The goal of antihypertensive therapy is to reduce the incidence of hypertension-related events. Many large-scale clinical trials have already demonstrated the benefits of antihypertensive treatment with drug therapy (*3, 4*). Based on these results, guidelines for the clinical management of hypertension such as "Japanese Society of Hypertension Guidelines for the Management of Hypertension (JSH 2004)" and the recommendations of the Seventh Report of the Joint National Committee on the Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC-7) have been established and used in the daily management of hypertension (*5, 6*). According to such guidelines, angiotensin converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs) are first-line agents for the treatment of hypertension, especially in hypertensive patients with diabetes.

Losartan potassium (losartan), a subtype 1 (AT1) selective angiotensin II (AII) receptor antagonist, has been widely prescribed worldwide. Several reports have suggested that losartan not only lowers the blood pressure (BP) values, but also has target organ protective effects. The Losartan Intervention for Endpoint Reduction (LIFE) study was a double-blind, prospective, parallel group trial that was designed to compare the effects of losartan with those of the β-blocker atenolol on cardiovascular morbidity and mortality in approximately 8,300 hypertensive patients with left ventricular hypertrophy. It demonstrated that losartan had a more favorable effect on cardiovascular events than atenolol (*7*). The Reduction of Endpoints in Non-Insulin-Dependent Diabetes Mellitus with the Angiotensin II Antagonist Losartan (RENAAL) study was a multinational, double-blind, randomized, and placebo-controlled trial that enrolled 1,513 patients with type 2 diabetes and nephropathy. It demonstrated a renoprotective effect of losartan (*8*).

Although studies conducted in Western countries have reported various beneficial effects of losartan therapy (*7–11*), the actual therapeutic benefit for Japanese patients has been unclear. Because the genetic and environmental background may differ between Japanese and Western patients (*12, 13*), an investigation of the effects of losartan in Japanese hypertensive patients would be of value. Accordingly, the Japan Hypertension Evaluation with Angiotensin II Antagonist Losartan Therapy (J-HEALTH) study was initiated in 2000 as a large-scale observational study of losartan therapy.

Recently, the significance of home BP monitoring has been an important topic in the management of hypertension (*14–16*). Since large-scale analysis of home BP data has not been performed in Japan, direct evidence of the significance of home BP values for future cardiovascular events is still lacking (*17*). Accordingly, the J-HEALTH study was performed to investigate the long-term antihypertensive efficacy and safety of losartan, and the incidence of cardiovascular events and mortality in this population. The study also aimed to investigate whether home BP monitoring would be effective for use in routine antihypertensive treatment.

Table 1. Exclusion Criteria

- Pregnant or could become pregnant, or breast-feeding
- Severe hepatic or renal disease
- Diseases of poor prognosis; malignant neoplasm, performing hemodialysis, or virus infections such as HIV
- Taking the study drug prior to the registration
- Recent stroke or myocardial infarction within 1 month
- Other inappropriate conditions judged by each investigator

HIV, human immunodeficiency virus.

Methods

Objectives

This study was designed to enroll 30,000 patients with hypertension throughout Japan, and the patients were treated with losartan on an open-label basis at a daily dose at 25–50 mg with standard clinical management for a maximum of 5 years. The aims of this study were to investigate the efficacy and safety profile of losartan during actual clinical use in the 5-year post-marketing period, the incidence of cardiovascular events and mortality, the value of lifestyle modification as antihypertensive therapy, and the relationship between the clinic BP and the home BP values in Japanese hypertensive patients primarily treated with losartan.

Patients Recruitment

The eligible patients were men or women ≥20 years of age who were diagnosed as having hypertension by their physicians and had not taken any antihypertensive agents within the previous 1 month. Patients who had previously take losartan were excluded. The other exclusion criteria are shown in Table 1. Each patient was informed of the purpose and methods of the study, as well as the effects and possible risks of losartan therapy, the right to withdraw from the study at any time, and the measures for privacy protection before they were enrolled. Patients provided their verbal informed consent and then underwent a complete medical history review, physical examination, and laboratory evaluation.

Drug Treatment and Study Procedure

The patients were initially treated with losartan at a dose of 25–50 mg once daily (usually in the morning), which is the approved dosage in Japan. The dose was increased up to a dose of 100 mg once daily, if necessary. Addition of other antihypertensive agents was allowed from 3 months after the start of losartan treatment, if required. No restrictions were placed on the treatment of complications.

The enrolled patients were registered in a central study registry that included the following information at baseline:

Table 2. Patients' Characteristics at Baseline

	Men	Women	Total
Number of patients (*n* (%))	13,737	17,311	31,048
Age (years old)	60.0±12.0	64.3±11.8	62.4±12.1
SBP (mmHg)	164.4±17.0	165.9±17.4	165.3±17.3
DBP (mmHg)	96.2±11.6	92.8±11.6	94.3±11.7
BMI (kg/m^2)	24.3±3.3	23.9±3.8	24.1±3.6
Alcohol drinkers (*n* (%))	9,147 (66.6)	2,674 (15.4)	11,821 (38.1)
Current smokers (*n* (%))	6,085 (44.3)	1,664 (9.6)	7,749 (25.0)
Complications			
Hyperlipidemia (*n* (%))	4,935 (35.9)	7,005 (40.5)	11,940 (38.5)
Diabetes mellitus (*n* (%))	2,170 (15.8)	1,883 (10.9)	4,053 (13.1)
Cardiovascular disease (*n* (%))	1,097 (8.0)	1,400 (8.1)	2,497 (8.0)
Cerebrovascular disease (*n* (%))	616 (4.5)	745 (4.3)	1,361 (4.4)
Hepatic disease (*n* (%))	1,901 (13.8)	1,069 (6.2)	2,970 (9.6)
Renal disease (*n* (%))	509 (3.7)	496 (2.9)	1,005 (3.2)
ECG abnormality (*n* (%))	2,119 (15.4)	2,234 (12.9)	4,353 (14.0)
Concomitant drugs			
Lipid-lowering drugs (*n* (%))	2,632 (19.2)	5,033 (29.1)	7,665 (24.7)
Antidiabetics (*n* (%))	1,452 (10.6)	1,336 (7.7)	2,788 (9.0)
UA lowering drugs (*n* (%))	1,371 (10.0)	239 (1.4)	1,610 (5.2)
Aspirin or antiplatelets (*n* (%))	1,102 (8.0)	1,188 (6.9)	2,290 (7.4)

Mean±SD. SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; UA, uric acid.

demographic data, physical data (height and body weight), history of hypertension, and use of antihypertensive drugs; BP values and pulse rate; complications and medical history (renal disease, hepatic disease, cerebrovascular disease, coronary heart disease, endocrine/metabolic disease, and other diseases); laboratory test results (complete blood count, biochemistry tests, and urinalysis); lifestyle modification if performed (physical exercise, restriction of alcohol consumption or salt intake, ceasing smoking, weight loss, *etc.*); and electrocardiograph findings.

The following patient information was recorded in the worksheets and collected every year after the start of losartan-based antihypertensive treatment: adverse events, clinic BP values, pulse rate, heart rate, weight, daily dose of losartan, concomitant drugs, laboratory tests, and ECG (if performed).

The clinic BP was measured by the routine method at each institution. At each time of measurement, one clinic BP value was reported at the discretion of the physician. The clinic BP data measured at a maximum of 3 different visits prior to starting losartan therapy was used for calculation of the mean baseline clinic BP. After starting losartan therapy, the clinic BP value was measured every 3 months. The clinic BP data thus obtained were used for analysis of the clinic BP values during treatment.

The home BP was measured during the study by patients who voluntarily agreed to monitor their BP themselves. Home BP was measured with an electronic automated sphygmomanometer based on the cuff-oscillometric principal (HEM-740A; Omron Healthcare Co., Ltd., Kyoto, Japan). Patients who had already been using another device and insisted on continuing its use were permitted to do so. Patients were asked to measure the home BP at rest in the sitting position once every morning just after waking and urinating, and before medication. Home BP was measured once at one opportunity of measurement. If the patient measured home BP twice or more at one opportunity, the first measured value was reported. Home BP values obtained prior to the start of losartan therapy were used to calculate the mean baseline home BP. As a rule, morning home BP values measured each month, usually on the day of attending hospital, were used for analysis of the mean home BP value during treatment.

Standard laboratory tests (including ECG recording) were performed with the routine methods used at each institution, so standardization of measuring methods and reference values was not carried out. A maximum of 2 results of standard laboratory tests measured prior to losartan therapy were used to calculate the baseline values. After the start of losartan therapy, standard laboratory tests were performed every 6 months.

To assess the complications and the medical history, physicians judged the existence of diseases indicated in the registration form prior to the start of the study at their discretion.

In addition, the patients who were receiving drug treatment for hyperlipidemia or diabetes mellitus and met the definition of either disease indicated in the relevant guidelines were defined as having hyperlipidemia or diabetes.

All adverse events were recorded by the investigators and were classified as definitely related, possibly related, or defi-

Table 3. Distribution of Age and WHO Hypertension Grade, and Mean Blood Pressure at Baseline

	Men (N=12,698)		Women (N=16,250)		Total (N=28,948)	
	n (%)	SBP/DBP (mmHg)	n (%)	SBP/DBP (mmHg)	n (%)	SBP/DBP (mmHg)
Age (years old)						
20–39	556 (4.4)	160.1±16.0/102.2±11.3	261 (1.6)	163.5±16.7/101.9±10.6	817 (2.8)	161.2±16.3/102.1±11.1
40–59	5,518 (43.5)	163.5±16.7/100.1±10.4	5,494 (33.8)	167.1±18.2/97.9±10.5	11,012 (38.0)	165.3±17.6/99.0±10.5
60–79	6,029 (47.5)	165.5±17.0/92.9±11.1	8,963 (55.2)	165.3±16.8/90.6±10.7	14,992 (51.8)	165.4±16.9/91.5±10.9
≥80	595 (4.7)	165.4±18.9/87.0±11.5	1,532 (9.4)	166.0±18.4/85.7±12.1	2,127 (7.4)	165.9±18.5/86.0±12.0
BP classification						
Optimal to High-normal	349 (2.7)	130.2±8.4/77.0±8.2	499 (3.1)	130.3±8.3/75.7±8.6	848 (2.9)	130.3±8.3/76.2±8.5
Grade 1	3,282 (25.9)	149.9±6.7/89.1±7.5	4,349 (26.8)	150.6±6.1/86.7±8.1	7,631 (26.4)	150.3±6.4/87.7±7.9
Grade 2	6,027 (47.5)	164.2±8.6/95.9±8.5	7,619 (46.9)	165.9±7.2/92.8±9.1	13,646 (47.1)	165.2±7.9/94.1±8.9
Grade 3	3,040 (23.9)	184.3±15.7/106.6±12.0	3,783 (23.3)	188.3±14.5/102.0±12.6	6,823 (23.6)	186.5±15.2/104.1±12.5

Mean±SD. BP, blood pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure.

nitely unrelated to losartan, or as unknown. All losartan-related adverse events were pooled and classified as adverse drug reactions (ADRs).

