Effects of fasudil in acute ischemic stroke: Results of a prospective placebo-controlled double-blind trial

Masato Shibuya a, Shunsaku Hirai b, Minoru Seto c, Shin-ichi Satoh c,*, Eiichi Ohtomo d

for the Fasudil Ischemic Stroke Study Group

Chukyo Hospital, Nagoya, Japan
Gunma University School of Medicine, Maebashi, Japan
Asahi Kasei Pharma Corporation, Shizuoka, Japan
Yokufukai Geriatric Hospital, Tokyo, Japan

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Abstract

Background: A multicenter, double-blind, placebo-controlled study was conducted to assess the efficacy and safety of fasudil, a Rho-kinase inhibitor (RKI), in the treatment of acute ischemic stroke.

Methods: A total of 160 patients, who were able to receive drug treatment within 48 h of acute ischemic stroke onset were enrolled. Patients received either 60 mg fasudil or a placebo (saline) by intravenous injection over 60 min, twice daily for 14 days. The primary end points were neurological status at 2 weeks after the start of treatment, and clinical outcome at 1 month after the onset of symptoms.

Results: Fasudil treatment resulted in significantly greater improvements in both neurological functions \((p=0.0013)\), and clinical outcome \((p=0.0015)\). There were no serious adverse events reported in the fasudil group. The average trough value (12 h values) of active metabolite hydroxyfasudil, another RKI, in healthy elderly volunteers receiving 60 mg of fasudil was 0.077 \(\mu\)M—a concentration well above that needed to inhibit Rho-kinase \((0.025–0.05 \mu\)M).

Conclusion: Treatment with fasudil within 48 h of acute ischemic stroke onset significantly improved the patient’s clinical outcome. This study found fasudil to be a useful and safe drug for patients with acute ischemic stroke. Further evaluations, for example, 3-month functional outcomes in a larger clinical trial, may help to define the efficacy of fasudil in acute ischemic stroke.

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Keywords: Acute ischemic stroke; Rho-kinase; Fasudil; Clinical trial

1. Introduction

Even though a large number of compounds have been proven to reduce the size of infarction in animal models, they have almost always failed in patients with acute ischemic stroke. The reasons for the unsuccessful clinical trials have been either the toxic side effects, or a limited therapeutic window [1]. Therefore, potent compounds with little or no side effects that can be administered within a few days after ischemic insult are being sought.

Ischemic stroke triggers secondary injury mechanisms such as inflammatory reactions, hemorheological abnormalities, vasoconstriction, and endothelial dysfunction. There is evidence that such secondary injury mechanisms contribute to the late stages in ischemic injury and results in the worsening of neurologic outcome. Therefore, intervention aimed at decreasing secondary injury mecha-
isms of the post-ischemic brain over a wide therapeutic window is an attractive therapeutic strategy for stroke.

Fasudil is a Rho-kinase inhibitor (RKI) that has shown clinical effectiveness on cerebral vasospasm in patients after clipping surgery for aneurysmal subarachnoid hemorrhage [2]. Fasudil significantly reduced angiographically demonstrable vasospasm, low-density regions on computerized tomography associated with vasospasm, symptomatic vasospasm, and the number of patients with poor clinical outcome associated with vasospasm. Fasudil was launched and has been widely used in Japan for that indication since 1995. Fasudil and its active metabolite, hydroxyfasudil, inhibit Rho-kinase more effectively than they inhibit other protein kinases; e.g., protein kinase C or myosin light chain kinase [3, 4].

Previous studies provide evidence that Rho-kinase or increased Rho-kinase activity is involved in the pathogenesis of various vascular lesions [5–8]: Rho-kinase is also thought to play a role in the mechanisms underlying the occurrence of hemodynamic dysfunctions and inflammatory processes [9–14]. Rho-kinase α (also called Rho-kinase, ROK α or ROCK I) is a Ser/Thr protein kinase that phosphorylates myosin phosphatase, inhibits myosin phosphatase activity, and contracts arterial smooth muscle. Rho-kinase β (AKA p160ROCK, ROK β, ROCK II) is a Ser/Thr protein kinase that phosphorylates myosin phosphatase, inhibits myosin phosphatase activity, and contracts arterial smooth muscle. Rho-kinase is one of the critical elements involved in: cell (neutrophil and monocyte) migration; endothelial injury through the down-regulation of endothelial nitric oxide synthase (eNOS) activity; production of O$_2^-$ in neutrophils and vessels; and hyperviscosity.

