Table 3
Electrocardiographic parameters during follow-up period

ECG Parameter (leads)	Early Period			Late Period		
	SCN5A-Positive Group (n = 8)	SCN5A-Negative Group (n = 36)	p Value	SCN5A-Positive Group (n = 8)	SCN5A-Negative Group (n = 36)	p Value
Heart rate (beats/min)	66 ± 11	64 ± 10	0.924	60 ± 6	67 ± 12	0.194
P-wave duration (II) (ms)	137 ± 21	110 ± 12	<0.001	155 ± 19†	119 ± 16†	<0.001
PQ interval (II) (ms)	227 ± 31	179 ± 18	<0.001	257 ± 22*	190 ± 22†	<0.001
QRS duration (II) (ms)	125 ± 22	102 ± 18	<0.001	142 ± 41‡	111 ± 19‡	<0.001
QRS duration (V_2) (ms)	135 ± 15	110 ± 13	<0.001	157 ± 28*	115 ± 16	<0.001
QRS duration (V_5) (ms)	130 ± 28	101 ± 15	<0.001	147 ± 42	108 ± 17	<0.001
S-wave duration (II) (ms)	65 ± 38	35 ± 24	<0.001	77 ± 54	43 ± 26‡	<0.001
S-wave duration (V_5) (ms)	69 ± 40	37 ± 19	<0.001	78 ± 50	49 ± 17*	<0.001
S-wave amplitude (II) (mV)	0.37 ± 0.23	0.23 ± 0.24	0.005	0.43 ± 0.24	0.21 ± 0.17	<0.001
S-wave amplitude (V_5) (mV)	0.83 ± 0.47	0.34 ± 0.25	<0.001	0.88 ± 0.48	0.47 ± 0.27†	<0.001
QRS axis (°)	44 ± 81	49 ± 43	0.954	10 ± 76‡	43 ± 41	0.001
QTc interval (II) (ms)	409 ± 37	396 ± 28	0.535	432 ± 40	410 ± 34	0.164
QTc interval (V_2) (ms)	427 ± 51	392 ± 37	0.038	471 ± 38‡	405 ± 38	<0.001
QTc interval (V_5) (ms)	401 ± 43	389 ± 29	0.593	408 ± 39	398 ± 36	0.746
JTc interval (II) (ms)	279 ± 32	290 ± 30	0.554	292 ± 44	293 ± 34	0.100
JTc interval (V_2) (ms)	285 ± 39	279 ± 35	0.960	316 ± 42	283 ± 38	0.044
JTc interval (V_5) (ms)	265 ± 26	286 ± 30	0.108	262 ± 42	283 ± 32	0.105
STJ amplitude (V_2) (mV)	0.42 ± 0.19	0.29 ± 0.13	0.014	0.37 ± 0.23	0.24 ± 0.17	0.011
STJ40 amplitude (V_2) (mV)	0.38 ± 0.14	0.23 ± 0.12	<0.001	0.34 ± 0.17	0.21 ± 0.15	0.006

Data are presented as means ± SD.
* $p < 0.001$ versus early period.
† $p < 0.01$ versus early period.
‡ $p < 0.05$ versus early period.
ECG = electrocardiographic; JTc = corrected JT; QTc = corrected QT; STJ amplitude = ST amplitude at J point; STJ 40 amplitude = ST amplitude 40 ms after J point.

Table 4
Comparison of the change of electrocardiographic parameters during follow-up

Change in ECG Parameter (leads)	SCN5A-Positive Group (n = 8)	SCN5A-Negative Group (n = 36)	p Value
Heart rate (beats/min)	−7 ± 10	3 ± 13	0.046
P-wave duration (II) (ms)	19 ± 12	9 ± 13	0.077
PQ interval (II) (ms)	30 ± 22	11 ± 14	0.004
QRS duration (II) (ms)	17 ± 22	8 ± 15	0.163
QRS duration (V_2) (ms)	22 ± 20	6 ± 11	0.003
QRS duration (V_5) (ms)	17 ± 29	8 ± 14	0.161
S-wave duration (II) (ms)	12 ± 17	8 ± 13	0.423
S-wave duration (V_5) (ms)	9 ± 15	12 ± 14	0.604
S-wave amplitude (II) (mV)	0.06 ± 0.10	−0.02 ± 0.14	0.152
S-wave amplitude (V_5) (mV)	0.05 ± 0.27	0.13 ± 0.18	0.331
QRS axis (°)	−34 ± 55	−6 ± 16	0.010
QTc interval (II) (ms)	22 ± 32	15 ± 34	0.562
QTc interval (V_2) (ms)	44 ± 49	13 ± 40	0.064
QTc interval (V_5) (ms)	6 ± 37	9 ± 30	0.845
JTc interval (II) (ms)	13 ± 27	3 ± 28	0.339
JTc interval (V_2) (ms)	31 ± 48	5 ± 38	0.094
JTc interval (V_5) (ms)	−3 ± 29	−3 ± 29	0.990
STJ amplitude (V_2) (mV)	−0.05 ± 0.18	−0.05 ± 0.12	0.949
STJ40 amplitude (V_2) (mV)	−0.04 ± 0.16	−0.02 ± 0.11	0.642

Abbreviations as in Table 3.

SCN5A mutation.[5,11] Smits et al[12] observed significantly longer PQ and HV intervals at baseline and a larger increase in PQ and QRS intervals after administration of sodium channel blockers in patients with BS with SCN5A mutations than in those without SCN5A mutations. Age-dependent variability in the conduction parameters was evidenced in SCN5A-positive patients with BS.[13,15] Moreover, this concept has been mechanistically investigated in vivo in heterozygous SCN5A mice, which showed progressive impairment with aging of atrial and

ventricular conduction associated with myocardial rearrangements and fibrosis.[16] Meregalli et al[17] showed prolongation of S-wave duration in leads II and III after administration of sodium channel blockers. Their group suggested that these electrocardiographic signs included reciprocal changes in the inferior leads, mirroring the conduction slowing in the RVOT,[17,18] which may progress with aging and relate to the pathogenesis of BS. In the present study, the P-wave, QRS, S-wave durations, and PQ intervals were all significantly longer, and the S-wave amplitude was significantly deeper in the SCN5A-positive group than in the SCN5A-negative group. In addition, the PQ interval and QRS duration in lead V_2 were more markedly prolonged, and the QRS axis deviated more to the left with aging in the SCN5A-positive group than in the SCN5A-negative group during the follow-up period. The results of previous clinical studies and the present study suggest that progressive depolarization abnormalities (i.e., conduction slowing) with aging may play a key role in the pathogenesis of BS.

It has been argued recently that arrhythmic events may occur when a sufficient degree of cell damage has been reached as a result of the severity of ion channel protein mutation. Frustaci et al[19] showed that myocyte apoptosis at the right and left ventricular myocardium was significantly higher in patients with BS with SCN5A mutations than in control subjects on histologic study. They suggested that abnormalities in the function of sodium channels may lead to cellular damage because intracellular sodium homeostasis has a relevant role in myocellular function.[19] Experimentally, Aiba et al[20] used a high-resolution optical mapping system in a pharmacologic BS model and demonstrated that depolarization abnormalities (i.e., conduction slowing) is required for the maintenance of VF in BS, although the initiating premature beats were a result of a phase 2 reentry mechanism. These histologic and experimental studies also support that progressive conduction abnormalities with aging may explain why an initial VF episode appears at middle to older ages, usually 40 to 50 years, in BS. It is generally accepted that SCN5A mutation is not associated with a higher risk of cardiac events, suggesting that genetic analysis is a useful diagnostic parameter but is not helpful for risk stratification.[7] Similarly, in the present study, the presence of SCN5A mutation did not predict subsequent arrhythmic events (Table 2). Most clinical studies have reported that induction of VF by programmed electrical stimulation did not predict the clinical outcome or clinical severity in patients with BS.[6,21,22] If the progressive conduction slowing with aging often observed in patients with BS, especially SCN5A-positve patients, are really linked to VF appearance, conduction parameters, such as QRS widening, late potentials, or inducibility of VF, may still have a potential to predict new or subsequent cardiac events.[23] A much larger patient population is required to make a definitive conclusion regarding the predictive value of SCN5A mutation and the conduction parameters for cardiac events.

Several clinical studies have suggested a localized QT prolongation, a repolarization parameter, in the right precordial leads (mainly lead V_2) in patients with BS.[24,25] Castro Hevia et al[25] have suggested that a QTc >460 ms in lead V_2 was a significant risk factor for subsequent cardiac events. We recently used 87-lead body surface ECGs and reported that a corrected recovery time, another repolarization parameter, was significantly longer in the right precordial body surface ECGs, reflecting the potentials of the RVOT, than in other body surface ECGs.[26] Similarly, in the present study, the longest QTc interval was observed in lead V_2 in most patients with BS with SCN5A mutation, who usually also had a coved-type ST-segment elevation and a terminal negative T wave. The fact that the QTc interval in lead V_2 was significantly longer in the SCN5A-positive patients than in the SCN5A-negative patients at the early and late periods can be explained by more frequent and higher coved-type ST-segment elevation with a terminal negative T wave in the SCN5A-positive patients. The QTc interval in lead V_2 was significantly prolonged from the early period to the late period in the SCN5A-positive patients; however, the JTc interval in lead V_2 did not change from the early period to the late period, suggesting that the significant QTc prolongation in lead V_2 with aging occurred mainly as a result of a significant prolongation of the QRS duration in lead V_2.