Endpoint Evaluation

The primary endpoint of the study was a composite of cardiovascular events, including fatal or non-fatal stroke (new occurrence or recurrence of cerebral hemorrhage, cerebral infarction, or subarachnoid hemorrhage diagnosed on the basis of typical clinical symptoms persisting for more than 24 h and/or computerized tomography/magnetic resonance imaging findings), transient ischemic attack defined as a focal neurological deficit presumed to be vascular in origin persisting for less than 24 h, fatal or non-fatal myocardial infarction (new occurrence or recurrence) diagnosed on the basis of typical clinical symptoms, ECG changes and elevation of cardiac enzymes, or sudden cardiac death. In addition, the independent event classification committee reviewed adjudicated endpoint events on the basis of all available information documented in the case report form by the physicians.

Statistical Considerations

Determination of the Sample Size

When performing life-table analysis combined with the log-rank test, a 30% difference in the incidence of the primary endpoint (stroke, transient ischemic attack, acute myocardial infarction, or sudden cardiac death) was assumed between a subgroup of patients that represented 60% of the total population with higher BP and the remaining patients with lower BP. The incidence of stroke, myocardial infarction and sudden cardiac death in the Japanese population is 6.8/1,000 patient-years in men and 4.8/1,000 patient-years in women according to the Hisayama study (*18*), and the mean follow-up period for the J-HEALTH was 2.7 years. Thus, a total of 28,000 patients were required to detect the assumed between-group difference with a 90% power at α=0.05 (2-sided). Therefore, the target sample size was set at 30,000 patients.

Statistical Analysis

For the present interim analysis, variables were compared using the t-test, the χ^2 test, or analysis of variance (ANOVA). Results were expressed as the mean±SD, and differences were considered statistically significant at $p<0.05$.

Statistical analysis of the overall results was based on survival analysis. Subgroups were classified by the BP values at baseline or during treatment. Differences between subgroups were assessed by the log-rank test or the χ^2 test. Relationships between the endpoints and the BP values, as well as prognostic factors, were assessed by using the Cox proportional hazards model with adjustment for gender, age, alcohol drinker, current smoker, coexisting of cardiovascular disease, cerebrovascular disease, diabetes mellitus, and hyperlipidemia. For analysis of safety data, the number of ADRs, drug-related ADRs and other ADRs were calculated. For efficacy analysis, the antihypertensive effect of losartan with respect to both clinic BP and home BP values was assessed, and subgroup analyses were performed as described for the safety analysis. Comparison of safety and efficacy among the subgroups was performed with the χ^2 test, t-test, or ANOVA. Results were expressed as the mean±SD and a 2-sided $p<0.05$ was considered statistically significant. Statistical analysis was conducted with the SAS package (version 8.02; SAS Institute Inc., Cary, USA).

Organization

The organization and the members of the committees of the J-HEALTH study are given in the Appendix. These committees were responsible for performing the study or analyzing the data. The Monitoring Committee determined the validity of continuing the study based on the safety and effectiveness of losartan therapy from an ethical point of view. The Event Assessment Committee reviewed the events of cerebrovascular disease and coronary heart disease reported during the

Table 4. Lipid Profiles at Baseline

	Men (N=13,737)	Women (N=17,311)	Total (N=31,048)
Hyperlipidemia (n)	4,935	7,005	11,940
TC (mg/dL)	216.4±37.2	230.2±35.3	224.4±36.7
HDL-C (mg/dL)	51.5±15.5	59.2±16.3	56.0±16.4
TG (mg/dL)	214.2±142.9	159.9±96.3	182.8±121.3
Without hyperlipidemia (n)	8,802	10,306	19,108
TC (mg/dL)	189.3±29.0	199.5±29.6	194.7±29.8
HDL-C (mg/dL)	56.5±14.5	61.1±15.0	58.9±14.9
TG (mg/dL)	126.8±89.1	110.0±58.4	117.8±74.7

Mean±SD. TC, total cholesterol; HDL-C, high density lipoprotein-cholesterol; TG, triglycerides.

study. The Safety Assessment Committee assessed the causal relationship between the ADRs that are reported and the drugs that are administered during the study. The Medical Expert Advisory and Publication Committee was responsible for reviewing the results and writing the paper.

Results

Baseline Patients' Characteristics

Between June 2000 and December 2001, patients were screened in all 47 prefectures throughout Japan. The number of patients enrolled in this study per prefecture ranged from 165 in Okinawa to 2,667 in Tokyo. The distribution of patient enrollment was similar to the recent Japanese population statistics (*19*), and there were no major regional differences of BP values among the prefectures (data not shown).

A total of 31,515 patients were screened at 3,755 institutions by 4,149 investigators. Among them, 31,048 patients were enrolled in this study and 467 patients were excluded according to the exclusion criteria shown in Table 1 or withdrew their consent before actual enrollment. The baseline characteristics of the 31,048 enrolled patients (13,737 men [44.2%] and 17,311 women [55.8%]) are summarized in Table 2. The mean age of the patients was 62.4±12.1 years and the mean clinic systolic/diastolic BP (SBP/DBP) values were 165.3±17.3/94.3±11.7 mmHg.

Concomitant medications and complications are also listed in Table 2. All complications and ECG abnormality were diagnosed by the study investigators. The prevalences of hyperlipidemia, diabetes mellitus, cardiovascular disease, cerebrovascular disease, and ECG abnormality were 38.5%, 13.1%, 8.0%, 4.4%, and 14.0% respectively. Subjects taking anti-diabetic agents or lipid-lowering drugs were defined as having diabetes or hyperlipidemia, respectively.

Table 3 shows distributions of age groups and grade in the World Health Organization (WHO), and the mean BP values at baseline. Young patients (20–39 years) accounted for 2.8%, middle-aged patients (40–59 years) accounted for 38.0%, and elderly patients (60–79 years) made up 51.8% of the total patients. It is worth noting that there were 2,127 (7.4%) very elderly patients (≥80 years). The age distribution was generally similar between men and women. Then we analyzed the BP values of each age group. As shown in Table 3, the SBP values increased with age, but the difference was relatively small. On the other hand, the DBP values decreased markedly with age. Grade 2 hypertensive patients (based on the WHO classification) were most frequent in our cohort (n=13,646, 47.1%), while the numbers of grade 1 and 3 patients were almost equal (n=7,631, 26.4% *vs.* n=6,823, 23.6%, respectively).

The mean total cholesterol (TC) level of all patients was 209.6 mg/dL, while the mean TC levels of hyperlipidemic patients (n=11,940) and non-hyperlipidemic patients (n=19,108) were 224.4 mg/dL and 194.7 mg/dL, respectively. Details of the lipid profile are given in Table 4.

A total of 11,135 patients agreed to measure their BP values at home. Although data were limited at the time of enrollment (n=9,182), the scatter plot (Fig. 1) demonstrates the relationship between clinic BP and home BP for both the SBP and DBP values. The Pearson's correlation coefficients were 0.62 and 0.69, respectively. "White-coat hypertension" (WCHT) was defined by the following criteria: clinic SBP ≥140 or clinic DBP ≥90 mmHg and home SBP <135 and home DBP <85 mmHg. Based on these criteria, 4.2% of our patient cohort had so-called WCHT.

Discussion

The JSH 2004 have been published and updated periodically based on mainly Western epidemiological and clinical results (*5*). Although many large-scale investigational studies have been conducted worldwide to explore the management of hypertension (*3, 4*), it is difficult to determine which studies are best applicable to each individual case in clinical practice. Therefore, it is very important to clarify the characteristics, clinical effects, and safety profiles of various drugs in clinical practice. Many studies with Japanese hypertensives have been conducted, but these have usually employed small cohorts in rural areas. Practical information from large-scale investigational studies in clinical practice is limited in Japan (*20*). The J-HEALTH is a large-scale (30,000 patients)

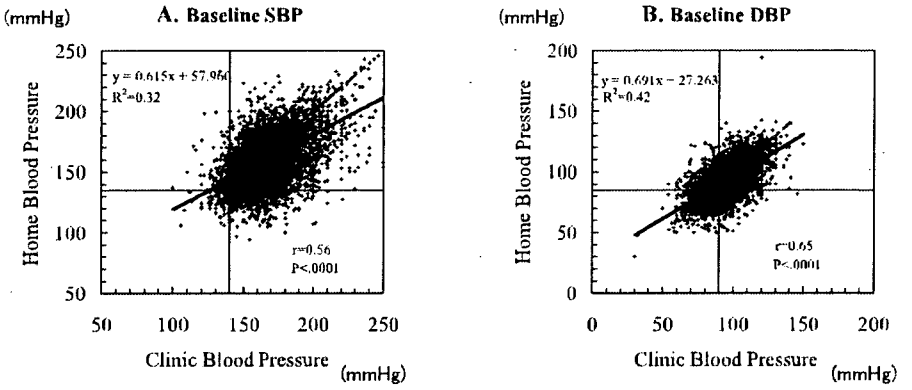

Fig. 1. *Scattered diagrams of clinic and home blood pressure at baseline (n=9,182). Each patient's clinic and home blood pressure was plotted for SBP (A) and DBP (B) with regression equations. SBP, systolic blood pressure; DBP, diastolic blood pressure.*

nationwide multicenter observational study, which may provide us valuable epidemiological information on Japanese patients with hypertension.

The distribution of the hypertensive patients enrolled in the J-HEALTH study was similar to that of the recent Japanese population statistics (*19*). Thus, the patients in this study can be regarded as being representative of the overall Japanese population. Geographical differences in the prevalence of hypertension have previously been noted in Japan (*21*), with a higher incidence in the primarily rural northern part of Japan and a lower rate in the Western region (*5*). One of the reasons for the higher BP values in rural northern Japan is the high salt intake of the local population. However, no major regional differences of the mean clinic BP values were observed among the prefectures in the J-HEALTH study. This may reflect recent lifestyle changes and/or the widespread acceptance of antihypertensive therapy in Japan.

It is well known that vascular mortality increases with age, but the contribution of BP values to vascular mortality differs between age groups. The Prospective Studies Collaboration has published a meta-analysis of individual data for one million adults in 61 prospective observational studies of BP values and mortality (*22*). Although the proportional difference in the risk of vascular death is associated with an absolute difference in BP values, the proportional difference in vascular mortality is only about half as large at 80–89 years compared with that at 40–49 years. Therefore, the age of the subjects is an essential factor when analyzing study endpoints. The mean age of the J-HEALTH cohort is 62.4±12.1 years. On the other hand, the mean age was 67 years in the LIFE study (*7*). This age difference between randomized trials and actual clinical practice should be taken into consideration when applying the results of randomized trials.

