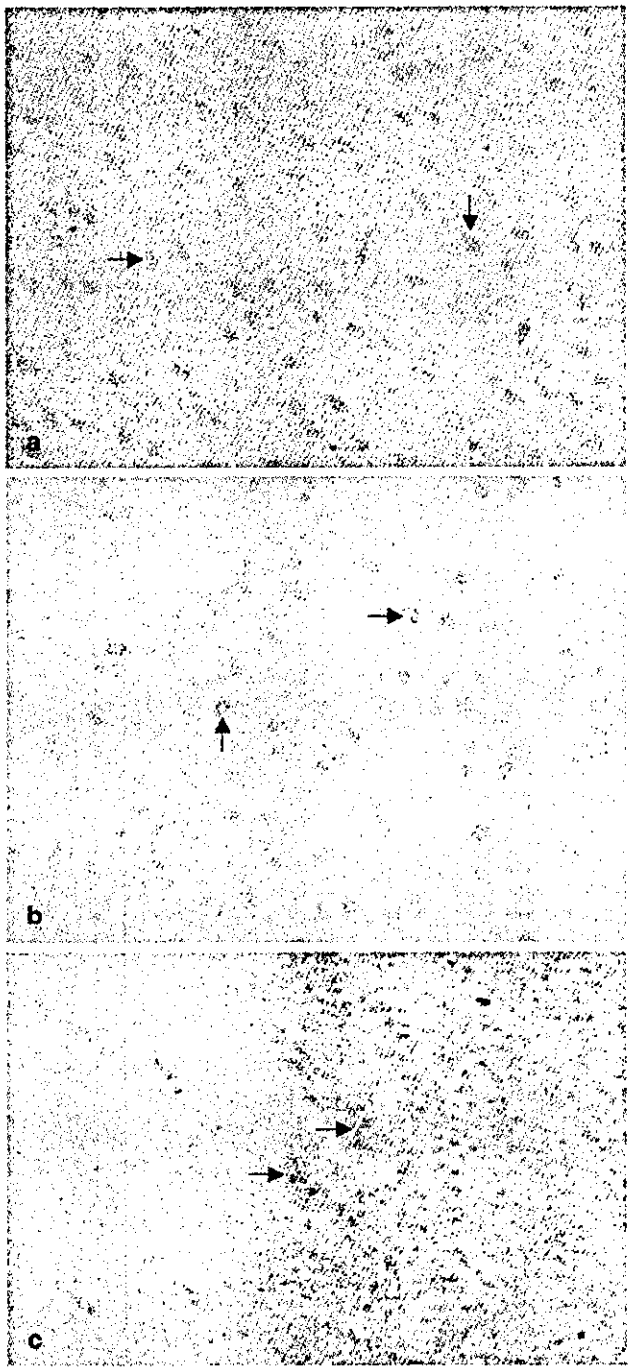


Fig. 1. An example of regions of interest (ROIs) on a coronal image. Twelve circular ROIs (2 mm in diameter) were determined on each hemisphere of the cortex symmetrically

Autoradiographic studies. The brain distributions of [^{123}I]IMZ and [^{125}I]IMP were determined using a dual-tracer autoradiographic technique. [^{123}I]IMZ (111 MBq/kg body weight) was first injected via the femoral vein 60 min before decapitation, to determine specific [^{123}I]IMZ distribution according to the methods reported by Toyama et al. [10, 11]. Then, 55 min later, [^{125}I]IMP (2.22 MBq/kg body weight) was injected via the contralateral femoral vein, to assess blood flow distribution [10, 11]. The rats were decapitated under chloral hydrate anaesthesia 5 min after the injection of [^{125}I]IMP, which was 2, 3, 4, 6, 8, 12 and 24 h after the ischaemic insult (n = four to six in each group). Their brains were removed quickly and carefully, and immersed in ice-cold saline. The brains were then sectioned at 6 mm caudal from the frontal pole using a brain matrix (RBM-4000C, ASI Instruments, Warren, MI) to obtain coronal sections. The brain samples were embedded in a medium (Tissue-Tek, Sakura Finetechnical Co., Ltd., Tokyo, Japan), frozen in isopentane-dry ice, and cut into 20-µm sections with a cryostat (Bright Instrument Co., Ltd., Cambridgeshire, England). The first autoradiographic exposure was performed for 3 h to detect the distribution of [^{123}I]IMZ. The second exposure was initiated 7 days later and carried out for 7 days to visualise the distribution of [^{125}I]IMP.