In animal models, fasudil and hydroxyfasudil showed promise in the treatment of acute ischemic stroke [15–17]. Benefits observed appear to be mediated by various mechanisms; for example, preventing inflammatory response by inhibiting neutrophil and monocyte infiltration [18, 19] and inhibiting the production of O$_2^-$ in neutrophils and vessels [20, 21]; and improving hemodynamic function by increasing regional cerebral blood flow [22, 23], preventing hyperviscosity [24], and upregulating eNOS activity in endothelial cells [9].

In a Phase IIb, multicenter, double-blind trial, patients with acute cerebral thrombosis and using a 48-h treatment window, received 10, 30 or 60 mg fasudil by intravenous infusion over 60 min, twice daily for 14 days. Fasudil treatment resulted in dose-dependent improvements in neurological functions and clinical outcome (published in Japanese). This Phase IIb trial demonstrated that doses up to 120 mg per day were well-tolerated in patients. We are reporting here the results of a randomized placebo-controlled double-blind (Phase III) trial of fasudil in patients with acute cerebral thrombosis. The endpoints of the trial were neurological status at 2 weeks after the start of treatment, and clinical outcome at 1 month after the onset of symptoms.

2. Subjects and methods

2.1. Randomized placebo-controlled double-blind (Phase III) trial

2.1.1. Study design and procedures

This was a multicenter, randomized, double-blind, placebo-controlled trial to evaluate the efficacy and safety of intravenous fasudil in patients with acute ischemic stroke. A total of 160 patients were enrolled at 29 neurological and neurosurgical institutions in Japan. The determination of sample size was calculated from the results of a Phase IIb trial of fasudil in patients with acute ischemic stroke and, based on a 0.9 power to detect a significant difference ($p=0.025$, single-sided), 80 patients should be required for each group. Informed consent was obtained from the patient or the nearest relative. This study was performed in accordance with Good Clinical Practice (Ministry of Health and Welfare of Japan), and the protocol was approved by the ethical committee in each hospital.

2.1.2. Patients

The target population was patients at least 20 years of age who possessed neurological deficits within 48 h of the onset of an acute cerebral ischemic stroke due to thrombosis (atherosclerosis and lacunar infarct). They had to exhibit a motor deficit (from moderate to severe) of the arms and/or legs, and have a Japan coma scale score of 0–3 at baseline (from clear level of consciousness to awake but disoriented). Table 1 lists inclusion and exclusion criteria.

2.1.3. Treatment

A random list was prepared by an independent statistical center. Patients were randomized to fasudil or placebo with the use of separate randomization lists balanced for each participating center by personnel unrelated to the study. Treatment with fasudil (60 mg/60 min) or placebo was started within 48 h after acute ischemic stroke and was continued twice daily for 14 days. Concomitant treatment

Table 1

<table>
<thead>
<tr>
<th>Inclusion criteria</th>
<th>Exclusion criteria</th>
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</thead>
<tbody>
<tr>
<td>Age ≥ 20 years</td>
<td>History of severe cardiopulmonary, hepato-renal or metabolic diseases</td>
</tr>
<tr>
<td>Male or female</td>
<td>Women of childbearing potential</td>
</tr>
<tr>
<td>Informed consent</td>
<td>Cerebral hemorrhage on the CT scan</td>
</tr>
<tr>
<td></td>
<td>Systolic blood pressure &lt; 90 mm Hg</td>
</tr>
</tbody>
</table>
| Acute ischemic stroke due to thrombosis | }

| Treatment onset within 48 h after stroke onset |
| Japan coma scale score of 0–3 |
| Motor deficit (from moderate to severe) of the arms and/or legs |
with other drugs, such as antiplatelets, steroids, low-molecular weight dextran and glycerol, was permitted. Administration of drugs approved for the treatment of acute stroke, such as sodium ozagrel (a thromboxane A2 synthetase inhibitor) and edaravone (a free radical scavenger), were prohibited. The use of thrombolytic therapy with urokinase or tissue plasminogen activator was also prohibited.