The risks and benefits of treatment with antihypertensive agents are uncertain in patients older than 80 years (*23*). Gueyffier *et al.* suggested that antihypertensive treatment could prevent stroke, major cardiovascular events, and heart failure, but not cardiovascular death based on a meta-analysis of data from 1,670 patients aged 80 years or older (*24*). The Hisayama study suggested that the Sixth Report of the Joint National Committee on the Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC-6) recommendations were not applicable to elderly Japanese persons over 80 years of age (*25*). Many very elderly hypertensive patients (≥80 years) were enrolled (595 men and 1,532 women) in the J-HEALTH study. Such a large number of elderly Japanese has not been studied before, so the results of the J-HEALTH study should be informative for this age group.

Of course, the BP is the most important characteristic of the patients in this study. The mean clinic SBP/DBP values of the J-HEALTH cohort were 165.3/94.3 mmHg. In contrast, the mean SBP/DBP values were 174.4/97.8 mmHg in the LIFE study. Based on their age and mean BP values, the J-HEALTH cohort is younger and has milder hypertension compared with the subjects of the LIFE study (*7*). Therefore, the J-HEALTH study may be able to assess the beneficial effects of ARB-based treatment for relatively low-risk hypertensive patients, who are the most common type encountered in clinical practice in Japan.

Not only the mean BP value itself, but also the grade of hypertension, is an important factor to be taken into consideration when evaluating a large-scale study. Grade 2 hypertension is the most frequent type in the J-HEALTH study population (47.1%). In their sub-analysis of the Hisayama study, Arima *et al.* excluded treated hypertensive patients and followed up 588 cardiovascular disease-free residents who were at least 60 years of age for about 30 years (from 1961 to 1993) to evaluate their cardiovascular risk. Among these patients, BP grade 1, 2, and 3 accounted for 27.2%, 18.6%, and 14.1%, respectively (they included normal BP and high normal BP subjects). Since the Hisayama study was an observational investigation of the general population, the hyperten-

sion stage distribution of the Hisayama population is different from that of the J-HEALTH cohort (*25*).

Complications represent another important background factor. The prevalences of ECG abnormality, cardiovascular disease, and cerebrovascular disease are 14.0%, 8.0%, and 4.4%, respectively, in the J-HEALTH cohort. These rates are lower than those in the LIFE study, again indicating that the J-HEALTH enrolled healthier subjects than the LIFE (*7*). The J-HEALTH cohort includes a high percentage of hyperlipidemic patients (38.5%). However, as shown in Table 4, the mean TC level of these hyperlipidemic patients is not extremely high, possibly because 24.7% of all the patients were taking lipid-lowering drugs (Table 3).

It has been emphasized that home BP values measured by ambulatory blood pressure (ABP) monitoring or self-measurement can be an important tool for the optimal management of hypertension with respect to cardiovascular risk (*26*). In the Pressioni Alteriose Monitorate e Loro Associazioni (PAMELA) study of 2,051 subjects who were representative of the general population, the clinic BP, home BP, and 24-h ABP values were measured. This study demonstrated that the risk of death increased more with a given increase in home BP or ABP than clinic BP values (*27*). Den Hond *et al.* investigated the diagnostic values of self-measured BP *vs.* ABP, and concluded that the specificity and sensitivity of ABP values for detecting WCHT were better than those of home BP values (*28*). While ABP values have better prognostic accuracy, the American Society of Hypertension Ad Hoc Panel recommends the use of home BP values for screening. Self-measurement of the BP is easy to repeat and is useful for patients to assess their own control (*29*). Hozawa *et al.* investigated the BP measured by home, ambulatory, and conventional methods in 1,174 Japanese subjects (150 with untreated hypertension, 399 with treated hypertension, and 625 normotensives). They also concluded that it was useful to measure the non-clinic BP values (*30*). We therefore determined the distribution and relation between clinic BP and home BP values at enrollment (Fig. 1).

WCHT is diagnosed by comparing the clinic BP and non-clinic BP values, and whether it causes target organ damage and cardiovascular events is one of the most important issues in the treatment of hypertension (*31*). The Ohasama study examined the prognostic significance of WCHT based on ABP monitoring, and concluded that the predictive power of the ABP values for subsequent mortality was stronger than that of the clinic BP values (*28*). The prevalence of WCHT is 4.2% in the J-HEALTH cohort, but the reported prevalence of WCHT in other studies varies widely because of differences in the definition of WCHT, method of BP measurement (self-measurement *vs.* ABP monitoring), and characteristics of the study population (untreated hypertension *vs.* treated hypertension). Masked hypertension (MHT) is also a topic of interest for antihypertensive therapy. The Self-Measurement of Blood Pressure at Home in the Elderly: Assessment and Follow-up (SHEAF) study demonstrated that about 9% of treated elderly patients had MHT and that the cardiovascular risk associated with MHT is significantly high (*32*).

A total of 9,182 patients measured their home BP values at the baseline in the J-HEALTH cohort. Therefore, we will use their data to identify WCHT and MHT and investigate the effect of home BP data on cardiovascular events. The home BP data obtained by self-measurement will display the time course effect of antihypertensive management and provide prognostic information for the hypertensive population.

The J-HEALTH study began in June 2000, and follow-up was completed in December 2005. The J-HEALTH study will clarify the long-term antihypertensive efficacy and safety of losartan-based therapy, and assess its preventive effect on hypertension-related diseases. It may provide new insights into therapeutic strategies for Japanese hypertensive patients.

Appendix

J-HEALTH Committees

Monitoring Committee: Takenori Yamaguchi (Chair), Tanenao Eto, Toshiharu Furukawa, Katsumi Yoshida.

Event Assessment Committee: Hiroaki Naritomi (Chair), Yoichiro Hashimoto, Uichi Ikeda, Mitsuaki Isobe, Toshio Kushiro, Ken Nagata, Kazuyuki Shimada, Takemori Yamawaki.

Safety Assessment Committee: Kendo Kiyosawa (Chair), Hiroshi Hirose, Sadayoshi Ito, Akinori Kasahara, Hiroshi Kawabe, Genjiro Kimura, Hirofumi Makino, Mitsuhiko Moriyama, Ikuo Saito, Hiromichi Suzuki, Eiji Tanaka.

Medical Expert Advisory and Publication Committee: Hiroaki Naritomi (Chair), Toshiro Fujita, Sadayoshi Ito, Toshio Ogihara, Kazuyuki Shimada, Kazuaki Shimamoto, Heizo Tanaka, Nobuo Yoshiike.

The Administrative Office: The Post-Marketing Surveillance Department of Banyu Pharmaceutical Co., Ltd. (Tokyo, Japan).

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Extremely Early Computed Tomography Signs in Hyperacute Ischemic Stroke as a Predictor of Parenchymal Hematoma

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Key Words
Acute stroke diagnosis · Computed tomography · Hemorrhagic transformation · Intracerebral hemorrhage · Ischemic stroke

Abstract
Background: In acute ischemic stroke, thrombolysis with intravenous recombinant tissue plasminogen activator is more effective when given within 90 min of onset compared to that given later than 90 min. However, the significance of early CT signs (ECTs) during such early periods has not yet been fully clarified. We investigated the usefulness of ECTs within 90 min for predicting parenchymal hematoma (PH) in patients without thrombolysis. ***Methods:*** We evaluated 212 consecutive patients with initial ischemic stroke in the anterior cerebral circulation who underwent the first CT within 6 h of onset. The patients were divided into 3 groups according to the interval from onset to CT: within 90 min (group A, n = 90), 91–180 min (group B, n = 76) and 181–360 min (group C, n = 46). Patients who had received thrombolytic therapy were excluded. ECTs were evaluated according to the Alberta Stroke Program Early CT Score (ASPECTS). The relationships between ECTs and the subsequent development of PH were compared among the groups. ***Results:*** In patients with ASPECTS values between 0 and 7, PH was developed more frequently in group A (35%) than in groups B (14%) or C (15%) (group A vs. B: p = 0.036, group A vs. C: p = 0.094). In group A, atrial fibrillation, elevated pretreatment blood pressure and ASPECTS ≤7 were independent predictors of PH. ***Conclusions:*** The manifestation of ECTs as represented by ASPECTS ≤7 within 90 min after stroke appears to indicate a high risk of PH.

Introduction

In hyperacute ischemic stroke, thrombolysis with intravenous recombinant tissue plasminogen activator (rt-PA) was found to improve the 90-day functional outcome when given within 3 h of symptom onset in the National Institute of Neurological Disorders and Stroke (NINDS) trial [1]. On the other hand, the frequency of intracranial hemorrhage (ICH) was shown to increase significantly in stroke patients treated with thrombolysis [1–3]. Thus, the pretreatment predictors of ICH are currently one of the most important issues in clinical practice. At present, computed tomography (CT) is the most widely available and convenient technique for evaluating stroke, and the prediction of ICH using CT would be exceedingly valuable. The European Cooperative Acute Sroke Study (ECASS) investigators suggested that baseline early CT signs (ECTs), according to the so-called one third rule, could be predictive of ICH [2]. However, there was no reference to ECTs in the NINDS trial [1]. The subanalysis of NINDS data could not confirm the relationship between ECTs and ICH either, although they found such a ten-

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dency that symptomatic hemorrhages develop more commonly in patients whose ECTs extend over more than one third of the middle cerebral artery territory [4]. In addition, a recent meta-analysis of 6 major intravenous thrombolytic therapy trials suggests that the benefit of rt-PA is greater if started within 90 min compared to that later than 90 min [5]. However, the significance of ECTs in such very early periods has not yet been fully clarified.

Recently, a new scoring system was developed to better locate and semiquantify the early ischemic changes on CT: the Alberta Stroke Program Early CT Score (ASPECTS) [3]. This semiquantitative scoring method improved the interobserver agreement ratio [6] and facilitated the detection of earlier and more subtle changes [7]. We hypothesized that the time from onset to the appearance of ECTs would correlate with the severity of cerebral ischemia and the frequency of subsequent parenchymal hematoma (PH). We investigated the usefulness of ECT evaluation using ASPECTS within 90 min for predicting the risk of spontaneous PH in the following acute or subacute phase in patients without thrombolysis.

Patients and Methods

Patients

Among 1,679 patients with ischemic stroke admitted to the stroke care unit of the National Cardiovascular Center, Japan, between January 1998 and June 2005, we retrospectively evaluated 655 consecutive patients who underwent the first CT within 6 h after onset. We excluded patients with a history of symptomatic stroke or intracranial surgery. We also excluded patients with unclear onset including stroke at awakening and those with infarction limited to the posterior circulation alone. Thirty-five patients who had received thrombolysis (intravenous or intra-arterial) were also excluded. In Japan, the use of intravenous rt-PA for acute ischemic stroke was approved in October 2005, by which time the present study had been completed. Therefore, during the study period, thrombolytic therapy was performed only in patients who were enrolled in other clinical trials. Consequently, 212 consecutive patients with initial ischemic stroke in the anterior cerebral circulation were enrolled in this study. The 212 patients were divided into 3 groups according to the interval from onset to the first CT: within 90 min (group A, n = 90), 91–180 min (group B, n = 76) and 181–360 min (group C, n = 46). Anticoagulants and/or antiplatelet agents were administered to the patients in case the attending physicians considered these therapies were sufficiently safe and valuable.