There are several limitations to the present study. First, because a small number of patients with BS with SCN5A mutation could be included in a single-center study, a larger number of patients with SCN5A mutation will be required to make a definitive conclusion. Second, the study population included 44 Brugada probands who could be prospectively followed up for average of 10 ± 5 years in our hospital. Therefore, the probands represent a severely affected population, but not a consecutively referred population. Third, Veltmann et al[27] recently reported the prevalence of fluctuations between diagnostic and nondiagnostic ECGs in patients with BS, which may influence the measurement of some electrocardiographic parameters, especially QT, JT interval, and ST amplitude, and should be taken into account. However, the influence of the fluctuations on depolarization parameters such as QRS duration is expected to be less pronounced.

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Effect of Sarpogrelate, a 5-HT_{2A} Antagonist, on Platelet Aggregation in Patients with Ischemic Stroke: Clinical-Pharmacological Dose-Response Study

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Key Words
Antiplatelet therapy · Platelet aggregation · Ischemic stroke · Stroke prevention · Sarpogrelate · Serotonin

Abstract
Background and Purpose: It is widely accepted that antiplatelet therapy is effective for secondary prevention of atherosclerotic vascular diseases. We performed a double-blind, controlled clinical-pharmacological study to investigate the antiplatelet efficacy of sarpogrelate, a selective 5-hydroxytryptamine (5-HT_{2A}) receptor antagonist, in patients with ischemic stroke, using a new assessment system employing combinations of 5-HT and epinephrine as agonists. ***Methods:*** Forty-seven patients with ischemic stroke were randomly assigned to three groups: 15 patients received 25 mg sarpogrelate (group L), 16 patients received 50 mg (group M), and 15 patients received 100 mg (group H) orally, three times daily for 7 days. The effect was expressed as maximum intensity of platelet aggregation on the last day of medication. Two combinations of agonists, 0.5 μmol/l 5-HT plus 3 μmol/l epinephrine, and 1 μmol/l 5-HT plus 3 μmol/l epinephrine, were used to induce platelet aggregation. ***Results:*** With both combinations of agonists, sarpogrelate treatment inhibited platelet aggregation dose-dependently ($p < 0.025$, Jonckheere test). In multiple-group comparison, the effect in group H was greater than that in group L or M ($p < 0.025$, Wilcoxon rank-sum test). ***Conclusion:*** Sarpogrelate treatment inhibited platelet aggregation dose-dependently in patients with ischemic stroke, as judged by a new assessment system employing combinations of 5-HT and epinephrine as agonists.

Introduction

Platelet activation plays an important role in the pathogenesis of atherothrombosis [1–5]. Platelets are activated in vivo by various agonists, such as thromboxane A_2, ADP, and serotonin (5-hydroxytryptamine; 5-HT), and antiplatelet therapy has been developed to block the metabolic or activation pathway related to each of these agonists, with good clinical outcomes in preventing vascular events [6–12]. For instance, aspirin, the first-line antiplatelet agent generally used throughout the world [13], reduces the risk of vascular events by inhibiting the production of thromboxane A_2 [6–10].

Recently, a number of reports have pointed out the importance of 5-HT in the pathogenesis of atherothrombosis [14–17]. 5-HT induces platelet activation, and 5-HT released from intracellular storage sites in activated platelets stimulates smooth muscle cell proliferation with vascular contraction, potentiating thrombus formation and

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vessel occlusion [18]. Furthermore, the finding that plasma 5-HT concentration is higher in patients with coronary artery diseases, diabetes mellitus, stroke and other disorders [14, 16, 19, 20] provides another line of clinical evidence that 5-HT is deeply involved in the development of atherothrombosis. It has been postulated that liberation of 5-HT from nerve terminals, as well as from platelets, results in a local increase in 5-HT concentration [16].

All the findings described above suggest that the 5-HT receptor could be a good target for antiplatelet and antithrombotic therapy. Thus, sarpogrelate {(±)-2-(dimethylamino)-1-[[o-(m-methoxyphenethyl) phenoxy]methyl] ethyl hydrogen succinate hydrochloride}, a selective 5-HT_{2A} receptor antagonist, has been developed as an inhibitor of platelet aggregation and vasoconstriction induced by 5-HT [18, 21–23]. It has been reported that extracellular release of 5-HT and P-selectin from platelets is associated with platelet aggregation, and these responses were suppressed by sarpogrelate in platelet-rich plasma (PRP) from healthy volunteers [24].

However, 5-HT alone is a mild platelet agonist which only induces shape change and reversible aggregation, and this renders the evaluation of its inhibitors extremely difficult if 5-HT alone is used to activate platelets [25]. Recently, a new method for monitoring the effects of sarpogrelate has been developed [26], and here we describe its application to evaluate the relationship between dosage of sarpogrelate and inhibition of platelet aggregation in patients with ischemic stroke.

Methods

Study Design

This randomized, double-blinded, intergroup comparison trial was conducted at 5 centers and was approved by the ethics review board at each institution in accordance with the Helsinki Declaration. Patients were enrolled between April 2004 and January 2005 after having given their written informed consent.

The primary endpoint was the inhibitory effect on platelet aggregation in patients with ischemic stroke.

Major inclusion criteria included: (1) ischemic stroke except cardioembolic stroke, based on the NINDS-III classification [27], with focal signs lasting >24 h, (2) defined onset of symptoms, and stable condition at the time of enrollment, (3) age >20 years, (4) systolic blood pressure <180 mm Hg and diastolic blood pressure <110 mm Hg, and (5) maximum intensity of platelet aggregation induced by serotonin (1 μmol/l) and epinephrine (3 μmol/l) above 15% on the day prior to the first medication.

Major exclusion criteria included: (1) modified Rankin Scale (mRS) score of 4 or more, (2) previous or planned vascular surgery, (3) history of intracranial hemorrhage, systemic bleeding, or other bleeding tendency or coagulopathy, (4) and serious complications such as cardiac, renal, hepatic, and blood disorders.

A wash-out period prior to study medication was given to patients who had been receiving antiplatelet agents, anticoagulants or fibrinolytic agents that were expected to affect the efficacy assessment, and these antithrombotic treatments were withheld during the study. For ethical reasons, the wash-out periods were set at the minimum required based on the duration of action of each antithrombotic treatment (e.g. aspirin, ticlopidine hydrochloride; 240 h [10 days], cilostazol; 48 h [2 days], sodium ozagrel, sarpogrelate; 24 h [1 day]). Moreover, we did not limit the use of drugs for the management of other risk factors of recurrence of stroke (e.g. antihypertensives, antidiabetics and antihyperlipidemics).

A limitation of this study is that it could not judge the relative efficacy of sarpogrelate to aspirin. At the planning stage of this study, we had confirmed that aspirin does not inhibit 5-HT-mediated platelet aggregation induced by the agonists that we used (data not shown), so that we could not include an aspirin control group.

Procedures

Treatment

Each patient was randomly allocated to one of three dosages of sarpogrelate, i.e. 25 mg (group L), 75 mg (group M), or 100 mg (group H), given three times daily for 7 days.

Measurement of Platelet Aggregation

Fasting blood samples were drawn on day 0, before the start of treatment, and on day 7 after treatment. Samples were drawn at fixed times (08:00 to 11:30 h, 90 min after administration of sarpogrelate). Platelet-rich plasma (PRP) anticoagulated with 0.1 mg/ml argatroban [28] was prepared and the platelet count in samples of PRP was adjusted to 2×10^{11}/l.

Platelet aggregation measurements were performed using a platelet aggregation analyzer (PA-20, KOWA, Japan), as previously described [26]. Briefly, aggregation was induced with two dosage combinations of agonists: 0.5 μmol/l 5-HT plus 3 μmol/l epinephrine (low-dose agonist) and 1 μmol/l 5-HT plus 3 μmol/l epinephrine (high-dose agonist). Results were expressed both as maximum intensity of platelet aggregation on the last day of the medication and as post-treatment percentage inhibition of platelet aggregation at baseline ([baseline – post-treatment]/baseline × 100 in each subject). Measurements of platelet aggregation are known to vary depending upon various factors, particularly inducers, and also analytical devices and techniques. In the present study, all reagents and devices were used under identical conditions throughout. In addition, analytical techniques were carefully standardized with the aid of a training program and a standard operating procedure. Platelet aggregation was thus determined under strict control.

Statistical Analysis

All measurements of platelet aggregation were run in duplicate for each patient at each point. Data are shown as mean ± SD, and as box and whiskers plots.