2.1.4. Assessments

The primary end points were neurological status at 2 weeks after the start of treatment and the clinical outcome at 1 month after the onset of symptoms. All assessments were blinded. Neurological status was assessed by the Motor System of Japan Stroke Scale (JSS) [25], measuring the severity of paresis in the hands, arms and legs. The JSS (motor system) score produced ordinal scores ranging from 0 (no neurological deficit) to 4 (severe) based on the ratings of 3 individual components of a standardized neurological examination. Neurological outcome was determined 2 weeks after the start of the treatment and defined as: “ΔJSS ≤ −3; marked improvement”, “ΔJSS = −2; improvement”, “ΔJSS = −1; minimal improvement”, “ΔJSS = 0; no change”, and “ΔJSS ≥ 1; worsening” (Table 2).

The clinical outcome was assessed according to a modified Rankin Scale (mRS) (0: no symptoms at all; 1: no significant disability despite symptoms; 2: slight disability; 3: moderate disability; 4: moderately severe disability; 5: severe disability). Clinical outcome was determined 1 month after the onset of symptoms and defined as: “ΔmRS ≤ −3; marked improvement”, “ΔmRS = −2; improvement”, “ΔmRS = −1; minimal improvement”, “ΔmRS = 0; no change”, and “ΔmRS ≥ 1; worsening” (Table 2).

Before opening the code, the study review committee checked the uniformity and appropriateness of each judgment by reviewing the data for each patient. An independent statistical center performed the statistical analyses in a blind manner.

2.2. Pharmacokinetic analysis

The pharmacokinetics of fasudil and its active metabolite hydroxyfasudil were assessed in 12 healthy elderly volunteers (6 men, 6 women; mean age: 69.4 years) after intravenous infusion of fasudil (60 mg/60 min).

Blood samples were taken from the cephalic vein 30, 60, 70, 80 and 90 min and 2, 3, 4, 6, 8 and 12 h after the administration of fasudil. The heparinized blood was immediately centrifuged at 3000 rpm for 10 min at 4 °C, and plasma was stored at −20 °C until assayed.

Concentrations of fasudil and hydroxyfasudil in plasma were measured by liquid chromatograph tandem mass spectrometry. The area under the plasma concentration–time curve from time 0 to infinity (AUC0–∞) was determined using the trapezoidal rule [26]. The maximum plasma concentration (Cmax) was derived directly from individual measurements. The elimination half-life (t1/2) was calculated by non-linear regression.

2.3. Kinase assay

Human recombinant truncated Rho-kinase α (1–543) and Rho-kinase β (1–727) as N-terminal His-tagged proteins were expressed in Sf9 cells with a baculovirus system (Invitrogen) and purified on a HiTrap chelating column charged with Ni2+ (Amersham Biosciences). The inhibitory effects of fasudil and hydroxyfasudil on Rho-kinase activities were examined by kinase assay based on an enzyme immunoassay system. Microtiter plates were coated with myosin binding subunit (MBS) of myosin phosphatase (500 ng/ml, 50 μl/well). The phosphorylation reaction started by adding 85 μl/well of a reaction mixture (10 ng/ml Rho-kinase α or Rho-kinase β/100 mM NaCl/5 mM dithiothreitol/5 mM MgSO4/30 μM ATP for Rho-kinase α or 10 μM ATP for Rho-kinase β assay/50 mM Tris–HCl (pH7.5)) in the absence or presence of various concentrations of either fasudil or hydroxyfasudil. After incubating for 10 min at 30 °C, the reaction was stopped by adding 85 μl of a 2% phosphoric acid. The phosphorylated MBS was detected with the anti-phospho-MBS antibody [4]. The inhibitory effects of fasudil and hydroxyfasudil were expressed as IC50 values.

