Effect of Sarpogrelate, a 5-HT<sub>2A</sub> 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<sub>2A</sub>) 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<sub>2</sub>, 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<sub>2</sub> [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...
stable condition at the time of enrollment, (3) age systolic blood pressure surgery, (3) history of intracranial hemorrhage, systemic bleeding, or (mRS) score of 4 or more, (2) previous or planned vascular sur-
above 15% on the day prior to the first medication.

Recently, a new method for monitoring the effects of sar-
opregrelate has been developed.

Platelet-rich plasma (PRP) from healthy volunteers
is associated with platelet aggregation, and these respons-
tracellular release of 5-HT and P-selectin from platelets
of atherothrombosis. It has been postulated that libera-
ma 5-HT concentration is higher in patients with coro-

Major exclusion criteria included: (1) modified Rankin Scale
age of sarpogrelate and inhibition of platelet aggregation
was conducted at 5 centers and was approved by the ethics re-
a clinical trial was conducted at 5 centers and was approved by the ethics re-

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 sur-
Sarpogrelate and Platelet Aggregation in Patients with Ischemic Stroke

A wash-out period prior to study medication was given to pa-
tients who had been receiving antiplatelet agents, anticoagulants or fibrinolytic agents that were expected to affect the efficacy as-
seessment, 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 hydro-
chloride; 240 h [10 days], cilostazol; 48 h [2 days], sodium ozagrel, sarpogrelate; 24 h [1 day]). Moreover, we did not limit the use 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 sar-
pogrelate).

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.

Fasting blood samples were drawn on day 0, before the start of
treatment, and on day 7 after treatment. Samples were drawn at
measured under strict control.

Each treatment group included 25 patients, and the study was
designed to include 75 patients in total (33 patients in the as-
pirin group, 25 patients in the sarpogrelate group, and 17 patients in the placebo group). Statistical analysis was performed using analysis of variance (ANOVA) for repeated measures with a post hoc comparison using the Tukey’s honestly significant difference (HSD) test. The significance level was set at p < 0.05.

The study was conducted in accordance with the Helsinki
Declaration. Patients were enrolled between April 2004 and Janu-
y 2005 after having given their written informed consent.

All measurements of platelet aggregation were run in dupli-
cate for each patient at each point. Data are shown as mean ± SD,
and as box and whiskers plots.
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 Table 1. The table presents demographic information such as age, gender, body weight, and medical history, along with neurological features including the size of infarct and modified Rankin scale at randomization. Additionally, it includes systolic and diastolic blood pressures, abnormal electrocardiogram rates, and platelet aggregation induction prior to medication.

**Table 1. Baseline characteristics of patients**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Sarpogrelate</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>L (n = 14)</td>
</tr>
<tr>
<td>Demography</td>
<td></td>
</tr>
<tr>
<td>Mean (SD) age, years</td>
<td>62 (9)</td>
</tr>
<tr>
<td>Men/women</td>
<td>12/2</td>
</tr>
<tr>
<td>Mean (SD) body weight, kg</td>
<td>62.1 (9.7)</td>
</tr>
<tr>
<td>History, n (%)</td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>10 (71.4)</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>6 (42.9)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>4 (8.6)</td>
</tr>
<tr>
<td>Prior ischemic stroke (before qualifying event)</td>
<td>1 (7.1)</td>
</tr>
<tr>
<td>Mean (SD) duration from the onset of ischemic stroke to medication, days</td>
<td>12.9 (3.6)</td>
</tr>
<tr>
<td>NINDS classification, n (%)</td>
<td></td>
</tr>
<tr>
<td>Atherothrombotic</td>
<td>6 (42.9)</td>
</tr>
<tr>
<td>Lacunar</td>
<td>6 (42.9)</td>
</tr>
<tr>
<td>Undetermined</td>
<td>2 (14.3)</td>
</tr>
<tr>
<td>Arterial system involved, n (%)</td>
<td></td>
</tr>
<tr>
<td>Internal carotid artery</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Verteobasilar artery</td>
<td>4 (28.6)</td>
</tr>
<tr>
<td>Anterior cerebral artery</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Middle cerebral artery</td>
<td>10 (71.4)</td>
</tr>
<tr>
<td>Posterior cerebral artery</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Size of infarct, n (%)</td>
<td></td>
</tr>
<tr>
<td>Small (diameter &lt;1.5 cm)</td>
<td>7 (50.0)</td>
</tr>
<tr>
<td>Medium</td>
<td>7 (50.0)</td>
</tr>
<tr>
<td>Large (&gt;1/2 of lobe)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Modified Rankin scale at randomization, n (%)</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>3 (21.4)</td>
</tr>
<tr>
<td>1</td>
<td>5 (35.7)</td>
</tr>
<tr>
<td>2</td>
<td>5 (35.7)</td>
</tr>
<tr>
<td>3</td>
<td>1 (7.1)</td>
</tr>
<tr>
<td>Mean (SD) blood pressure, mm Hg</td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>138.1 (9.4)</td>
</tr>
<tr>
<td>Diastolic</td>
<td>84.9 (8.8)</td>
</tr>
<tr>
<td>Abnormal electrocardiogram, n (%)</td>
<td></td>
</tr>
<tr>
<td>0 (0.0)</td>
<td>4 (25.0)</td>
</tr>
<tr>
<td>Mean (SD) maximum platelet aggregation prior to medication (%) induced by 1 µmol/l 5-HT plus 3 µmol/l epinephrine</td>
<td>52.57 (20.72)</td>
</tr>
</tbody>
</table>

* KW = Kruskal-Wallis test; Fi = Fisher’s exact 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 ±
ischemic stroke prevention of recurrence in 1,510 patients with recent efficacy and safety of sarpogrelate with those of aspirin for trial has been conducted to evaluate and compare the ef-

In multiple-group comparisons, significant differ-
ences 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 com-
pletion): 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 treat-
ment in another patient). Both complications of micro-
scopic 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 treat-
ment of peripheral artery diseases (PAD) [29], and its clinical potential has been suggested for the treatment of atherosclerotic cardiovascular disease and diabetes mel-
litus [15, 30, 31]. A double-blind, randomized controlled trial has been conducted to evaluate and compare the ef-
cacy 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 pre-
defined criterion of noninferiority to aspirin for efficacy against recurrence of cerebral infarction, because the re-
currence rates of cerebral infarction were 72 (6.09% per year) with sarpogrelate and 58 (4.86% per year) with as-
pirin (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-re-
lated 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 ther-
apeutic 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 ensured 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 prevalence of ‘aspirin resistance’ in 10–40% of patients un-
der aspirin therapy [10, 33, 34]; patients with aspirin re-

sistance 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 spe-
cific antagonist for 5-HT2A, 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 in-
duces shape change and reversible aggregation, it is dif-
cult to assess the effects of its inhibitors if 5-HT alone is used to activate platelets [25]. 5-HT synergistically ampli-
ifies platelet aggregation induced by ADP, collagen, or epi-

nephrine, and thus the effects of 5-HT receptor antago-
nists have been conventionally evaluated by using the combination of 5-HT with a low concentration of a plate-
let agonist such as collagen, which by itself does not in-
duce platelet aggregation [22]. However, as platelet re-

sponses to low concentrations of agonists differ consider-
ably among individuals, the threshold concentration of the agonist (e.g. collagen) has to be determined separately for each platelet preparation. The whole process is in-
vitably 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 assess-
ment of platelet aggregation, based on combined stimula-
tion with 5-HT and epinephrine [26]. Epinephrine is a phisiological platelet agonist, which induces platelet ag-
gregation 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 the 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.

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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