Table 1. Baseline characteristics of patients

Characteristic	Sarpogrelate			Statistical probability value*
	group L (n = 14)	group M (n = 16)	group H (n = 15)	
Demography				
Mean (SD) age, years	62 (9)	67 (9)	70 (8)	0.0340 KW
Men/women	12/2	10/6	9/6	0.2849 Fi
Mean (SD) body weight, kg	62.1 (9.7)	61.1 (15.6)	58.4 (8.5)	0.5516 KW
History, n (%)				
Hypertension	10 (71.4)	10 (62.5)	11 (73.3)	0.8498 Fi
Hyperlipidemia	6 (42.9)	4 (25.0)	6 (40.0)	0.5391 Fi
Diabetes mellitus	4 (8.6)	4 (25.0)	4 (26.7)	1.0000 Fi
Prior ischemic stroke (before qualifying event)	1 (7.1)	1 (6.3)	3 (20.0)	0.5002 Fi
Mean (SD) duration from the onset of ischemic stroke to medication, days	12.9 (3.6)	17.4 (6.8)	19.6 (12.0)	0.0449 KW
NINDS classification, n (%)				
Atherothrombotic	6 (42.9)	9 (56.3)	5 (33.3)	
Lacunar	6 (42.9)	7 (43.8)	9 (60.0)	0.4772 Fi
Undetermined	2 (14.3)	0 (0.0)	1 (6.7)	
Arterial system involved, n (%)				
Internal carotid artery	0 (0.0)	2 (12.5)	0 (0.0)	0.3192 Fi
Vertebrobasilar artery	4 (28.6)	6 (37.5)	3 (20.0)	0.6008 Fi
Anterior cerebral artery	0 (0.0)	0 (0.0)	1 (6.7)	0.6444 Fi
Middle cerebral artery	10 (71.4)	8 (50.0)	11 (73.3)	0.3386 Fi
Posterior cerebral artery	0 (0.0)	0 (0.0)	0 (0.0)	–
Size of infarct, n (%)				
Small (diameter <1.5 cm)	7 (50.0)	13 (81.3)	10 (66.7)	
Medium	7 (50.0)	3 (18.8)	5 (33.3)	0.1999 Fi
Large (>1/2 of lobe)	0 (0.0)	0 (0.0)	0 (0.0)	
Modified Rankin scale at randomization, n (%)				
0	3 (21.4)	1 (6.3)	0 (0.0)	
1	5 (35.7)	7 (43.8)	10 (66.7)	0.3018 Fi
2	5 (35.7)	4 (25.0)	4 (26.7)	
3	1 (7.1)	4 (25.0)	1 (6.7)	
Mean (SD) blood pressure, mm Hg				
Systolic	138.1 (9.4)	137.9 (14.1)	138.7 (12.1)	0.8440 KW
Diastolic	84.9 (8.8)	79.6 (12.0)	78.8 (9.0)	0.2814 KW
Abnormal electrocardiogram, n (%)	0 (0.0)	4 (25.0)	4 (26.7)	0.0999 Fi
Mean (SD) maximum platelet aggregation prior to medication (%) induced by 1 μmol/l 5-HT plus 3 μmol/l epinephrine	52.57 (20.72)	47.78 (14.50)	46.60 (18.19)	0.6519 KW

* KW = Kruskal-Wallis test; Fi = Fisher's exact test.

Efficacy was evaluated on the per-protocol-set basis, whereas safety analysis was performed on all randomized patients. For baseline characteristics of enrolled patients, comparisons between treatment groups were made with Fisher's exact test or the Kruskal-Wallis test for heterogeneity of variance, and the criterion of significance was set at $p < 0.15$ (two-tailed). The Jonckheere test was used to test for dose-response relationship, and the Wilcoxon rank-sum test was used to conduct multiple-group comparison, with the criterion of significance set at $p < 0.025$ (one-tailed). Statistical comparisons of safety data were made using the chi-square test, with the criterion of significance set at $p < 0.05$ (two-tailed).

Results

Forty-seven patients were enrolled and randomly assigned to three groups (L, M, and H). Of these patients, 2 were excluded from the efficacy analysis; 1 (group H) withdrew due to recurrent cerebral infarction and the other (group L) took a medication affecting coagulation during the study.

Baseline characteristics of randomized patients who were included in the efficacy analysis are summarized in

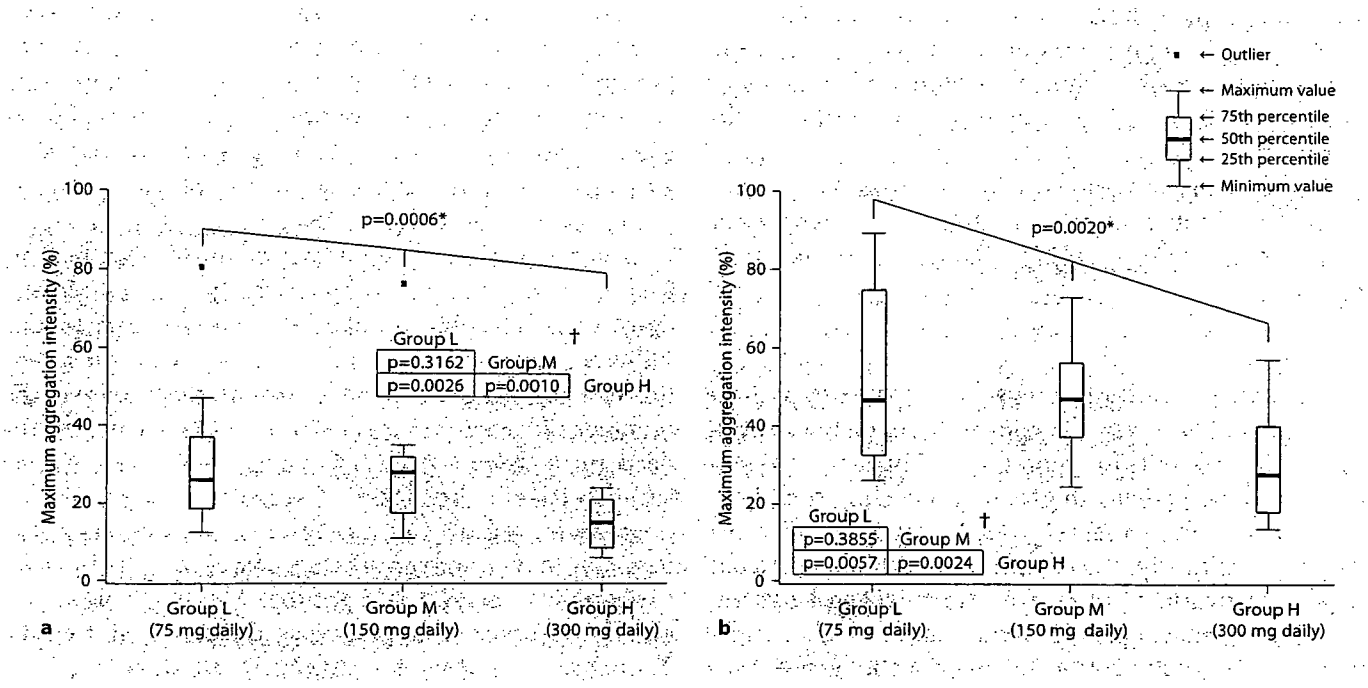

Fig. 1. Maximum intensity of platelet aggregation on the last day of the medication. **a** Agonist: low-dose (0.5 μmol/l 5-HT + 3 μmol/l epinephrine). **b** Agonist: high-dose (1 μmol/l 5-HT + 3 μmol/l epinephrine). The horizontal line in the center of the box represents the 50th percentile, the ends of the box represent the 25th and 75th percentiles, the tips of the whiskers represent the minimum and maximum percentiles and closed squares indicate individual outliers. * Jonckheere test; † Wilcoxon rank-sum test.

table 1. An adjusted analysis (analysis of variance) was performed with background factors of patients for which heterogeneity of variance ($p < 0.15$) was found among groups (age, time from onset to study medication, abnormal findings on standard ECG) as covariates, and no effect was found on the efficacy results (maximum intensity of platelet aggregation [%]).

Antithrombotic therapy was performed in 34 patients (11 patients in group L, 11 patients in group M, and 12 patients in group H). Specifically, sodium ozagrel was used in 25 patients, sarpogrelate in 6, cilostazol in 2, and ticlopidine hydrochloride in 1 patient, and all of these patients had conformed to the required wash-out periods (sodium ozagrel and sarpogrelate: 24 h; cilostazol: 48 h; ticlopidine hydrochloride: 10 days). The longest drug-free period was 10 days in 1 patient (treated with ticlopidine hydrochloride). The physician in charge of this patient considered it ethically acceptable to suspend the antiplatelet agent because (1) the antiplatelet drug was discontinued 48 days after the onset of ischemic stroke, when this patient was in the chronic stage, with a stable platelet activation status, and (2) antihypertensive and antihyperlipidemic drugs were being used to manage risk factors for recurrence.