2.4. Statistics

Characteristics at baseline were compared using Fisher’s test, χ2 test and Wilcoxon rank sum test. Comparisons of proportions of efficacy end-point variables between treat-

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Table 2 Improvement index in the Motor System of Japan Stroke Scale and modified Rankin Scale

<table>
<thead>
<tr>
<th>Improvement of neurological status</th>
<th>ΔJSS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marked improvement</td>
<td>≤ −3</td>
</tr>
<tr>
<td>Improvement</td>
<td>−2</td>
</tr>
<tr>
<td>Minimal improvement</td>
<td>−1</td>
</tr>
<tr>
<td>No change</td>
<td>0</td>
</tr>
<tr>
<td>Worsening</td>
<td>1≤</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Improvement of clinical outcome</th>
<th>ΔmRS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marked improvement</td>
<td>≤ −3</td>
</tr>
<tr>
<td>Improvement</td>
<td>−2</td>
</tr>
<tr>
<td>Minimal improvement</td>
<td>−1</td>
</tr>
<tr>
<td>No change</td>
<td>0</td>
</tr>
<tr>
<td>Worsening</td>
<td>1≤</td>
</tr>
</tbody>
</table>

Efficacy on neurological functions and clinical outcome were evaluated by the changes in JSS score and mRS score. JSS = Motor System of Japan Stroke Scale; mRS = modified Rankin Scale.
ment groups were made using Wilcoxon rank sum test. Tests were one sided, with a significance level of \( p = 0.025 \) for the efficacy end points. Adverse events were compared using Fisher’s exact test and \( \chi^2 \) test.

3. Results

3.1. Randomized placebo-controlled double-blind (Phase III) trial

3.1.1. Demographic and clinical data

Between July 1998 and February 2002, 160 patients were enrolled in the trial. The trial profile is shown in Fig. 1. A total of 149 patients completed the treatment. Treatment was discontinued in 11 patients (4 in the fasudil group, 7 in the placebo group). Reasons for treatment discontinuation were similar in both groups and included adverse event and request of patient or investigator. All patients enrolled in this trial were included in the assessment of efficacy and safety. The demographic and background data of patients, including age, sex, neurological status and time from onset to treatment, were compared and no statistically significant differences were found between the treatment groups (Table 3). The number of patients with hypertension, diabetes mellitus or hyperlipidemia was similar in the two groups. Lacunar infarct was frequent (more than 60%) in patients enrolled in this trial, and there was no significant difference in the types of stroke between the two groups.

3.1.2. Neurological status

Fasudil treatment resulted in significantly greater improvement in neurological status at 2 weeks after the start of treatment \( (p = 0.0013) \) (Fig. 2). Treatment with fasudil

![Fig. 1. Trial profile.](image)
Increased the number of markedly improved and improved patients from 15 cases (19%) of the placebo group to 24 cases (30%) of the fasudil group, and reduced the number of worsened patients from 14 cases (18%) of placebo group to 2 cases (2%) of the fasudil group.

3.1.3. Clinical outcome at 1 month

Fasudil treatment resulted in significantly greater improvement in clinical outcome at 1 month after the onset of symptoms ($p = 0.0015$) (Fig. 3). Treatment with fasudil increased the number of markedly improved and improved patients from 35 cases (44%) of the placebo group to 51 cases (63%) of the fasudil group, and reduced the number of worsened patients from 8 cases (10%) of placebo group to 1 case (1%) of the fasudil group.

Twenty-nine patients (35.8%) out of 81 in fasudil group and 22 patients (27.8%) out of 79 in placebo group showed no symptoms at all (mRS = 0), and 4 patients (4.9%) in fasudil group and 8 patients (10.1%) in placebo group showed severe disability (mRS = 5) on their 1-month clinical outcome.

### 3.1.4. Safety

No serious adverse events were observed in both groups. The adverse events occurring more than 5% in fasudil-treated patients are summarized in Table 4. The number of patients with positive urinary occult blood significantly increased the number of markedly improved and improved patients from 15 cases (19%) of the placebo group to 24 cases (30%) of the fasudil group, and reduced the number of worsened patients from 14 cases (18%) of placebo group to 2 cases (2%) of the fasudil group.

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### Table 4

<table>
<thead>
<tr>
<th>Adverse event</th>
<th>Fasudil (n = 81)</th>
<th>Placebo (n = 79)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myalgia</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Headache</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>Insomnia</td>
<td>18</td>
<td>13</td>
</tr>
<tr>
<td>Constipation</td>
<td>31</td>
<td>32</td>
</tr>
<tr>
<td>Hepatic function abnormal</td>
<td>17</td>
<td>12</td>
</tr>
<tr>
<td>GOT increased</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Sugar blood level increased</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>Triglyceride increased</td>
<td>13</td>
<td>10</td>
</tr>
<tr>
<td>Common cold syndrome</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>Anemia</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Urinary occult blood positive</td>
<td>5*</td>
<td>14</td>
</tr>
<tr>
<td>Urinary tract infection</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Fever</td>
<td>9</td>
<td>13</td>
</tr>
</tbody>
</table>

Adverse events are presented if they occurred in >5% of fasudil-treated patients. Values represent number of patients. The number of patients with urinary occult blood positive was significantly decreased with fasudil. No statistically significances were found between the two groups in other adverse events. *$p < 0.05$ vs. placebo group.