CT Studies

All patients underwent noncontrast CT scans using an X-Vigor (Toshiba, Tokyo) on arrival. The scanning parameters were nonhelical, 120 kV, 170 mA, 10-mm collimation, matrix size of 512 × 512 and 2- or 3-second scan time. Routine photography was performed at window level and a width of 30 and 70–80 Hounsfield units (HU), respectively. Follow-up CTs were performed at 24 h and 7–14 days after stroke, as well as at the discretion of treating neurologists and reviewed in this study when performed within 14 days. All baseline CTs and follow-up CTs were retrospectively evaluated by 2 expert neurologists (S.O. and H.M.) with knowledge of the side of the affected hemisphere but blinded to treatment assignment, stroke severity or other clinical details. ECTs were scored according to ASPECTS [3, 6]. ASPECTS was assessed by scoring 10 regions (caudate nucleus, internal capsule, lentiform nucleus, insula and 6 cortical regions) systematically on the CT scan. A score of 1 was assigned for normal and 0 for a region showing ECTs. A normal CT scan has ASPECTS values of 10 points. Score 0 indicates diffuse ischemia throughout the territory of the middle cerebral artery. The ASPECTS study group suggested that baseline ASPECTS values in 2 categories (≤7 and >7) discriminated poor and good functional outcome, or high and low risk of ICH [3]. Therefore, we compared the incidence of ICH between a group with ASPECTS ≤7 and one with ASPECTS >7 in each period.

ECTs were defined as X-ray hypoattenuation, loss of the gray-white boundary or effacement of cortical sulci [3, 6]. ICH was classified as hemorrhagic infarction and PH; hemorrhagic infarction was defined as petechial infarction without space-occupying effect, and PH was defined as hemorrhage with mass effect. PH was subdivided into PH1, as blood clots in ≤30% of the infarcted area with some slight space-occupying effect, and PH2, as blood clots in >30% of the infarcted area with a substantial space-occupying effect, corresponding to ECASS II criteria [2]. In cases of disagreement in the findings, these 2 observers reviewed the CTs together and discussed the findings until a consensus was established.

Clinical Evaluation

Risk factors for ischemic stroke (i.e. age, sex, hypertension, diabetes mellitus, hyperlipidemia, atrial fibrillation and smoking) were evaluated in all patients. According to the exclusion criteria of NINDS [1], we defined 'elevated pretreatment blood pressure' as systolic blood pressure >185 mm Hg or diastolic blood pressure >110 mm Hg. Because the second analysis of NINDS showed that patients >75 years of age or with National Institutes of Health Stroke Scale (NIHSS) scores >20 were associated with poor outcome and high risk of ICH [9], we decided on the cutoff points of age >75 years and NIHSS score >20 on univariate analysis. The trial of ORG10172 in Acute Stroke Treatment criteria [10] were used to classify stroke subtypes.

Data Analysis

We determined the predictive value of ASPECTS referring to the findings of follow-up CT obtained at 24 h as a standard. ECTs were judged 'true positive' if a definite infarct was correctly located on the follow-up CT. If it was absent on the second CT, the reading was 'false positive'. The readings were judged 'true negative' if both baseline and follow-up CT did not reveal any infarcted changes. The readings were defined as 'false negative' if ECTs were not detected on the baseline CT and a definite infarct was visible on the follow-up CT. On the basis of these definitions, we assessed the predictive value in each of the 10 regions in each patient, then summed the results all together and calculated the sensitivity and specificity.

Table 1. Baseline characteristics of the 3 groups classified by time from stroke onset to initial CT

Variables	Group A (0–90 min)	Group B (91–180 min)	Group C (181–360 min)	p value
Patients	90	76	46	
Age, years	71 ± 11	70 ± 11	70 ± 12	0.783
Male	61 (68)	56 (74)	31 (67)	0.655
Hypertension	64 (71)	54 (71)	31 (67)	0.889
Diabetes mellitus	21 (23)	22 (29)	15 (33)	0.480
Hyperlipidemia	27 (30)	26 (34)	17 (37)	0.690
Atrial fibrillation	45 (50)	37 (49)	22 (48)	0.968
Smoking	25 (28)	23 (30)	12 (26)	0.588
Pretreatment, mm Hg				
Systolic blood pressure	162 ± 26	160 ± 26	171 ± 25	0.072
Diastolic blood pressure	87 ± 15	87 ± 13	90 ± 14	0.377
Elevated pretreatment blood pressure	20 (22)	12 (16)	9 (20)	0.578
NIHSS scores, median	12	9	6	0.097
1–5	20 (22)	24 (32)	21 (46)	
6–10	22 (24)	18 (24)	10 (22)	
11–15	14 (16)	7 (9)	5 (11)	
16–20	20 (22)	19 (25)	6 (13)	
>20	14 (16)	8 (11)	4 (9)	
ASPECTS, median	9	8	8	0.111
≤7	34 (37)	35 (46)	20 (44)	

Categorical values are expressed as numbers with percentages in parentheses, continuous variables as means ± SD. The χ^2 test was conducted for categorical variables, ANOVA for continuous variables and the Kruskal-Wallis test for scoring variables.

Statistical Analysis

Continuous data are expressed as means ± SD. For analysis of baseline characteristics, categorical variables were compared by χ^2 test, continuous variables by 1-way ANOVA and scoring variables by Kruskal-Wallis test. For univariate analysis of the predictors of PH, categorical variables were compared by χ^2 test, continuous variables by unpaired t test and scoring variables by Mann-Whitney U test. Multivariable analysis was undertaken using a logistic regression model. The results were considered significant at $p < 0.05$. Dr. SPSS II for Windows 11.0.1J was used for all calculations.

Results

Two hundred and twelve patients (148 men, 64 women) with ages ranging from 41 to 101 years (70 ± 11) were enrolled into this study. The stroke subtype was cardiogenic embolism in 118 patients, large-artery atherosclerosis in 29, small-vessel disease in 16 and other or unknown etiology in 49. As shown in table 1, the baseline characteristics were not significantly different between groups A, B and C.

ECTs were positive in 142 (67%) of the 212 patients. The predictive value for any ECTs with ASPECTS in each group was as follows: sensitivity 60% and specificity 95% in group A, sensitivity 79% and specificity 91% in group B, and sensitivity 75% and specificity 90% in group C. PH was observed in 25 (12%) of the 212 patients. PH1 occurred in 14 patients and PH2 in 11. There were no differences in baseline CT findings and clinical characteristics between PH1 and PH2. Antithrombotic agents were used in 188 patients (89%) within 2 weeks from onset; 143 patients (68%) were treated with intravenous heparin and 79 patients (37%) with antiplatelet agents. Among the 188 patients with antithrombotic therapy, only 15 (8%) developed PH. On the other hand, 10 (42%) of the 24 patients without antithrombotic therapy developed PH. Figure 1 shows the proportion of PH by ASPECTS category in each group. When the ASPECTS values were between 0 and 7, PH was developed in 12 (35%) of the 33 patients in group A, showing a significantly higher frequency as compared with group B (14%) or group C (15%) (group A vs. group B: $p = 0.036$, group A vs. group C: $p = 0.094$).

Table 2 details the univariate analysis of predictors of PH within 90 min. Atrial fibrillation, elevated pretreatment blood pressure, NIHSS score and ASPECTS ≤7

Table 2. Univariate analysis of PH predictors in cases of CT examination within 90 min

Variables	PH (16 patients)	no PH (74 patients)	p value	Odds ratio
Age	73 ± 11	70 ± 11	0.33	
>75 years	7 (44)	24 (32)	0.40	1.62 [0.54, 4.87]
Male	12 (75)	49 (66)	0.57	1.53 [0.45, 5.24]
Hypertension	14 (88)	50 (68)	0.14	3.36 [0.71, 15.98]
Diabetes mellitus	6 (38)	15 (20)	0.19	2.36 [0.74, 7.53]
Hyperlipidemia	5 (31)	22 (30)	1.00	1.07 [0.33, 2.65]
Smoking	8 (50)	39 (53)	1.00	0.90 [0.30, 2.65]
Atrial fibrillation	13 (81)	32 (43)	0.011	5.69 [1.49, 21.66]
Elevated pretreatment blood pressure	7 (44)	13 (18)	0.042	3.65 [1.15, 11.58]
NIHSS scores, median	16.5	10	0.006	
>20	4 (25)	10 (14)	0.26	2.13 [0.57, 7.93]
ASPECTS, median	5	9	<0.001	
≤7	12 (75)	21 (28)	0.001	7.36 [2.14, 25.33]

Categorical values are expressed as numbers with percentages in parentheses, continuous variables as means ± SD. Figures in square brackets are 95% confidence limits. Elevated pretreatment blood pressure is defined as systolic blood pressure >185 mm Hg or diastolic blood pressure >110 mm Hg. Antiplatelet therapy includes aspirin, cilostazol, ticlopidine, argatroban and sodium ozagrel. Anticoagulant therapy includes heparin and warfarin.

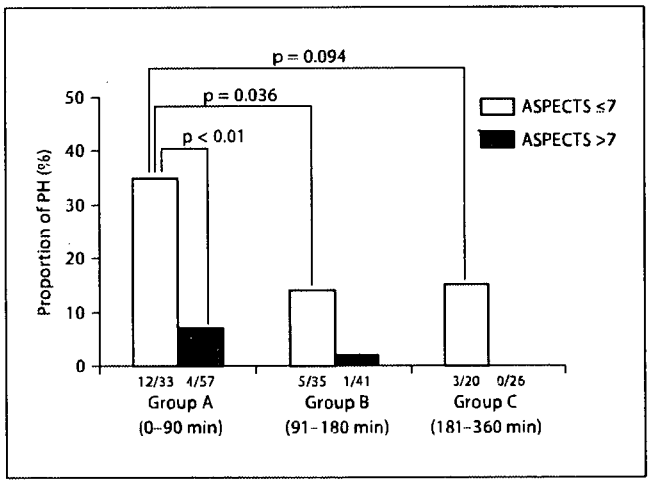

Fig. 1. Incidence of PH after ischemic stroke by categories of time from stroke onset to CT, comparing ASPECTS ≤7 with ASPECTS >7. Values are numbers of patients with PH divided by the total patients in each group (n/n).

were associated with PH. Multivariate logistic regression analysis for prediction of PH is presented in table 3. ASPECTS ≤7, elevated pretreatment blood pressure and atrial fibrillation were independent predictors of PH within 90 min of onset.