Primary Endpoint

The results are shown in figure 1. Values of maximum intensities of platelet aggregation induced by low-dose agonist and by high-dose agonist on the last day of medication are shown in figures 1a and b, respectively. The maximum intensities of platelet aggregation were 30.57 ± 17.76 (group L), 28.09 ± 14.96 (group M), and 14.67 ± 6.41 (group H) with low-dose agonist, and 51.36 ± 22.71 (group L), 46.78 ± 15.10 (group M), and 29.67 ± 12.25 (group H) with high-dose agonist. Clear dose-response relationships were observed (low-dose agonist, $p = 0.0006$; high-dose agonist, $p = 0.0020$; Jonckheere test) (fig. 1a, b).

Values of post-treatment percentage inhibition of baseline platelet aggregation induced by low-dose agonist and by high-dose agonist are as follows. The values of percentage inhibition of platelet aggregation were 3.61 ± 35.33 (group L), 12.56 ± 29.97 (group M), and 43.04 ± 26.39 (group H) with low-dose agonist, and −2.29 ±

30.94 (group L), −0.50± 26.72 (group M), and 30.19 ± 28.79 (group H) with high-dose agonist. Again, clear dose-response relationships were obtained (low-dose agonist, p = 0.0008; high-dose agonist, p = 0.0014; Jonckheere test).

In multiple-group comparisons, significant differences were found between groups L and H, as well as between groups M and H ($p < 0.025$; Wilcoxon rank-sum test) (fig. 1a, b).

Safety

There were 27 adverse events in 16 patients, and no difference in frequency was apparent among the groups; 7 events in 5 patients in group L, 9 in 5 patients in group M, and 11 in 6 patients in group H. Bleeding complications occurred in 2 patients (group H, day 8 at study completion): specifically, positive urinary occult blood (from 1+ prior to treatment to 2+ after treatment in 1 patient, and from − (minus) prior to treatment to 1+ after treatment in another patient). Both complications of microscopic hematuria disappeared within 1 month without treatment. There were three serious adverse events in 2 patients. These were an episode of cerebral infarction (group H, day 3 during treatment), paroxysmal atrial fibrillation and fever of unidentified cause (group L, day 2 after treatment); none of these events were considered to be related to the study medication.

Discussion

Sarpogrelate has been proven effective for the treatment of peripheral artery diseases (PAD) [29], and its clinical potential has been suggested for the treatment of atherosclerotic cardiovascular disease and diabetes mellitus [15, 30, 31]. A double-blind, randomized controlled trial has been conducted to evaluate and compare the efficacy and safety of sarpogrelate with those of aspirin for prevention of recurrence in 1,510 patients with recent ischemic stroke [32]. Sarpogrelate did not meet a predefined criterion of noninferiority to aspirin for efficacy against recurrence of cerebral infarction, because the recurrence rates of cerebral infarction were 72 (6.09% per year) with sarpogrelate and 58 (4.86% per year) with aspirin (hazard ratio 1.25 [95% CI 0.89–1.77], p = 0.19). However, the effects on serious vascular events including stroke, acute coronary syndrome, or vascular event-related death were comparable, i.e. 90 (7.61% per year) with sarpogrelate and 85 (7.12% per year) with aspirin (hazard ratio 1.07 [95% CI 0.80–1.44], p = 0.65), and sarpogrelate was better tolerated than aspirin, with significantly fewer bleeding events (11.9% with sarpogrelate and 17.3% with aspirin [p = 0.004]). This favorable feature of a lower rate of bleeding complications may open up a number of therapeutic options for sarpogrelate, e.g. as an alternative to aspirin in aspirin-resistant or aspirin-intolerable patients, or in combination with aspirin.

In order to ensure best efficacy of antiplatelet therapy, it is most desirable to evaluate whether an antiplatelet agent actually inhibits the platelet function of a particular individual ex vivo. This concept has attracted the attention of a number of clinicians, particularly because of the presence of 'aspirin resistance' in 10–40% of patients under aspirin therapy [10, 33, 34]; patients with aspirin resistance have higher rates of vascular accidents if not properly treated with other regimens. Analogously, it is desirable to monitor the efficacy of sarpogrelate in order to obtain information as to appropriate dose, compliance and the presence of 'sarpogrelate resistance', if it exists. Therefore, we consider it clinically useful to establish methods for evaluation of sarpogrelate as an antiplatelet agent.

To examine the clinical effect of sarpogrelate, a specific antagonist for 5-HT_{2A}, it is essential to evaluate its effect on platelet aggregation induced by 5-HT. However, since 5-HT alone is a mild platelet agonist which only induces shape change and reversible aggregation, it is difficult to assess the effects of its inhibitors if 5-HT alone is used to activate platelets [25]. 5-HT synergistically amplifies platelet aggregation induced by ADP, collagen, or epinephrine, and thus the effects of 5-HT receptor antagonists have been conventionally evaluated by using the combination of 5-HT with a low concentration of a platelet agonist such as collagen, which by itself does not induce platelet aggregation [22]. However, as platelet responses to low concentrations of agonists differ considerably among individuals, the threshold concentration of the agonist (e.g. collagen) has to be determined separately for each platelet preparation. The whole process is invariably time-consuming, and it also suffers frequent criticism concerning the use of different doses of agonists among individuals for evaluation of the inhibitory effects of a particular agent.

In the present study, we used a new method for assessment of platelet aggregation, based on combined stimulation with 5-HT and epinephrine [26]. Epinephrine is a physiological platelet agonist, which induces platelet aggregation in platelet-rich plasma (PRP) anticoagulated with sodium citrate, a system conventionally and widely used to assess platelet aggregation; sodium citrate is

known to lower Ca^{2+} concentration, thereby inhibiting the coagulation process. However, it is of interest that in the presence of the physiological concentration of Ca^{2+}, epinephrine does not induce the formation of platelet aggregates, although it does potentiate platelet responses [35]. The new assessment method takes advantage of this phenomenon that epinephrine alone even at high concentrations does not induce the formation of platelet aggregates at the physiological Ca^{2+} concentration. Argatroban (0.1 mg/ml), a synthetic thrombin inhibitor which is unaffected by Ca^{2+} concentration, was used instead of sodium citrate as an anticoagulant for PRP preparation.

In this system, 5-HT or epinephrine alone did not induce platelet aggregation even at the highest concentration examined (100 μmol/l epinephrine, or 100 μmol/l 5-HT) [26], whereas the use of the combined agonists invariably induced full platelet aggregation, irrespective of individual differences in relevant factors. A preliminary study in healthy volunteers showed that stable platelet aggregation was induced by the combination of 0.5 or 1 μmol/l 5-HT and 3 μmol/l epinephrine [unpubl. data], and thus, we chose these combinations of stimuli as agonists in the present study.

Using the same protocol for the measurement of platelet aggregation in the hands of different technicians in 5 centers, consistent inhibitory effects of sarpogrelate on platelet aggregation in 45 patients with ischemic stroke were observed. These findings demonstrate that the new assessment system can be used to monitor the effect of sarpogrelate under ex vivo conditions in the clinical setting, and that the system permits valid inter-laboratory comparisons of the antiplatelet efficacy of sarpogrelate.

Our results revealed a dose-dependent inhibitory effect of sarpogrelate on platelet aggregation in patients with ischemic stroke; the efficacy of the total dose of sarpogrelate 300 mg/day is significantly superior to that of 150 or 75 mg/day. This dosage is consistent with the recommended dosage of sarpogrelate in clinical practice [29, 32].

In conclusion, we confirmed that sarpogrelate shows a dose-dependent inhibitory effect on platelet aggregation in patients with ischemic stroke, using a new assessment method. This observation may account for the observed clinical benefit of sarpogrelate in patients with cerebrovascular disease, and provides support for sarpogrelate as a therapeutic option in patients with atherosclerotic vascular disease.

Appendix

The following persons and institutions participated in the present study: J. Nakagawara, Nakamura Memorial Hospital, Hokkaido; A. Suzuki, Research Institute for Brain and Blood Vessels Akita, Akita; T. Katsumata, Nippon Medical School Hospital, Tokyo; K. Kashihara, Okayama Kyokuto Hospital, Okayama; K. Fukuyama, Fukuoka Wajiro Hospital, Fukuoka.

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Efficacy of anti-coagulant treatment with argatroban on cardioembolic stroke

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Abstract Argatroban, which is a thrombin inhibitor, has an indication as a treatment in the acute phase on atherosclerotic ischemic stroke in Japan. Howeve, in cardioembolic stroke, argatroban is considered to be contraindicated with the side effect of hemorrhage, though there is no clear clinical evidence to show that argatroban increases hemorrhagic compared with heparin. The efficacy of anti-coagulant treatment with argatroban on cardioembolic stroke was evaluated retrospectively in this study. We identified 3,113 patients from the Japan Standard Stroke Registry Study who had had a cardioembolic ischemic stroke. We excluded patients with the anti-platelet treatment or the combination therapy of anti-coagulation. Our analyses are therefore based on a cohort of 2,529 patients who were treated either with heparin, and argatroban, or with no anti-coagulation treatment. With multivariable regression, hemorrhagic it was shown that hemorrhage was significantly reduced in heparin and argatroban treatments in the patients with mild severity. There was no significant difference in the recurrence of ischemic stroke between the treatments. Both argatroban and heparin showed dramatic improvement compared with the no treatment standard, but only heparin achieved statistical significance for mortality and change in NIHSS score (admission to discharge) in the moderate stroke subgroup [NIHSS 11–22]. Both heparin and argatroban [more so than heparin alone] have a significantly reduced mortality risk. From the present study, it is suggested that argatroban may be useful on cardioembolic stroke, increasing the improvement of recovery of stroke severity without increasing the risk of hemorrhage. Further prospective studies are awaited for evaluating better the efficacy of argatroban on cardioembolic stroke.