### Table 5

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Fasudil</th>
<th>Hydroxyfasudil</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{max}$ (ng/ml)</td>
<td>299.2 ± 62.0</td>
<td>256.7 ± 60.4</td>
</tr>
<tr>
<td>AUC (ng h/ml)</td>
<td>278.9 ± 72.5</td>
<td>1040.9 ± 205.8</td>
</tr>
<tr>
<td>$t_{1/2}$ (h)</td>
<td>0.78 ± 0.16</td>
<td>4.66 ± 0.49</td>
</tr>
<tr>
<td>Trough value (ng/ml)</td>
<td>0.0 ± 0.0</td>
<td>25.4 ± 6.3</td>
</tr>
</tbody>
</table>

Pharmacokinetic parameters are expressed as the mean ± S.D. of 12 healthy elderly volunteers.
decreased with fasudil (14 of 79 patients in placebo group, 5 of 81 patients in fasudil group; \( p < 0.05 \)). No statistically significant differences were found between the placebo and fasudil group for other adverse events.

### 3.2. Pharmacokinetic parameter

The pharmacokinetic parameters for fasudil and hydroxyfasudil are listed in Table 5. Hydroxyfasudil was found following intravenous infusion of fasudil, and the maximum plasma concentration of hydroxyfasudil was approximately 86% of the parent drug. The elimination half-life of hydroxyfasudil was longer than that of fasudil. After intravenous infusion of fasudil, \( t_{1/2} \) of fasudil and hydroxyfasudil was 0.78 and 4.66 h, respectively. The AUC\(_{0\rightarrow\infty} \) value of hydroxyfasudil was 3.7 times higher than value of fasudil. The trough value (12-h value) of hydroxyfasudil was 25.4 ng/ml (0.077 μM).

![Graph A](image1.png)

![Graph B](image2.png)

![Graph C](image3.png)

![Graph D](image4.png)

**Fig. 4.** Inhibitory effects of fasudil and hydroxyfasudil on Rho-kinase α or β activities. Each data point represents the mean±S.E.M. of four experiments. The lowest concentration of fasudil or hydroxyfasudil which inhibited Rho-kinase α or β was 0.025–0.05 μM.
The repeated doses did not seem to produce an accumulation of both fasudil and hydroxyfasudil in plasma (data not shown).

3.3. Rho-kinase inhibition

Fasudil (0.05–1.6 μM) or hydroxyfasudil (0.025–1.6 μM) dose-dependently inhibited Rho-kinase α or β (Fig. 4). The lowest concentration of fasudil or hydroxyfasudil at which inhibition of the Rho-kinase α or β was 0.025–0.05 μM. Fasudil and hydroxyfasudil potently inhibited both Rho-kinase α and Rho-kinase β in the same degree (Table 6).

4. Discussion

In this trial of 160 patients using a 48-h treatment window, the RKI fasudil produced statistically significant improvements in neurological status and clinical outcome; especially, in reducing the number of worsening patients. This data suggest that the therapeutic window for fasudil at the current dose level is at least 48 h after the onset of an acute ischemic stroke. Acute diseases, such as stroke, are pathophysiological events that initiate a deleterious cascade, which include hemodynamic dysfunctions, inflammatory processes, edema, and finally, neuronal cell death. In acute stroke, the brain is damaged by primary impact, cerebral ischemia, and secondary injury; for example, continuous hemodynamic dysfunction in cerebral circulation and inflammatory processes potentially contribute to the onset and maintenance of ischemic neuronal damage [16,27–29]. These events can last from several hours to weeks in duration.