Table 3. Predictors of PH in cases of CT examination within 90 min: multivariable logistic regression analysis

	p value	OR	95% CI
NIHSS (per 1 score increase)	0.308	1.05	0.95–1.17
ASPECTS ≤7	0.013	6.14	1.47–25.66
Elevated pretreatment blood pressure	0.022	5.45	1.28–23.25
Atrial fibrillation	0.045	5.02	1.03–24.37

Elevated pretreatment blood pressure is defined as systolic blood pressure >185 mm Hg or diastolic blood pressure >110 mm Hg. OR = Odds ratio; CI = confidence interval.

Discussion

We conducted the present study to evaluate the clinical significance of ECTs in the hyperacute phase of ischemic stroke and found that the manifestation of ECTs within 90 min after stroke indicates a high risk of subsequent PH. We evaluated PH as a type of ICH which may be more closely related with symptomatic deterioration than hemorrhagic infarction [11]. In our study, PH was observed in 12% of the patients. The frequency of PH was higher than that in ECASS II (3.1%) [2]. Okada et al. [12] reported that PH was found in 10% of 160 Japanese patients with acute

cerebral embolism. Because our study enrolled more patients with cardiogenic embolism than the ECASS II trial, the frequency of PH may become higher. Another explanation for the dissociation of PH frequency between our study and ECASS II is the racial difference in blood coagulation-fibrinolysis factors, such as fibrinogen and factor XII, between Japanese and Caucasians [13].

Several factors related to ECTs were reported to be important. CT density decreases linearly over time, describing the course of water uptake after ischemia [14]. The severity of perfusion impairment also correlates with the degree of CT density reduction [15]. In a recent study using single-photon emission CT, the time from onset to CT and residual cerebral blood flow were independent factors that contributed to the presence of ECTs [16]. Another study using positron emission tomography also shows that ECTs reflect the coupling of the severity of ischemia [17]. These findings suggest that the earlier the manifestation of ECTs, the severer the depth of brain ischemia. ECTs in the extremely early stage likely indicate severe ischemic brain damage associated with poor collaterals, leading to damage to the integrity of the blood-brain barrier [18]. Disruption of the blood-brain barrier contributes to the risk of ICH [19]. Our study showed that the sensitivity of ECTs was relatively low during the initial 90 min, however, the specificity was sufficiently high in each period. These findings were remarkably similar to the results of the study which assessed the significance of X-ray hypoattenuation at CT using the ECASS II population [20]. Careful detection of these subtle findings in the very early periods of ischemic stroke may facilitate the prediction of ICH.

The dichotomized score of ASPECTS 0–7 versus 8–10 was previously validated and shown to have prognostic value among patients treated with intravenous rt-PA for acute ischemic stroke within 3 h of onset [3, 6]. Recently, Dzialowski et al. [21] examined 800 patients within 6 h of stroke onset in the subanalysis of ECASS II and reported that ASPECTS ≤ 7 was a substantially increased risk of thrombolysis-related PH. These results are compatible with our study showing that in patients without thrombolysis, ASPECTS ≤ 7 was an independent predictor of PH, a sign of poor prognosis.

Several previous studies of intravenous rt-PA therapy suggested that ECTs were not associated with increased risk of ICH [4, 22]. These studies did not describe the interval between onset and CT, and the relationship between the ECT manifestation and the subsequent hemorrhagic transformation was not clarified. However, Demchuk et al. [22] concluded on the basis of such results that there was no evidence of treatment effect modification from the baseline ASPECTS value by thrombolysis. Data from animal models and human clinical trials have demonstrated that earlier stroke treatment is associated with better outcome [5, 23, 24]. There is a possibility that thrombolysis in the very early stage of ischemic stroke may diminish the disruption of the blood-brain barrier and reduce the risk of ICH.

The present study has several limitations. First, it was designed prior to the government approval of intravenous rt-PA in Japan. Therefore, we could not compare the neuroradiological and functional outcome between patients with thrombolysis and those without in spite of the fact that such a comparison may allow us to definitively define a subgroup of patients receiving benefit from thrombolysis. Second, we defined the ECTs according to ASPECTS criteria which include parenchymal hypoattenuation and brain swelling. There is increasing evidence that brain swelling without parenchymal hypoattenuation may be reversible [25]. On the other hand, brain swelling theoretically can represent early stages in a process that manifests finally as PH [26]. The actual relationship between brain swelling and PH remains unclear.

In conclusion, the manifestation of ECTs as represented by ASPECTS ≤ 7 within 90 min after stroke appears to indicate a high risk of parenchymal hemorrhage even when thrombolysis was not performed. Whether or not thrombolysis is indicated for such cases should be clarified in the future by a large-scale study.

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Delayed Postischemic Treatment With Fluvastatin Improved Cognitive Impairment After Stroke in Rats

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Background and Purposes—Recent clinical evidences indicate that statins may have beneficial effects on the functional recovery after ischemic stroke. However, the effect of delayed postischemic treatment with statins is still unclear. In the present study, we evaluated the effects of fluvastatin in the chronic stage of cerebral infarction in a rat model.

Methods—Rats exposed to permanent middle cerebral artery occlusion were treated for 3 months with fluvastatin beginning from 7 days after stroke. MRI, behavioral analysis, and immunohistochemistry were performed.

Results—Two months of treatment with fluvastatin showed the significant recovery in spatial learning without the decrease in serum total cholesterol level and worsening of infarction. Microangiography showed a significant increase in capillary density in the peri-infarct region in fluvastatin-treated rats after 3 months of treatment. Consistently, BrdU/CD31-positive cells were significantly increased in fluvastatin-treated rats after 7 days of treatment. MAP1B-positive neurites were also increased in the peri-infarct region in fluvastatin-treated rats. In addition, rats treated with fluvastatin showed the reduction of superoxide anion after 7 days of treatment and the reduction of Aβ deposits in the thalamic nuclei after 3 months of treatment.

Conclusions—Thus, delayed postischemic administration of fluvastatin had beneficial effects on the recovery of cognitive function without affecting the infarction size after ischemic stroke. Pleiotropic effects of fluvastatin, such as angiogenesis, neuritogenesis, and inhibition of superoxide production and Aβ deposition, might be associated with a favorable outcome. (***Stroke***. **2007;38:3251-3258.**)

Key Words: angiogenesis ■ cerebral infarct ■ microcirculation ■ statins

Despite conflicting data correlating cholesterol level with stroke, 2 early trials of HMG-CoA reductase inhibitors (statins) in patients after myocardial infarction patients showed a reduction in stroke risk as a secondary end point.[1] A meta-analysis of 9 statin intervention trials, which enrolled patients with coronary artery disease or those at high risk for coronary disease, demonstrated a 21% relative risk reduction for stroke after 5 years of treatment.[2] Another clinical evidence suggests that the commencement of statins within 4 weeks of a stroke results in a favorable 90-day outcome.[3] To clarify the effects of postischemic statin treatment, previous studies in which atorvastatin was started 1 day after stroke in rodents showed improvement of sensory motor deficit through induction of angiogenesis, neurogenesis, and synaptogenesis.[4,5] These pleiotropic effects of statins were shown to be the result of induction of vascular endothelial growth factor or brain-derived neurotrophic factor.[4] Additionally, the microvascular dysfunction in the posttreatment of stroke with recombinant human tissue-type plasminogen activator could be reduced by statins in rodent model.[6] However, the effect of delayed treatment with statins after ischemic stroke is still unknown. From this viewpoint, we investigated whether chronic statin treatment beginning 7 days after ischemic stroke had influences on neurological deficits and pathophysiology after the permanent middle cerebral artery occlusion (MCAo) model in rats.

Materials and Methods

Surgical Procedure

Male Wistar rats (270 to 300 grams; Charles River; Kanagawa, Japan) were used in this study. The right MCA was occluded by placement of poly-L-lysine–coated 4-0 nylon, as described previously.[7]

Protocol for Treatment and Behavioral Tests

Ten rats were only anesthetized (sham operation) and 32 rats were subjected to MCAo (day 1). Based on neuromuscular function on day 7, the rats were divided equally into saline-treated (n=16) or fluvastatin-treated (n=16) groups. Fluvastatin (5 mg/kg per day;

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provided by Novartis Pharma) or saline was given by gavage from day 7 to 100. We chose the dose (5 mg/kg per day), because a previous report showed that this dose could effectively induce angiogenesis in ischemic limb.[8] On day 55, neuromuscular function and locomotor activity were evaluated in the surviving rats. Then, cognitive function was examined by Morris water maze from day 56 to 63, because the effects of neuronal regeneration could be detected not in the early stage but in the chronic stage of ischemic brain such as 49 to 53 days after the insult.[9] On day 96, MRI was performed. On day 100, microangiography was performed.

MRI

High-resolution T1-weighted fast spin echo sequence images (repetition time [TR]=1500 ms; echo time [TE]=10.3 ms; field of view [FOV]=4× 3 cm; matrix=256×192; slice thickness=1.5 mm; slice gap=0.5 mm; number of slices=16; number of excitation=10; total time=9.39 min) were obtained using a 3-T MRI scanner (Signa LX VAH/I; GE).

Sensory Motor Deficit and Locomotor Activity

Although there are various batteries for testing sensory motor deficit, we used a simple protocol.[10] For forelimb flexion, rats were held by the tail on a flat surface. Paralysis of the forelimbs was evaluated by the degree of left forelimb flexion. For torso twisting, rats were held by the tail on a flat surface. The degree of body rotation was checked. For lateral push, rats were pushed either left or right. Rats with right MCA occlusion showed weak or no resistance against a left push. For hind limb placement, one hind limb was removed from the surface. Rats with right MCA occlusion showed delayed or no replacement of the hind limb when it was removed from the surface.

Spontaneous activity was measured via the open field (0.69 m^2). We set the sensor, which also put beams on the field, at 30 cm above the field. The number of count, which is when the animal crosses the beam, was measured for 30 minutes.

Morris Water Maze Task

A cylindrical tank 1.5 m in diameter was filled with water (25°C), and a transparent platform 15 cm in diameter was placed at a fixed position in the center of 1 of the 4 quadrants (O'Hara & Co, Ltd). In the hidden platform trials, we performed the tests 4 times per day for 4 days. When the rat could not reach the platform, the latency was set at 60 sec. In the visible platform trials, the tests were performed 4 times per day for 4 days. The acquired data were averaged per day.

Evaluation of Capillary Density

Using a recently developed microangiographic technique,[11] capillary density and blood–brain barrier leakage were evaluated in the cerebral cortex after MCA occlusion. The area or length of vessels was analyzed with an angiogenesis image analyzer (version 1.0; Kurabo).