Key words cardioembolic stroke · heparin · argatroban · hemorrhagic formation

Introduction

Stroke is a major cause of serious long-term disability and death [15]. Acute ischemic stroke accounts for approximately 80% of all strokes, and is caused by embolic or thrombotic occlusion in the cerebral vessels. In this regard, cardioembolic stroke generally results in more severe disability, since it typically

involves a larger ischemic area than the other types of ischemic stroke.

Heparin was once widely used as a treatment in the acute phase of cardioembolic stroke to prevent recurrence. However, after the International Stroke Trial Collaborative Group reported that heparin did decrease the recurrence of cardioembolic stroke due to atrial fibrillation but increased hemorrhage [5], the use of heparin for preventing the recurrence of ischemic stroke has waned.

Argatroban is a direct thrombin inhibitor that provides effective parenteral anticoagulation, with an acceptably low bleeding risk, in a variety of clinical settings associated with increased thrombotic risk. Unlike heparin, the direct thrombin inhibitor argatroban effectively inhibits thrombin that is either free or bound to fibrin or clots [1] including clots that are aged or treated with thrombolysis [4]. Argatroban is available for use in the prophylaxis or treatment of thrombosis in heparin induced thrombocytopenia [12], acute atherothrombotic ischemic stroke [8], and chronic arterial occlusion [13]. However, in cardioembolic stroke, argatroban is considered as contraindiced because of the side effect of hemorrhagic formation in Japan, though there is no clear clinical evidence to show that argatroban increases hemorrhage compared with heparin. In preclinical studies, argatroban causes less bleeding than heparin, when used in combination with thrombolysis in patients with acute myocardial infarction [7]. It has been reported that argatroban decreased mortality, stroke index score, cell injury, and cerebral edema in an experimental study [17] and extended activated partial thromboplastin time stably and showed lower side effect of hemorrhagic formation [11]. Therefore, argatroban may effectively reduce the recurrence rate of cardioembolic stroke with less risk of hemorrhag than heparin.

The aim of the present study was to evaluate the efficacy of argatroban and its safety relating to hemorrhagic formation in cardioembolic stroke. Our study is retrospective, utilizing data from the Japan Standard Stroke Registry Study.

Methods

Subjects

Our report is based on data from the Japan Standard Stroke Registry Study (JSSRS), maintained by the Japan Stroke Association. Sixty acute stroke hospitals throughout Japan participated in the Japan Standard Stroke Registry Study, documenting the in-hospital course of 16,630 consecutive patients with acute stroke from January 2001 to March 2004. Data from all patients with acute stroke presenting at the participating hospitals were included in the registry, with the exceptions of patients with cerebral tumor, sub- or epidural hemorrhage or other conditions imitating ischemic stroke. This study was approved by the investigational review board of the Japan Stroke Association.

For all acute stroke patients, detailed data forms were completed on admission and at discharge by attending physicians. The following data were recorded:

1. Baseline characteristics: Age, sex, blood pressure, oral anticoagulant or antiplatelet usage before stroke, family history of stroke, smoking habit, alcohol consumption.
2. Vascular risk factors and co-morbid conditions: Coronary artery disease, other heart disease (dilated or hypertrophic cardiomyopathy, valvular heart disease, congenital heart disease), previous stroke, hypertension, diabetes mellitus, chronic obstructive pulmonary disease, smoking, alcohol consumption, malignancy and dementia.

   Hypertension, diabetes mellitus, and hyperlipidemia were diagnosed by a physician. Patients were designated as hypertensive if they were taking anti-hypertensive agents or had a systolic blood pressure of > = 140 mmHg, diastolic blood pressure of > = 90 mmHg or both. They were designated as having diabetes mellitus if they were treated with oral hypoglycemic agents or insulin, their serum fasting blood sugar level was > = 126 mg/dl or both. Hyperlipidemia was designated if patients were taking anti-hyperlipidemic drugs, their serum cholesterol level was > = 220 mg/dl or triglyceride > = 150 mg/dl, or both.
3. Diagnosis, severity and outcome: For diagnosis of stroke subtype, we used National Institute of Neurological Disorders and Stroke (NINDS) criteria [16]. The basis for the clinical diagnosis of cardioembolic stroke is the demonstration of a cardiac-transcardiac source of embolus and no evidence of other causes of stroke. Cardiac conditions that may produce emboli include intermittent or continuous atrial fibrillation or flutter, recent myocardial infarction, congestive heart failure, and mitral or aortic valve disease. When the source is transcardiac by way of a right-to-left cardiac shunt (paradoxical embolus), the source of the clot is usually a peripheral venous thrombus.

   Severity of stroke at admission and hospital discharge was evaluated using the National Institutes of Health Stroke Scale (NIHSS) [2, 3], and disability from stroke at discharge was evaluated with the modified Rankin scale [19]. From these scales, we investigated three clinical outcome measures: the difference of NIHSS scores between admission and discharge (admission - discharge), a measure of recovery; mortality [mRS 6 at discharge], and a Rankin-related favorable outcome: mRS 0 to 2 at discharge.
4. Imaging diagnosis: Computed tomography and magnetic resonance image findings were available for most patients. Angiographic data were obtained using cerebrovascular angiography, magnetic resonance angiography, 3D-computed tomography angiography, or carotid ultrasonography. In the present study, hemorrhage was designated when the patients had symptomatic cerebral hematoma with worsening symptoms and an extended hospitalization period. When patients' symptoms worsened, CT was carried out to check for additional brain disease (e.g., ischemic stroke recurrence or hemorrhagic stroke). Otherwise, CT was performed 1 week and 1 month after the onset of cardioembolic stroke in follow-up assessments.
5. Acute treatment against stroke: Argatroban, heparin, ozagrel hydrochloride, aspirin, and/or edaravone.

   The clinical use of argatroban has been approved for the treatment of chronic peripheral arterial obstructive disease and acute ischemic stroke in Japan. Its typical mode of administra-

tion is via continuous infusion at 2.5 mg/hour for the first 48 hours, and then 10 mg infused over 3 hours twice a day on days 3–7.

We identified 3,113 patients from the Japan Standard Stroke Registry Study who had cardioembolic ischemic stroke. From this cohort, we excluded all individuals less than 21 years of age, as well as the patients who had incomplete (missing) data on treatments. We further excluded patients with concurrent anti-platelet or anti-coagulation treatment at stroke onset. Our analyses are thus based on a cohort of 2,529 patients who were treated either with heparin, or argatroban, or no anti-coagulation treatment.

Statistical analysis

We stratified patients into three categories representing mild, moderate, and severe stroke according to NIHSS admission scores: 0–10, mild; 11–22, moderate; 23–42, severe. We present summary statistics at baseline [time of hospitalization] separately for patients in these subgroups. Summary statistics are reported as means ± standard deviations or medians (and interquartile ranges) for continuous variables, and frequencies for categorical variables. Simple group comparisons were undertaken with parametric or nonparametric analyses of variance. We investigated six clinical outcomes: recurrence, hemorrhagic, a composite of recurrence or hemorrhagic and the three outcomes relating to mRS scores and NIHSS scores at discharge described previously [mortality, that is, mRS = 6; "favorable" outcome, mRS of 0 to 2 at discharge; and, change in NIHSS score {admission - discharge)]. We first screened for potential predictors of the clinical outcomes from the variables listed in Table 1 using univariate regression techniques; we found only age to be consistently related to outcomes within each of the NIHSS severity subgroups. We then investigated the effects of argatroban and heparin on clinical outcomes with multivariable logistic regression for the five dichotomous outcomes, mortality and "favorable" mRS outcome, 0–2 vs. 3–6, and multiple linear regression for change in NIHSS (admission - discharge). We undertook separate regressions for each of the NIHSS admission subgroups, and adjusted for age in these analyses.