Histological studies. Immunoreactivity to COX-2 and microtubule-associated protein-2 (MAP-2) were detected in frozen sections (10 µm thick) adjacent to those used for the autoradiographic studies, using a standard immunostaining procedure [23]. Briefly, after fixation in a cold 1:1 acetone-to-methanol mixture, the sections were incubated with a polyclonal anti-COX-2 antibody (Cayman Chemical; dilution 1:2,000) or a purified mouse monoclonal anti-MAP-2 antibody (BD Pharmingen, San Diego; dilution 1:400). The bound antibody was visualised by staining with avidin/biotin conjugate immunoperoxidase (Vector Laboratories, Inc., CA, USA) and 3,3'-diaminobenzidine tetrahydrochloride (DAB; Vectastain Elite Kit, Vector Laboratories, CA).

DNA fragmentation was also detected on the adjacent sections by incorporation of digoxigenin-dUTP using DNA polymerase-I according to the method previously described [18, 23]. To confirm the nuclear localisation of the label, some sections were counterstained with haematoxylin.



Data analysis. The autoradiograms were analysed using a computerised imaging analysis system (Bio-imaging Analyser BAS-5000, Fuji Photo Film, Tokyo, Japan). To quantitatively evaluate the distributions of [^{123}I]IMZ and [^{125}I]IMP, 12 circular regions of interest (ROIs, 2 mm in diameter) were determined on each hemisphere of the cerebral cortex in the autoradiograms symmetrically from the longitudinal fissure to the temporal lobe (Fig. 1). Lesion to normal ratios (LNRs) were defined as the ratios of values for an ROI in the lesioned hemisphere to those for the contralateral homologous ROI.

Based on the LNRs for [^{123}I]IMZ and [^{125}I]IMP, ROIs determined on the lesioned hemisphere were classified into three groups as shown in Table 1: group 1, LNRs for both [^{125}I]IMP and [^{123}I]IMZ were equal to or larger than 0.8; group 2, the LNRs for [^{125}I]IMP were less than 0.8 and those for [^{123}I]IMZ were equal to or larger than 0.8; group 3, LNRs for both [^{125}I]IMP and [^{123}I]IMZ were less than 0.8. A threshold LNR of 0.8 was chosen, considering the lesion detectability by the autoradiographic methods [11].

The ROIs determined on the lesioned hemisphere were also classified into four groups based on histological findings as follows (Fig. 2): group A, impaired MAP-2 immunostaining; group B, preserved MAP-2 immunostaining and positive for dUTP incorporation; group C, preserved MAP-2 immunostaining, negative for dUTP incorporation and positive for COX-2; group D, no histological evidence of an ischaemic injury.

All values are expressed as means or means ± standard deviation. A Z test was used to assess the significance of difference in the percentage of ROIs with impaired [^{125}I]IMP or [^{123}I]IMZ accumulation. One-way ANOVA and post-hoc tests (Fisher's method) were used to assess the significance of difference in the LNRs among the four groups classified on the basis of histological findings. A two-tail value of $P<0.05$ was considered to indicate statistical significance.

Results

Figure 3 shows representative autoradiograms for [^{125}I]IMP and [^{123}I]IMZ. The accumulation of [^{125}I]IMP decreased in a wide region in the MCA territory 2 h after occlusion, which extended with time. The region with

Fig. 2a–c. Representative images of **a** COX-2 immunostaining (×200), **b** dUTP incorporation (×200) and **c** MAP-2 immunostaining (×200). **a** Expression of COX-2 protein was occasionally observed (*arrows*). **b** The ring-like appearance (*arrows*) of dUTP incorporation shows the neuron on the way to apoptotic cell death. **c** Positive MAP-2 immunostaining (*arrows*) shows cellular integrity

Table 1. Histological findings in the three ROI groups classified on the basis of LNRs

Group	LNR_{IMP}	LNR_{IMZ}	Number (%) of ROIs		
			COX-2 (+)	dUTP (+)	MAP-2 (–)
Group 1 (n=14)	≥0.8	≥0.8	0 (0%)	0 (0%)	0 (0%)
Group 2 (n=24)	<0.8	≥0.8	4 (16.7%)	0 (0%)	0 (0%)
Group 3 (n=238)	<0.8	<0.8	79 (33.2%)	59 (24.8%)	176 (73.9%)

LNR_{IMP}, LNR for [^{125}I]IMP; LNR_{IMZ}, LNR for [^{123}I]IMZ; COX-2 (+), positive immunostaining for COX-2; dUTP (+), positive dUTP incorporation; MAP-2 (–), negative immunostaining for MAP-2