Immunohistochemical Study: Bromodeoxyuridine Labeling

To identify newly formed DNA, saline-treated (n=5) and fluvastatin-treated (n=5) rats received injections of bromodeoxyuridine (BrdU, 50 mg/kg; Sigma-Aldrich, Saint Louis, Mo) intraperitoneally starting on day 7 twice per day until day 13. Rats were euthanized on day 14. After the sections (8-μm thickness) was fixed in 10% formaldehyde/MeOH neutral buffer solution and blocked, they were incubated with mouse monoclonal anti-rat CD31 antibody (1:100; BD Biosciences; San Jose, Calif), goat polyclonal anti-doublecortin (anti-DCX; Santa Cruz) antibody (1:100; Santa Cruz, Calif), mouse monoclonal anti-NeuN antibody (1:1000; Chemicon, Temecula, Calif), or mouse monoclonal anti-MAP1B antibody (1:100; Sigma-Aldrich), followed by anti-mouse goat fluorescent antibody (1:1000 for NeuN and MAP1B, 1:400 for CD31, Alexa Flour 546, Molecular Probes; Eugene, Ore) or anti-goat donkey fluorescent antibody (1:1000 for DCX Alexa Fluor 546). For double immunostaining, these sections were fixed again and incubated in 2 N HCl at 37°C for 30 minutes. After blocking, they were incubated with rat monoclonal anti-BrdU antibody (1:200; Abcam, Cambridge, UK) followed by anti-rat goat fluorescent antibody (1:1000, Alexa Fluor 488). For immunohistochemical staining for Aβ, sections were pretreated for 30 minutes with hot (85°C) citrate buffer as described before.[12] Confocal images were acquired using an FV-300 (Olympus).

Table. Infarction Volume Calculated by MRI, Blood Pressure, and Serum Total Cholesterol

	Sham	MCAo+S	MCAo+F	*P*
Infarction volume in total rats (mm^3)	...	283.8±23.9	278.4±26.4	0.851
Type of infarction in Figure 1a (N of rats)				0.828
A	...	12	11	...
B	...	3	3	...
C	...	1	2	...
Infarction volume (mm^3) in type A rats	...	322.8±15.0	327.0±18.8	0.758
Systolic blood pressure (mm Hg) in type A rats				
Day 7	116.1±5.4	123.7±6.0	115.5±7.3	0.654
Day 56	146.5±4.7	148.3±2.7	136.1±5.2	0.132
Serum total cholesterol (mg/dl) in type A rats on day 56	85.9±5.6	75.3±3.5	73.5±2.7	0.949

Type A, low-intensity area seen in the dorsolateral and lateral portions of the neocortex and the entire caudate putamen; type B, low-intensity area seen in the dorsolateral and lateral portions of the neocortex and in part of the caudate putamen; type C, low-intensity area seen in part of the lateral neocortex and caudate putamen. MCAo+S, saline-treated rats after MCAo; MCAo+F, fluvastatin-treated rats after MCAo.

P, saline vs fluvastatin.

Quantitative Histological Analysis

To quantify the immunoreactivity for MAP1B and Aβ, the acquired image was analyzed by Image J (version 1.32; NIH).

Detection of Superoxide Anion in Brain Sections

Superoxide anion was detected on day 14 as described previously.[13] Because intact cortex showed red fluorescence, we calculated the ratio of fluorescence as follows: ratio of fluorescence=[fluorescence intensity in ischemic core or peri-infarct region]/[fluorescence intensity in intact region].

Statistical Analysis

All values are expressed as mean±SEM. To analyze the differences in the type of cerebral infarction, χ^2 test was performed. The latency, path length, and mean speed in Morris water maze and sensory motor deficits were analyzed by a 2-factor repeated-measure ANOVA. Post hoc analyses were performed, and the Scheffe test was applied to control the inflation in type I error. The value of the serum total cholesterol, the blood pressure, and the spontaneous activity was analyzed by Scheffe rules. The differences in the immunohistochemistry and the volume of infarction were assessed by Mann–Whitney *U* analyses. In all cases, $P<0.05$ was considered significant.

Results

Effects of Fluvastatin on Cognitive Impairment

To confirm the severity of cerebral infarction, all rats were examined by T1-weighted MRI after 89 days of treatment. Although the total volume of infarction calculated in T1-weighted images was not different between rats treated with

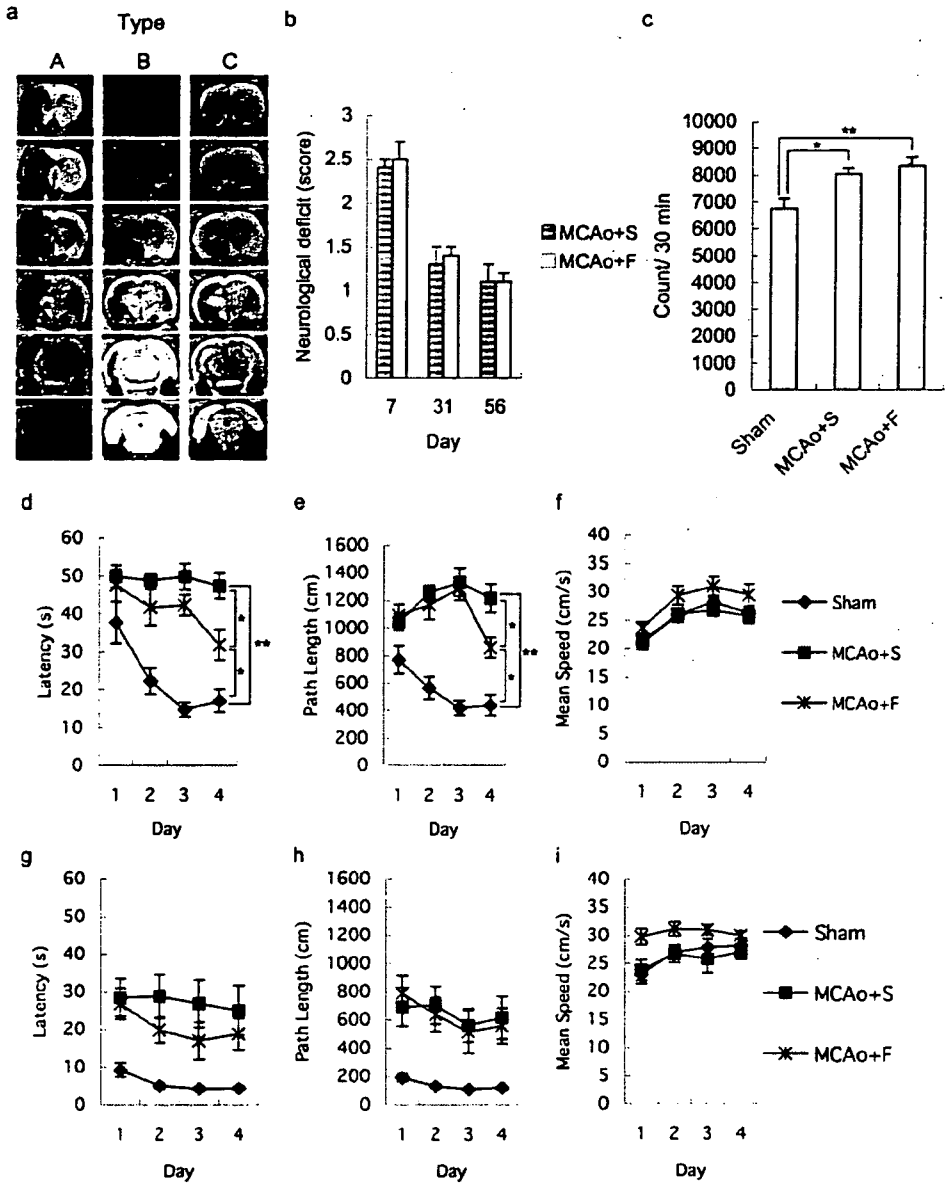

Figure 1. Typical T1-weighted image of coronal section of rat brain (a). The images were divided into 3 groups. Type A, low-intensity area seen in the dorsolateral and lateral portions of neocortex and the entire caudoputamen; type B, low-intensity area seen in the dorsolateral and lateral portions of neocortex and in part of the caudatoputamen; and type C, high-intensity area seen in part of the lateral neocortex and caudatoputamen. Sensory motor deficit (b). Spontaneous locomotor activity (c). Hidden platform test in Morris water maze. Each figure showed latency (d), path length (e), and mean speed (f). Days 1 to 4 indicate the trial day in the hidden platform test (56 to 59 days after middle cerebral artery occlusion). Visible platform test in Morris water maze. Each figure showed latency (g), path length (h), and mean speed (i). Days 1 to 4 indicate the day in the visible platform test (60 to 63 days after middle cerebral artery occlusion). MCAo+S indicates rats treated with saline after middle cerebral artery occlusion; MCAo+F, rats treated with fluvastatin after middle cerebral artery occlusion.

saline and fluvastatin (Table), the pattern of cerebral infarction was divided into 3 groups: type A, low-intensity area seen in the dorsolateral and lateral portions of the neocortex and the entire caudate putamen; type B, low-intensity area seen in the dorsolateral and lateral portions of the neocortex and in part of the caudate putamen; type C, low-intensity area seen in part of the lateral neocortex and caudate putamen (Figure 1a). In type C, most of the lateral neocortex was intact. To exclude the influence of the pattern of cerebral infarction on cognitive function, we focused on type A rats in the present study. The volume of cerebral infarction in type A rats was not different between the groups (Table). Blood pressure and serum total cholesterol also showed no difference among the groups (Table).