Results

The Japan Standard Stroke Registry Study includes 3,113 patients with cardioembolic stroke between January 2001 and March 2004. After exclusions for age, missing data on treatments, or concurrent anti-platelet or anti-coagulant therapy as described above, our study cohort comprised 2,529 adult patients treated with heparin, argatroban, or no anti-coagulant treatment. Relevant demographics and clinical characteristics of these patients are listed in Table 1. There were statistically significant differences in sex, age, hypertension, drinking, smoking, atrial fibrillation, myocardial infarction, patent foramen ovale, pacemaker, and edaravone usage among the stroke severity subgroups. Ages, and the proportion of females, both increased with increasing severity of stroke (NIHSS) at admission ($p < 0.001$). We remark that females were significantly older than males (77 ± 10 and 72 ± 11 respectively, $p < 0.001$); this 4 to 5 year age differential was observed in each of the severity subgroups. In addition, patterns of risk factors differed between males and females, with differences most pronounced in smoking and drinking habits.

As would be expected, clinical outcomes varied substantially among the stroke severity subgroups (Table 2). Clinical patterns are most evident with the modified Rankin scale. Favorable mRS outcomes (0 to 2) are common with mild stroke [NIHSS 0 to 10], but tend to be increasingly rare as severity at admission increases. The converse pattern obtains with mortality (mRS 6 at discharge): maximal mortality with the most severe strokes, minimal mortality with mild strokes. In comparison, changes in NIHSS score seem much more diffuse across the severity subgroups. Patients seem to be under constant risk of recurrence across the severity subgroups, whereas hemorrhage tends to increase with increasing severity of stroke. The statistical significance of the composite outcome recurrence or hemorrhage is driven solely by the differential rate of hemorrhage across the severity subgroups.

We first screened the demographic and history variables in Table 1 in univariate analyses, to identify possibly predictive variables related to the clinical outcomes in each of the NIHSS admission categories. Among them, we found only age to be consistently related to outcome, with increasing age being associated with less favorable outcome. In particular, edavarone treatment was not found to be significantly related to any of the clinical outcomes. With univariate analysis, both argatroban and heparin were associated with increased favorable outcome in the moderate stroke subgroup ($p < 0.001$) and decreased mortality in the moderate and severe stroke subgroups ($p = 0.004$, $p = 0.01$; respectively, Figure 1). In the mild and moderate stroke subgroups, the change in NIHSS score (admission - discharge) was significantly greater than no anti-coagulant treatment ($p = 0.016$, < 0.001; respectively, Figure 2).

We then used multivariable regression models to investigate whether argatroban and heparin were related to clinical outcomes, while adjusting for age. Logistic regressions were appropriate for the dichotomous outcomes [favorable vs. unfavorable, mortality vs. discharge alive, recurrence of ischemic stroke, hemorrhagic formation, and recurrence or hemorrhage], and multiple linear regression was used for change in NIHSS score (admission - discharge). The findings are presented in Table 3. In the mild stroke subgroup [NIHSS 0–10], both argatroban and heparin tended to be associated with favorable outcomes, but neither achieved significance at the conventional 0.05 level with the singular exception of

Table 1 Patient Characteristics

	NIHSS at Admission				
	Overall	0–10	11–22	23–42	P-value
Patients: n (%)	2529 (100)	1137 (45)	628 (25)	509 (20)	
Sex (F/M): n (%female)	1129/1400 (45)	410/727 (36)	313/315 (50)	281/228 (55)	<0.001
Age: median (25%, 75%)(tears)	75 (68, 82)	72 (64, 80)	77 (70, 83)	79 (73, 86)	<0.001
Hypertension: yes/no (%)	1328/1144 (53)	576/544 (51)	349/261 (56)	279/211 (55)	0.028
Diabetes Mellitus: yes/no (%)	460/2022 (18)	218/908 (19)	115/501 (18)	86/402 (17)	0.711
Hyperlipidemia: yes/no (%)	352/2042 (14)	181/902 (16)	85/514 (14)	56/408 (11)	0.052
Family History of Stroke: yes/no (%)	364/1632 (14)	174/735 (15)	81/426 (13)	60/294 (12)	0.294
Drinking: yes/no (%)	871/1342 (34)	462/541 (41)	197/355 (31)	120/297 (24)	<0.001
Smoking: yes/no (%)	450/1746 (18)	253/747 (22)	99/444 (16)	62/349 (12)	<0.001
Atrial Fibrillation: yes/no (%)	1799/648 (71)	778/327 (68)	470/143 (75)	373/108 (73)	0.002
Valvular Disease: yes/no (%)	273/2096 (11)	123/945 (11)	66/534 (11)	57/412 (11)	0.842
Myocardial Infarction: yes/no (%)	189/2180 (7)	81/987 (7)	39/561 (6)	52/417 (10)	0.017
Patent Foramen Ovale: yes/no (%)	62/2307 (2)	38/1030 (3)	8/592 (1)	2/467 (0)	<0.001
VSD + ASD: yes/no (%)	14/2355 (1)	6/1062 (1)	4/596 (1)	3/466 (1)	0.937
Cardiomyopaty: yes/no (%)	87/2282 (3)	44/1024 (4)	20/580 (3)	17/452 (3)	0.706
Pacemaker: yes/no (%)	45/2324 (2)	11/1057 (1)	17/583 (3)	16/453 (3)	0.003
Edaravone: yes/no (%)	1091/1437 (43)	462/675 (41)	310/317 (49)	241/268 (47)	<0.001
Argatroban/Heparin/No Anti-Coagulant	326/998/1205 (13/39/48)	190/483/464 (17/42/41)	72/246/310 (11/39/49)	39/151/319 (8/30/63)	<0.001

Statistical analyses (p values) were made among the stroke. Severity groups

Table 2 Outcome variation among NIHSS at admission

	NIHSS at Admission				
	Overall	0–10	11–22	23–42	P-value
mRS at Discharge: n (%)	2218 (100)	1112 (50)	611 (28)	495 (22)	
0–2: n (%)	894 (40)	796 (71)	82 (13)	16 (3)	
3–5: n (%)	1033 (47)	285 (26)	451 (74)	297 (60)	<0.001
6: n (%)	291 (13)	31 (3)	78 (13)	182 (37)	
NIHSS (admission-discharge): median (25%, 75%)	2 (−42, 42)	2 (−42, 10)	4 (−31, 22)	0 (−19, 42)	<0.001
Recurrence of Ischemic Stroke: yes/no (%)	135/1932 (5)	58/884 (5)	38/498 (6)	27/373 (5)	0.772
Hemorrhage: yes/no (%)	86/2284 (3)	19/1046 (2)	25/571 (4)	32/439 (6)	<0.001
Recurrence or Hemorrhage: yes/no (%)	212/1808 (8)	74/850 (7)	59/465 (9)	58/326 (11)	<0.001

Statistical analysis (p values) were made among the stroke severity groups

hemorrhage recurrence. In the moderate stroke subgroup [NIHSS 11–22], the individual estimates of treatment effects for both argatroban and heparin indicate dramatic improvement over the no treatment standard relative to the mRS and NIHSS outcome measures, but only heparin achieves significance at alpha level 0.05 for mortality and change in NIHSS score (admission – discharge). In the severe stroke subgroup [NIHSS 23–42], it is perhaps unrealistic to expect a "favorable" outcome of mRS 0–2; note, however, that both heparin and argatroban [more so than heparin] have a significantly reduced mortality risk. Their favorable effects on change in NIHSS score (admission - discharge) are less pronounced than with the moderate stroke subgroup. As with the moderate stroke subgroup, neither treatment is significantly associated with recurrence or hemorrhagic formation.

Discussion

The efficacy of anti-coagulant treatment with argatroban on cardioembolic stroke was evaluated retrospectively in this study. Hemorrhage was significantly reduced with heparin and argatroban treatments in the patients with the mild severity. There was no significant difference in the recurrence of ischemic stroke among the treatments. Both argatroban and heparin indicate dramatic improvement over the no treatment standard, but only heparin achieves statistical significance for mortality and change in NIHSS score (admission - discharge) in the moderate stroke subgroup [NIHSS 11–22]. Both heparin and argatroban [more so than heparin] have a significantly reduced mortality risk.