Sensory motor deficit had spontaneously recovered to some extent by 8 weeks in both groups, and there was no difference (Figure 1b). Locomotor activity in rats subjected to MCAo was increased as compared with that in sham-operated rats, as described before,[14] but there was no significant difference between fluvastatin-treated and saline-treated rats (Figure 1c). In Morris water maze (Figure 1d-i), which examines spatial learning, there were significant differences

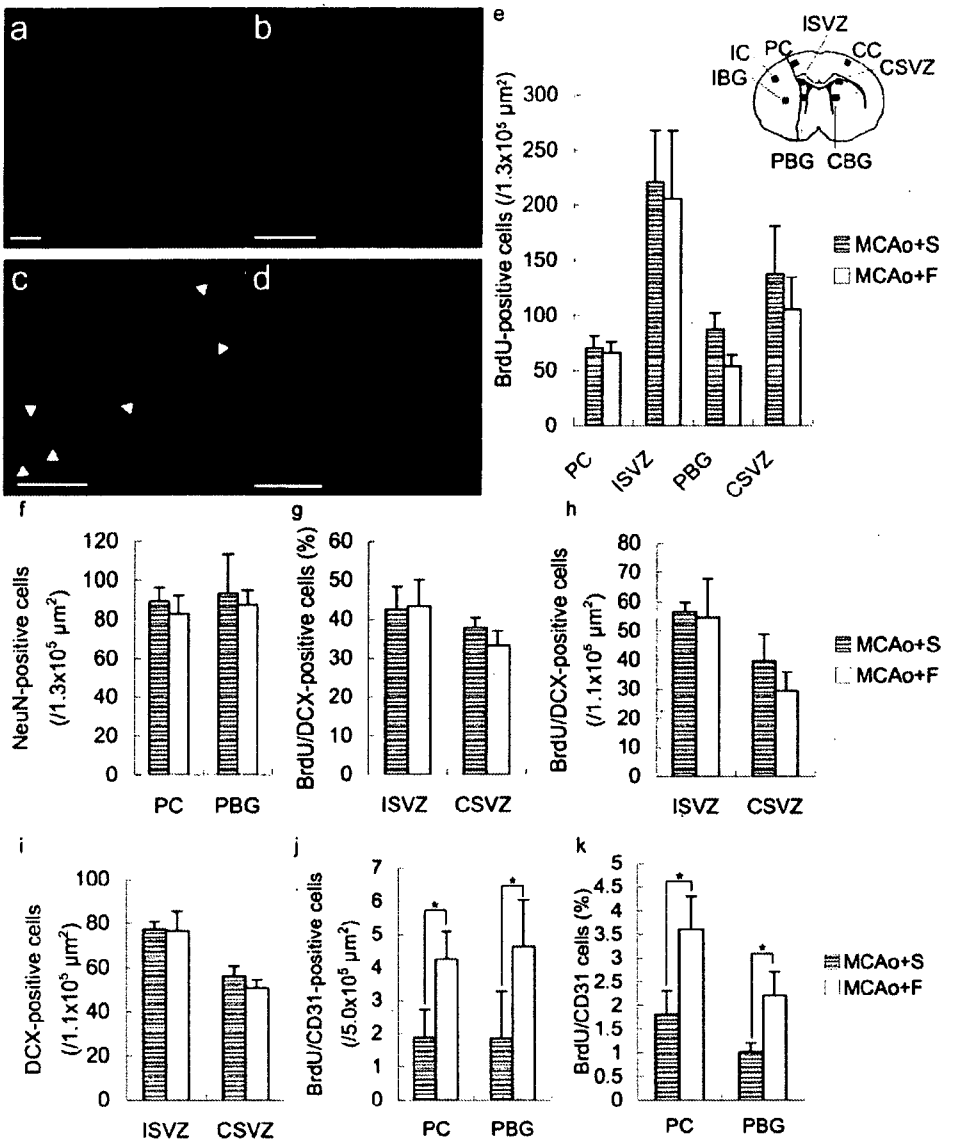

Figure 2. Representative images of immunohistochemical staining on day 14. Rats treated with fluvastatin (a through c), rats treated with saline (d). Although BrdU-positive cells were observed in the peri-infarct cortex (a), peri-infarct basal ganglia, and subventricular zone, these cells did not express NeuN (a), but expressed DCX in the subventricular zone (b). Fluvastatin-treated rats showed some BrdU/CD31-positive cells (arrows, c), although most BrdU-positive cells were negative for CD31 in saline-treated rats (d). The number of BrdU-positive cells (e), NeuN-positive cells (f), BrdU/DCX-positive cells (h), DCX-positive cells (i), and BrdU/CD31-positive cells (j); the percentage of BrdU/DCX-positive cells (g) or BrdU/CD31 cells (k) in total BrdU-positive cells. PC indicates peri-infarct cortex; PBG, peri-infarct basal ganglia; IC, infarcted cortex; IBG, ischemic basal ganglia; ISVZ, subventricular zone on infarcted side; CC, contralateral cortex, CSVZ, subventricular zone on contralateral side; CBG, contralateral basal ganglia ($n=5$ in each group, $^*P<0.05$, bar=100 μm).

in the latency and path length in hidden platform test among the groups (supplemental Table I, available online at http://stroke.ahajournals.org). A significant difference was observed on day 4 between fluvastatin-treated and saline-treated rats (supplemental Table I). Also, there was a significant difference between sham and saline-treated rats (supplemental Table I). There was no significant difference both in swimming speed and visible platform test, which excluded the possible influence of visual loss, sensory motor deficit, and motivation on the results.[15] These data suggest that impaired spatial learning was improved by fluvastatin.

Histological Changes by Fluvastatin

Next, we studied whether fluvastatin had some influences on the histology. Initially, we focused on neurogenesis and angiogenesis. To examine neurogenesis, we measured BrdU-incorporated cells after injecting BrdU from day 7 to day 13. Although BrdU-positive cells were observed in the subventricular zone and peri-infarct region (Figure 2a to 2d), the total number did not differ between the groups (Figure 2e). Similarly, the density of NeuN-positive cells, as a marker of adult neurons, also did not differ between the groups (Figure 2f), whereas there were no BrdU/NeuN-positive cells in the

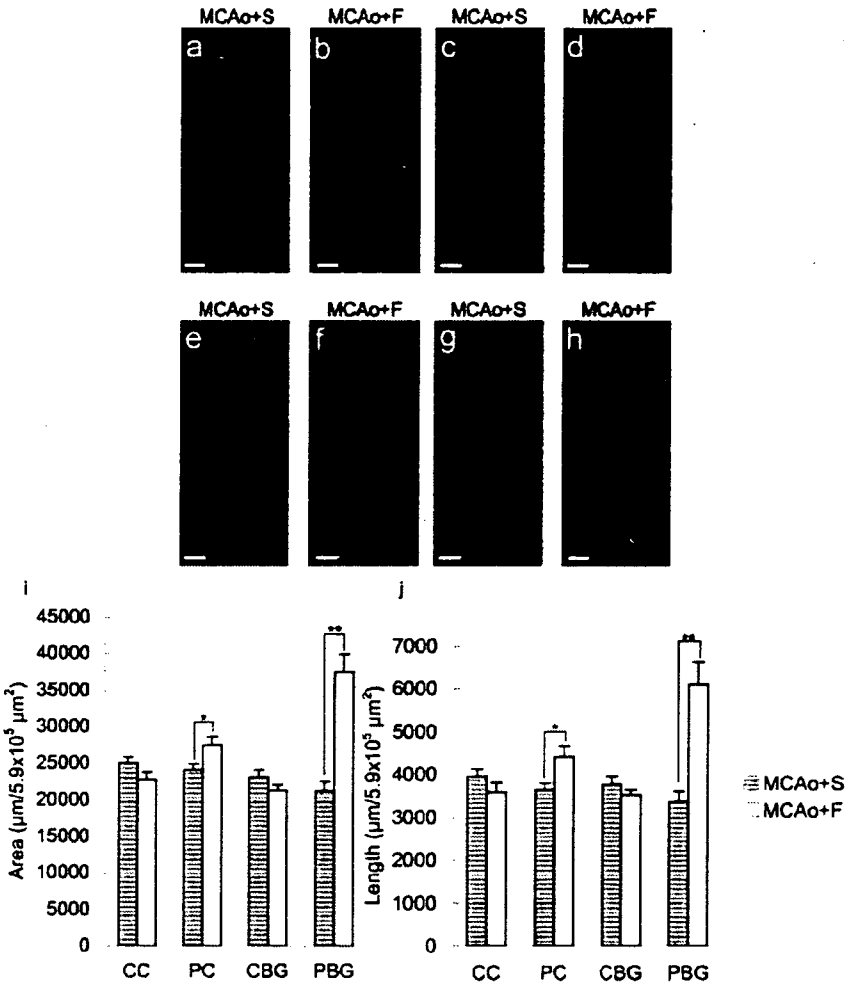

Figure 3. Microangiographic images using albumin-fluorescence isothiocyanate on day 100: (a and b) peri-infarct cortex; (c and d) contralateral cortex; (e and f) peri-infarct basal ganglia; (g and h) contralateral basal ganglia (bar=100 μm). Quantitative analysis (i and j) of microangiography. Rats treated with fluvastatin showed increased microvessels in the peri-infarct region (n=4 in each group, $^{*}P<0.05$, $^{**}P<0.01$).

peri-infarct cortex and subventricular zone (Figure 2a). Although some BrdU-positive cells expressing DCX, a marker for migrating neuroblasts, could be detected in subventricular zone (Figure 2b), the percentage in total BrdU-positive cells (Figure 2g) and the number (Figure 2h) did not differ between the groups. Also, the number of DCX-positive cells was same in the both groups (Figure 2i). There were no BrdU-positive cells expressing DCX in the cerebral cortex. Unexpectedly, these data suggest that neurogenesis was not enhanced by fluvastatin.

Thus, we further examined whether angiogenesis was affected by fluvastatin. In the peri-infarct cortex and basal ganglia, BrdU-positive cells that were positive for CD31 as a marker of endothelial cells could be detected (Figure 2c,2d). The number of BrdU/CD31-double-positive cells was significantly increased in fluvastatin-treated rats (Figure 2j). The percentage of BrdU/CD31-double-positive cells in total BrdU-positive cells was also increased in fluvastatin-treated rats (Figure 2k). Consistently, microangiography using FITC-conjugated albumin[11] also showed that microvessels were significantly increased in fluvastatin-treated rats only in the peri-infarct cortex and basal ganglia, without destruction of the blood–brain (Figure 3a to 3h). Quantitative analysis showed that the length and area of microvessels were also increased in the peri-infarct region, but not in the contralateral cortex and contralateral basal ganglia, in rats treated with fluvastatin, at 3 months after stroke (Figure 3i,j).

Because recent reports showed that neurite outgrowth was observed in the peri-infarct region from 7 to 14 days after cerebral infarction,[16,17] we next examined the effect of fluvastatin on neurite outgrowth. Immunohistochemical staining showed that treatment with fluvastatin significantly increased the immunoreactivity of MAP1B, a marker of neurite outgrowth, in neurites[16,18] (Figure 4), although the number of MAP1B-positive cells was the same in both groups. These data implied that the fluvastatin might promote angiogenesis, resulting in improvement of the microcirculation, and neurite outgrowth.

One possible explanation for the enhanced angiogenesis and neurite outgrowth is a decrease in oxidative stress by fluvastatin. To assess oxidative stress, we evaluated superoxide production using dihydroethidium staining (Figure 5a to 5e). Superoxide anion was increased in the ischemic core as compared with the contralateral region at 2 weeks after MCA occlusion (Figure 5a,5c). However, rats treated with fluvastatin showed a significant reduction in superoxide anion especially in the ischemic core region, but not in the peri-infarct cortex and basal ganglia (Figure 5b,5d,5e).