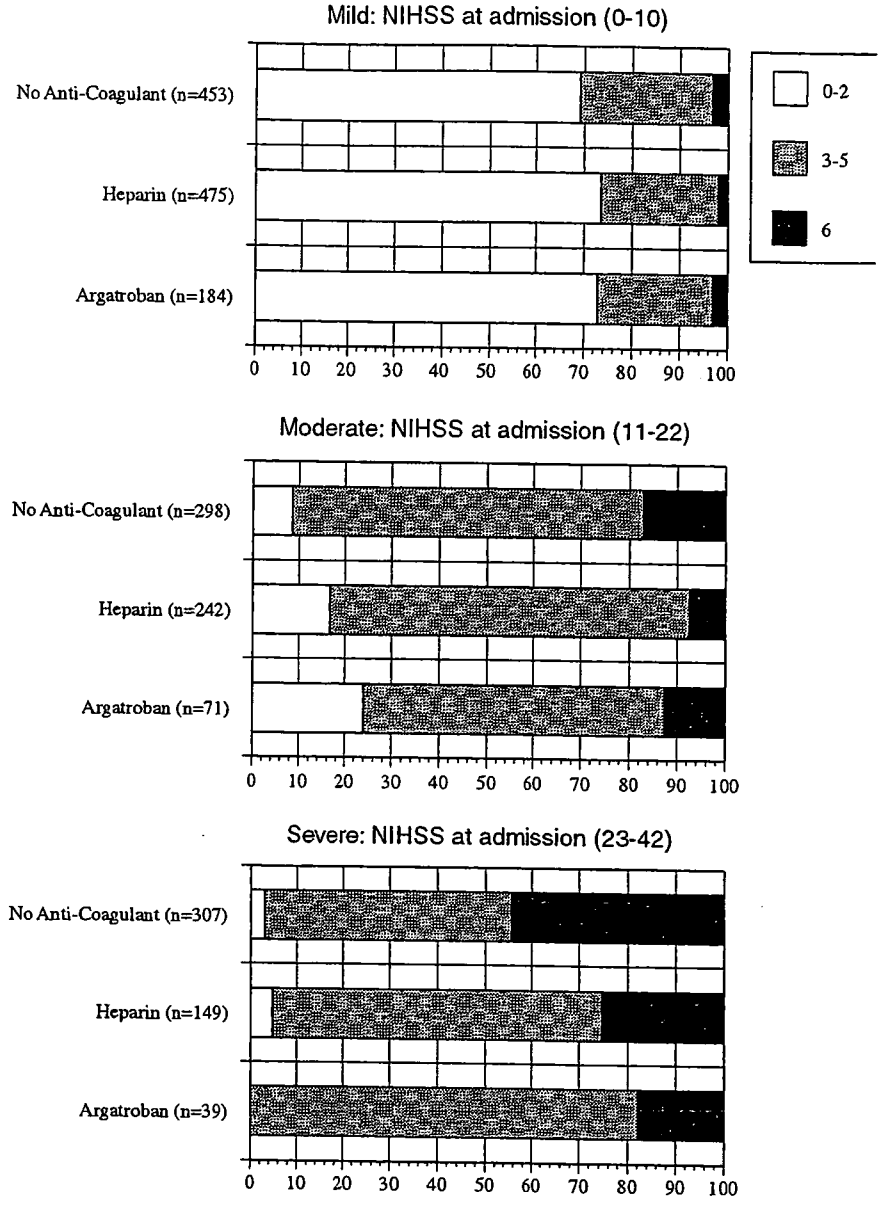

Fig. 1 The distribution of patients in modified Rankin Scale (0–2, 3–5, and 6) at hospital discharge. There was a significant difference in the all severity among the treatments

Anti-coagulants are highly effective for preventing cardioembolic stroke. From injury through to healing, thrombin has several important functions in blood clotting, subsequent clot lysis, and tissue repair. These include edema, inflammation, cell recruitment, cellular releases, transformations, mitogenesis, and angiogenesis. Thrombin also participates in disease states, such as venous thrombosis, coronary thrombosis, stroke, and pulmonary emboli, among others and is implicated in atherosclerosis, the growth and metastasis of certain cancers, Alzheimer's disease, and perhaps other conditions.

Argatroban, a synthetic peptidomimetic antithrombin agent, is the first clinical anticoagulant solely to target thrombin. Hemostatic activation, occurring in acute ischemic stroke, was effectively blocked by argatroban [6]. In the North American randomized, double-blinded, placebo-controlled study of direct thrombin inhibition in acute ischemic stroke, argatroban at each dose evaluated significantly prolonged aPTTs without increasing symptomatic intracranial hemorrhage or major bleeding [11]. On the other hand, there is another study, which showed that both 30 patients with cardioembolic infarction and 30 patients with atherothrombotic infarction showed significant

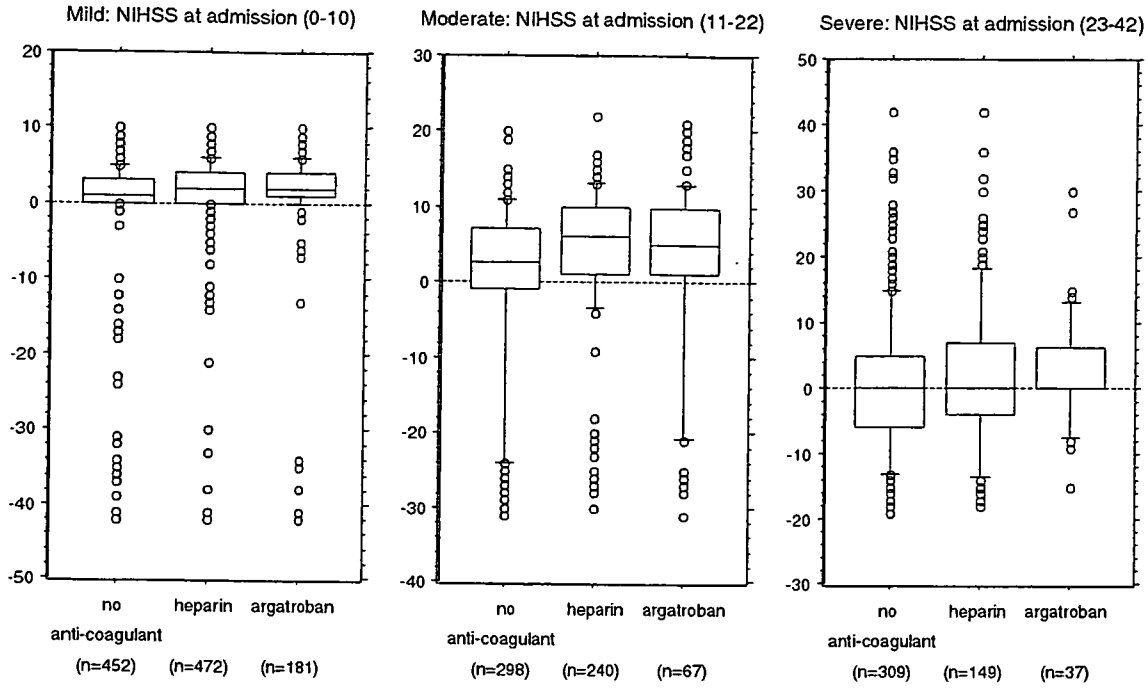

Fig. 2 The change in NIHSS score (admission – discharge) in each severity. There was a significant difference in the mild and moderate severity among the treatments

improvement of the Hemispheric Stroke Scale after 7 days of argatroban therapy ($p < 0.05$) [18]. Hemorrhage developed in 8 of the patients with cardioembolic infarction, but no worsening of symptoms was noted in any of these patients. There was no significant prolongation of activated partial thromboplastin time or prothrombin time after 7 days. Argatroban, given as adjunctive treatment to alteplase, is tolerated well in patients with acute myocardial infarction. Safety and efficacy of the combination alteplase and argatroban are similar to those of alteplase and heparin [20]. In the experimental study, the combination of argatroban and recombinant tPA extends the window of opportunity for treatment of stroke to 4 hours without increasing hemorrhagic transformation in experimental embolic stroke [14]. Also argatroban significantly decreased cerebral edema following severe forebrain ischemia in gerbil [17].

Argatroban is also a synthetic direct thrombin inhibitor indicated for parenteral use in the prevention and treatment of thromboembolism in patients with heparin-induced thrombocytopenia [9]. Its elimination half-life is approximately 40–50 minutes, and it is primarily eliminated by hepatic metabolism and biliary secretion. Compared with historical controls, argatroban-treated patients with heparin-induced thrombocytopenia or heparin-induced thrombocytopenia with thrombosis experienced lower rates of the composite end point of death, amputation, and new thrombosis [9].

Recently, the effect of argatroban on stroke has been retrospectively evaluated in patients with heparin induced thrombocytopenia [10], and its safety has been prospectively evaluated in acute ischemic stroke [11]. In ARGIS-1 study [11], symptomatic intracranial hemorrhage at 30 days was not significantly different between high-dose argatroban, low-dose argatroban, and placebo group. There were no significant differences between the treatment and placebo groups in asymptomatic intracranial hemorrhage, major systemic hemorrhage, or 90 days mortality.