Finally, we examined Aβ deposition in the thalamic nuclei, because previous reports showed that Aβ deposits in the

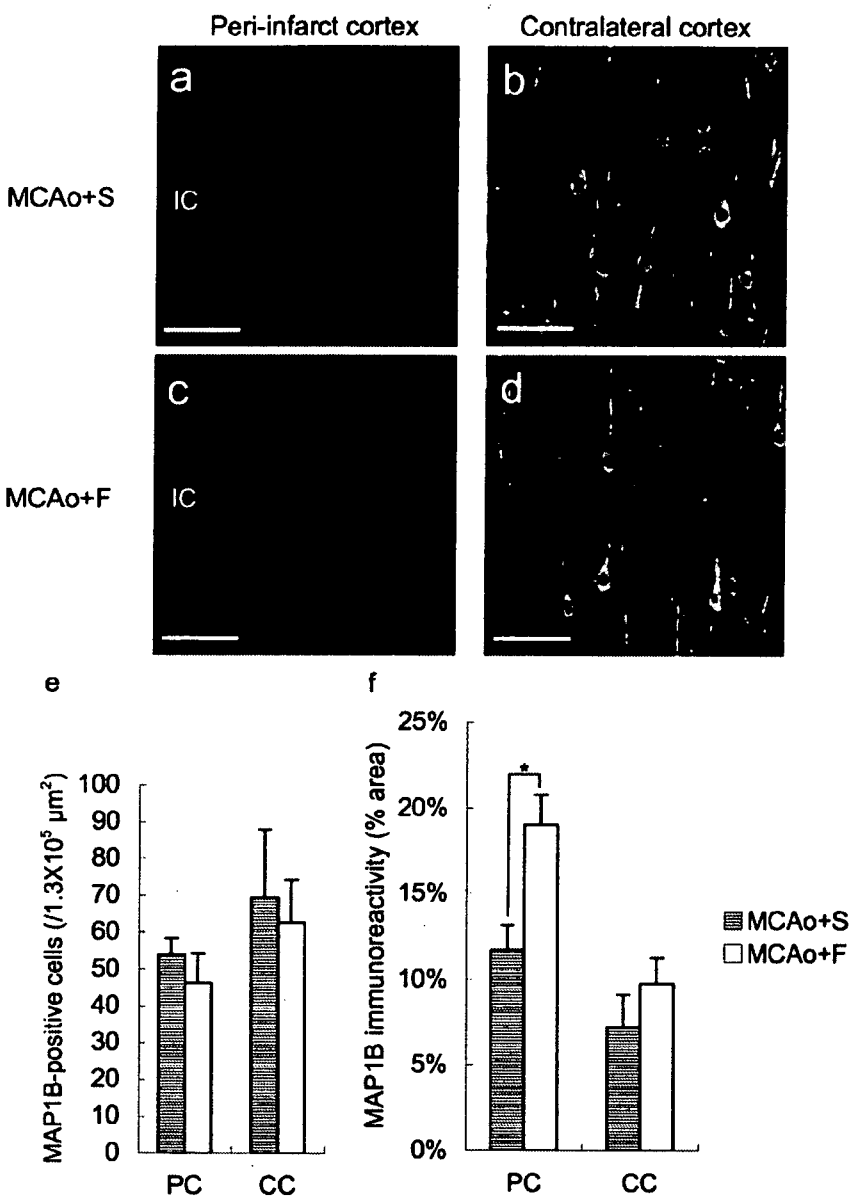

Figure 4. Typical images of immunohistochemical staining for MAP1B in peri-infarct cortex (a and c) and contralateral cortex (b and d) on day 14 (bar=100 μm). Although the number of MAP1B-positive cells was the same in both groups (e), immunoreactivity was higher in the peri-infarct region in fluvastatin-treated rats (f) (n=4 in each group, $^*P<0.05$).

thalamic nuclei persisted as long as 9 months after focal cerebral ischemia.[12] Although immunohistochemical staining showed marked deposition of Aβ in the ventrolateral and ventromedial thalamic nuclei at 3 months after stroke, the area of Aβ deposits was significantly decreased in fluvastatin-treated rats (Figure 5f to 5h). In other regions, such as cortex or basal ganglia, there was no Aβ deposits in both groups as reported before.[12]

Discussion

Although several laboratories have shown that long-term pretreatment with a statin reduces infarct size in rodents,[19] no articles have reported the effects of delayed postischemic treatment with statins. The present study demonstrated that statin treatment beginning 7 days after ischemic stroke resulted in significant improvement of spatial learning at 8 weeks after stroke, without any change in the plasma cholesterol level and infarct size.

Fluvastatin-treated rats showed a significant increase of MAP1B in neurites in the peri-infarct region. Considering that MAP1B is especially prominent in extending neurites[20] and related to functional recovery after ischemic stroke,[17] one of the possible effects of fluvastatin is to enhance neurite outgrowth, "neuritogenesis," in the early stage of treatment. This speculation might be supported by the recent study demonstrating that neurite outgrowth is accelerated by pravastatin via inhibiting the activity of geranylgeranylated proteins such as RhoA.[21]

As BrdU/CD31-positive cells were increased 14 days after MCAo and microvessels were also increased in the peri-infarct region 100 days after MCAo, fluvastatin enhanced angiogenesis and resulted in improvement of microcirculation in the peri-infarct region. Although the relationship between the improved microcirculation and behavior is still unclear, a recent report demonstrated that the restoration of perfusion by collateral growth and new capillaries in the

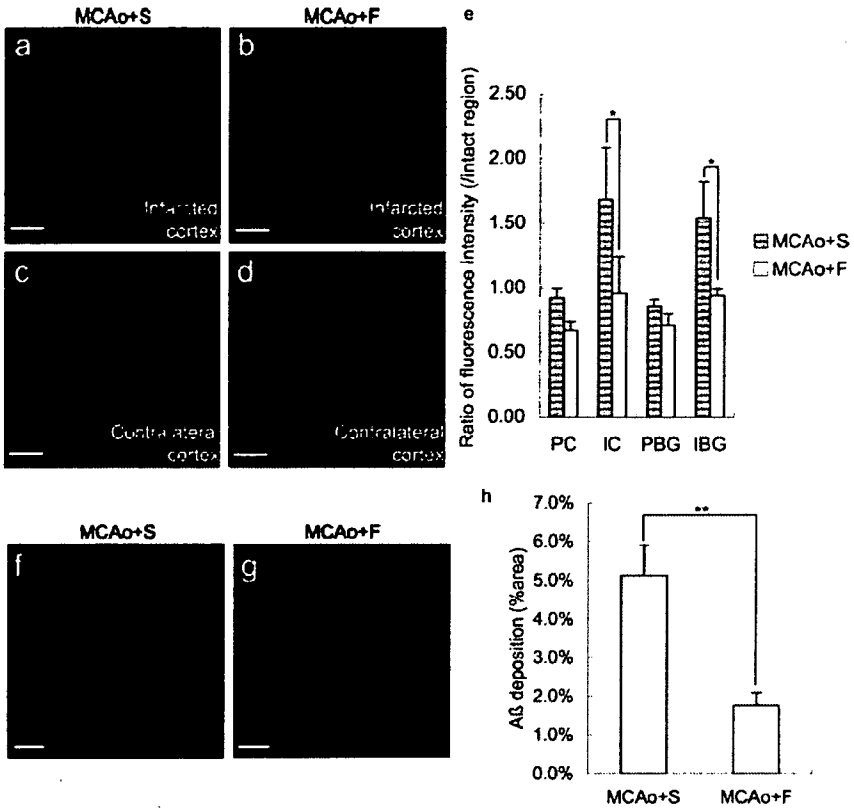

Figure 5. a through e, Superoxide anion detected by dihydroethidium staining on day 14. Red spots show the existence of superoxide anion. Fluorescence intensity was higher in the infarcted cortex (a) compared with the contralateral cortex (c). Fluvastatin-treated rats showed decreased fluorescence intensity in the infarcted cortex (b), although there was no difference in the peri-infarct cortex and basal ganglia (e) (n=4 in each group, $^{*}P<0.05$, bar=100 μm). Deposition of Aβ in thalamus on day 100 after middle cerebral artery occlusion. Although deposition of Aβ was observed in the thalamic nuclei (f and g), there was no deposition in other regions such as the cortex and basal ganglia. Quantitative analysis showed decreased Aβ deposition in fluvastatin-treated rats (h) (n=6 in each group, $^{**}P<0.01$, bar=200 μm).

ischemic border zone around a cortical infarct supported long-term functional recovery in rats.[22] Additionally, others reported that some patients who received tissue plasminogen activator therapy with no immediate clinical improvement despite early recanalization showed delayed clinical improvement.[23] From these viewpoints, it is likely that the improvement of microcirculation is an important factor for the functional recovery.

Of importance, fluvastatin reduced deposition of Aβ in the ventrolateral–ventromedial thalamic nuclei in the chronic stage of ischemic stroke, although rats subjected to focal cerebral ischemia develop deposition of Aβ in the ventroposterior lateral and ventroposterior medial nuclei for as long as 9 months.[12] This might be similar with precious reports showing that statins reduced the production of Aβ in Alzheimer disease.[24] The mechanism of the reduction of Aβ by fluvastatin should be further investigated.

Thus, the rats treated with fluvastatin showed enhancement of angiogenesis and neurite outgrowth in the peri-infarct cortex and reduced deposition of Aβ in the ventrolateral–ventromedial thalamic nuclei. Because those regions are important sites for spatial learning,[25,26] we speculate that the enhancement of functional recovery by fluvastatin might be dependent on those regions.

The other histological difference was the reduction of superoxide anion in the ischemic core in fluvastatin-treated rats. Because cerebral blood flow in the ischemic cortex remained to be reduced for 48 hours and restored to some extent 9 days after permanent MCAo,[27] we speculate that fluvastatin could reach the ischemic core and show the antioxidative effects. On the contrary, in the peri-infarct region, superoxide anion was not detected even in the control group and no effect of fluvastatin might be observed. This effect of statin is similar with the previous report showing that cerivastatin prevented the production of superoxide anion in the cerebral parenchyma in stroke-prone spontaneously hypertensive rats.[28] Also, fluvastatin is reported to possess antioxidative properties in other cells.[29,30]

The association of neurogenesis is also the center of interest, because previous reports showed an increase in neurogenesis after atorvastatin treatment beginning at 1 day after stroke.[5] However, we speculate that neurogenesis might not have contributed to the favorable outcome in the present study, because the volume of infarction was not decreased by fluvastatin, and the density of mature neurons (NeuN-positive cells) and proliferative immature neurons (BrdU/DCX-positive cells) was the same in both groups. From the viewpoints, the timing of treatment seems important for the enhancement of neurogenesis and the beginning of statin 7 days after MCAo might be too late to enhance neurogenesis.

The limitation of the present study is that there is no data demonstrating that fluvastatin crossed over the blood–brain barrier and acted on neurons directly. Blood–brain barrier permeability differs among statins and correlates in part with their respective lipophilicity.[31] Considering that pretreatment with pravastatin and rosuvastatin, whose lipophilicity is 0.84 and 0.33, respectively, shows significant effects on reducing infarction volume,[31] fluvastatin, whose lipophilicity is 1.27, might penetrate blood–brain barrier and have some direct effects on neurons. Otherwise, fluvastatin could penetrate the brain because of the disruption of blood–brain barrier after MCAo. One of other limitations in the present study is no