From this analysis we found that argatroban did not increase the risk of hemorrhage on compared with the heparin treatment. Furthermore, argatroban may have the effect of decreasing the severity of stroke in the patients with NIHSS less than 22 at admission and mortality in the patients with NIHSS more than 23 at admission. From the present study, it is suggested that argatroban may be useful in cardioembolic stroke, increasing the recovery of stroke severity without increasing the hemorrhage

Table 3 Effects of Argatroban and Heparin on Clinical Outcomes

	NIHSS at Admission		
	0–10	11–22	23–42
Favorable Outcome [1]			
Argatroban	1.12 (0.75, 1.68)	3.34 (1.67, 6.68)	NA
Heparin	1.13 (0.84, 1.53)	2.09 (1.23, 3.57)	1.46 (0.52, 4.09)
No Treatment	1	1	1
Mortality [2]			
Argatroban	1.05 (0.40, 2.77)	0.75 (0.35, 1.62)	0.27 (0.12, 0.63)
Heparin	0.68 (0.30, 1.53)	0.46 (0.27, 0.80)	0.43 (0.28, 0.66)
No Treatment	1	1	1
Change in NIHSS (admission – discharge)			
Argatroban	0.35 (−0.94, 1.63)	2.76 (−0.29, 5.81)	1.81 (−1.91, 5.53)
Heparin	0.91 (−0.04, 1.85)	3.94 (1.98, 5.89)	1.28 (−0.85, 3.42)
No Treatment	1	1	1
Recurrence of Ischemic Stroke			
Argatroban	1.10 (0.46, 2.43)	0.72 (0.21, 1.98)	0.98 (0.22, 3.05)
Heparin	1.54 (0.85, 2.83)	0.66 (0.31, 1.34)	0.38 (0.11, 1.04)
No Treatment	1	1	1
Hemorrhagic Formation			
Argatroban	0.15 (0.01, 0.76)	1.45 (0.40, 4.33)	NA
Heparin	0.19 (0.04, 0.57)	0.94 (0.38, 2.27)	0.52 (0.20, 1.18)
No Treatment	1	1	1
Recurrence or Hemorrhage			
Argatroban	0.62 (0.27, 1.28)	0.87 (0.34, 1.96)	0.37 (0.09, 1.10)
Heparin	0.99 (0.59, 1.65)	0.70 (0.38, 1.26)	0.44 (0.21, 0.85)
No Treatment	1	1	1

Notes: [1] A "favorable" outcome consists of a modified Rankin scale score of 0 to 2 at discharge. [2] Mortality was designated by mRS = 6 at discharge. Multivariate odds ratios and corresponding 95% confidence intervals are presented for Argatroban and Heparin for the dichotomous "favorable" and "mortality" outcomes, and for the recurrence and hemorrhagic formation outcomes. These odds ratios are relative to the referent. "No Treatment" group, and were determined from logistic regressions including age as an independent variable. Regression coefficients and corresponding 95% confidence intervals are presented for Argatroban and Heparin for change in NIHSS score (admission – discharge) dependent variable, as determined from multiple linear regressions including age as an independent variable. Argatroban and Heparin usage were coded with 0–1 indicator variables, so the regression coefficients immediately denote increments (or decrements) in change in NIHSS score (admission – discharge) relative to no treatment

risk. Further prospective study will be awaited for evaluating the efficacy of argatroban on cardioembolic stroke.

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治療

超急性期脳梗塞の治療

山脇 健盛

Question & Answer

Q：t-PA 静注療法はどのような脳梗塞が適応になるのですか？

A：発症から3時間以内の脳梗塞です．発症時刻であって，発見時刻ではないことに注意が必要です．起床時にすでに症状がある場合，起床時刻が発症時刻ではありません．

Keyword：超急性期脳梗塞(*J1*)，血栓溶解療法，t-PA，頭蓋内出血

Case

心房細動を有し突然右片麻痺が出現した1例

症例：61歳の女性．

主訴：右片麻痺．

既往歴：5年前より拡張型心筋症，心房細動にてアスピリン内服中．

現病歴：某年某月，18時10分頃，台所仕事中に突然右片麻痺と構音障害が出現，18時55分に救急車で来院．

主訴：血圧 142/78 mmHg，脈拍 66/分不整．神経学的には，軽度意識障害，軽度失語，構音障害，右上肢は完全麻痺，右下肢は膝立不能を認めた．NIHSS(National Institute of Health Stroke Scale, *J2*)15点．頭部CTでは異常なし．

心原性脳塞栓症と考え，t-PA 静注療法を発症後2時間20分後より開始した．投与中より右上下肢の挙上が可能となり，1時間後の投与終了時にはNIHSSは7点まで改善した．その後さらに改善し，1カ月後にはNIHSS 3点となり，歩行自立，日常会話に問題ない程度まで改善した．

1995年米国 National Institute of Neurological Disorders and Stroke (NINDS)による大規模臨床試験で，発症3時間以内の超急性期脳梗塞患者に対する経静脈的血栓溶解療法(*J3*)である tissue plasminogen activator (t-PA)静注療法の有効性が証明され[1]，t-PAは米国では初めての脳梗塞急性期治療薬として翌年認可された．t-PAの急性期脳梗塞患者における二重盲検試験はそれ以前にわが国で行われ，有効性が示されたが，残念ながら特許の関係で開発が中止されたという経緯が

用語ノート

J1 超急性期脳梗塞

超急性期の定義はないが，一般には3時間または6時間とされることが多い．ペナンブラ(血流は低下しているが神経細胞はまだ死んでいない領域)を救える可能性のある時間帯と考えられている．

J2 NIHSS

1994年にNIH (National Institute of Health)により考案された世界で最も頻用されている脳卒中における神経症候評価尺度．15の評価項目があり，最低は0点(神経脱落症状なし)で，最高(最重症)は42点．

J3 血栓溶解療法

血栓溶解薬としては，ウロキナーゼ，t-PAがあるが，前者は流血中のプラスミノゲンも活性化するのに対し，後者は血栓上で同様の効力を発揮する．投与方法として，経動脈(領域動脈内，選択的)と経静脈がある．

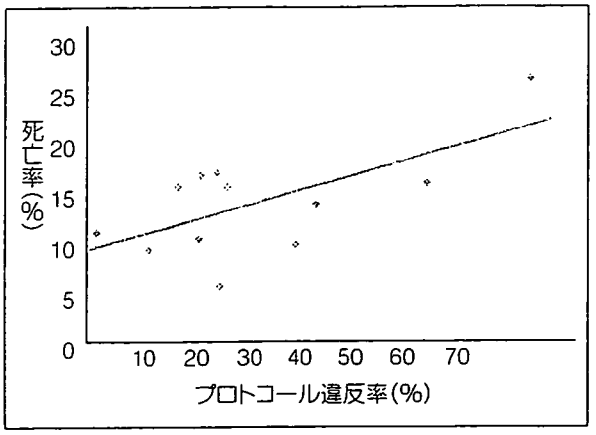

図1 アルテプラーゼ市販後臨床成績のメタアナリシス―プロトコール逸脱と死亡率の関連
(Graham GD. Stroke 34 : 2847-50, 2003)

ある．1995年にNINDS試験が発表され，米国に続きカナダ，欧州，アジア諸国でt-PAは脳梗塞急性期治療薬として認可されたが，先進国では唯一日本で認可されていなかった．2002年にプラセボを対照としないt-PAのオープン試験(J-ACT)がわが国で行われ[2]，その結果NINDS試験のt-PA投与群と同等の成績が得られ，2005年10月にようやく認可されるに至った．

医療における影響

t-PA（アルテプラーゼ)静注療法は確かにきわめて有効な治療法であるが，その恩恵にあずかれるのはごく一部の例である．内外の報告をみると脳梗塞全体の数%とされる．2005年10月以降のわが国でのアルテプラーゼ市販後調査では，脳梗塞全体の約1%にしか使用されていない．3時間以内に治療を開始するには，最低2時間で病院に到着する必要がある．脳卒中を発症して3時間以内に病院に到着する例は，最近のわが国のデータでは，大体36%とされる．2時間以内となると20%前後と考えられる．さらにそのうち厳格な適応基準に適合するのは，多くて10%と考えられる．

適応症例の少ない最も大きな要因は受診の遅れ(patient's delay)である．今後さらに一般市民への脳卒中の症状，早期受診の重要性を啓蒙していくとともに，各地域における脳卒中救急医療体制の確立が急務である．そして医療機関における治療開始の遅れ(doctor's delay)が決してあってはならない．各医療機関における脳卒中診療体制の整備も大きな課題といえる．

患者さんに説明する際のポイント

t-PA静注療法は，超急性期脳梗塞患者においてきわめて有効な治療法であるが，頭蓋内出血という重大な副作用を引き起こす可能性があり，いわば「両刃の剣」の治療法といえる．適応基準を逸脱した例ほど頭蓋内出血が多いことが明らかとなっている(図1)[3]．社会復帰が可能なまで改善する例が増加する一方で，生命予後に影響する重大な頭蓋内出血を惹起する可能性がある(5～6%)．そのため，本療法による利益・不利益を本人・家族(意識障害や失語が存在する場合も多く，実際には家族であることが多い)に十分説明し，理解を得たうえでの同意が必要である．しかし，本療法は1分でも早くに行うことが必要であり，実際の臨床現場では説明のための時間的余裕を十分に確保することは難しい．可能な限り複数で対応し，診察・検査と同時進行で説明する必要がある．当然のことながら説明を行う医師は，本療法による利益・不利益はもちろん，脳梗塞全般の内科的・外科的治療を含めた幅広い知識が必要であることはいうまでもない．

2005年の認可に合わせ，日本脳卒中学会により「rt-PA（アルテプラーゼ)静注療法適正治療指針」が作成され発表された．これは本治療法が広くかつ安全に実施されることを目指したきわめて実践的なガイドラインである．その中には，患者説明用資料も入っており，有用であることはもちろん，本治療法を行う可能性がある医師は，本指針を熟読しておく必要がある．この指針は，日本