Inflammatory responses promote the migration and recruitment of neutrophils and macrophages into the area of ischemic injury. Neutrophils and macrophages release proteases and oxygen radicals, and worsen pathology by damaging tissue that survives the initial ischemic insult [27,30]. Akopov et al. reported that neutrophils accumulate at 6 to 12 h after stroke onset, and remain at high levels for 6 to 9 days in patients [27]. This neutrophil accumulation correlates with the severity of the brain tissue damage and poor neurological outcome [27,31]. After a week, monocyte accumulation occurs and persists for many weeks [27]. Fasudil and hydroxyfasudil were reported to have a direct inhibitory effect on neutrophil and monocyte migration in vitro [18,19]. In a cerebral microembolism model in rats, neutrophil accumulation in untreated ischemic brain was observed, while in the treatment group, fasudil and hydroxyfasudil prevented neutrophil accumulation [16,18].

Vasoconstriction, hyperviscosity and endothelial damage are thought to be contributory factors in hemodynamic dysfunction. Fasudil and hydroxyfasudil improve hemodynamic function by increasing regional cerebral blood flow [22,23] as well as preventing hyperviscosity [24], and upregulating eNOS activity in endothelial cells [9]. Rho-kinase is thought to play a role in the mechanisms underlying the occurrence of hemodynamic dysfunctions and inflammatory processes [5–14]. Fasudil and hydroxyfasudil inhibit Rho-kinase α with a potency similar to that for Rho-kinase β. Fasudil’s inhibition of Rho-kinase α and β is similarly potent to hydroxyfasudil’s inhibition of Rho-kinase α and β. In our study discussed here, fasudil significantly improved neurological dysfunction and clinical outcome in acute cerebral thrombosis. Clinical effectiveness of fasudil in patients with acute stroke may be due to the reduction of the various secondary brain damage such as the inflammatory reactions and hemodynamic abnormalities by inhibiting Rho-kinases. In the present study, inhibitory effects of fasudil on Rho-kinase α and β, and hydroxyfasudil on Rho-kinase α were measured at concentrations of 0.05 to 1.6 μM. Inhibitory effect of hydroxyfasudil on Rho-kinase β was measured at concentrations of 0.025 to 0.8 μM. Fasudil (0.05 μM) inhibited Rho-kinase α and β by 15–16%. Hydroxyfasudil (0.05 μM) also inhibited Rho-kinase α by 16%, and hydroxyfasudil (0.025 μM) inhibited Rho-kinase β by 16%. To determine the inhibitory efficacy of fasudil or hydroxyfasudil on Rho-kinase α or β, it may be useful to examine the effects of fasudil or hydroxyfasudil at lower concentrations.

Immediately after an intravenous infusion of fasudil (60 mg/60 min) in elderly healthy volunteers, its active metabolite, hydroxyfasudil, was observed, having a maximum plasma concentration of approximately 86% of the parent drug. Hydroxyfasudil was eliminated slowly. AUC values of hydroxyfasudil were 3.7 times higher than those of fasudil. Hydroxyfasudil may contribute to the potency and long term action of fasudil. Intravenous infusion of fasudil provided a minimum trough plasma concentration for its active metabolite, hydroxyfasudil of 0.077 μM—a concentration well above that needed to inhibit Rho-kinase (0.025–0.05 μM). These results suggest that clinical effectiveness of fasudil in patients with acute stroke is associated with maintaining a total effective plasma concentration of fasudil plus hydroxyfasudil over the 14-day treatment period. This can be accomplished by a twice daily treatment.

From June 1995 through June 2001, approximately 52,000 patients with aneurysmal subarachnoid hemorrhage have been treated with fasudil for cerebral vasospasm. It has demonstrated an excellent tolerability profile (published in Japanese). In the present clinical trial, fasudil was also well-tolerated in patients with acute stroke. These results indicate that fasudil offers a safe option for the treatment of cerebral infarction in patients with acute stroke.
cerebral thrombosis as well as cerebral vasospasm in patients with subarachnoid hemorrhage.

Several limitations can be raised in the present trial. First, this Phase III trial was performed in a relatively small number of patients, and modified Rankin Scale was used to assess the clinical outcome with 1-month follow-up. The determination of sample size was calculated from the results of a Phase IIb trial of fasudil and to be the minimum number necessary to detect a clinical meaningful difference. Further evaluation, for example 3-month functional outcomes in a larger clinical trial, may help to define the efficacy of fasudil in acute ischemic stroke. Second, to assess the neurological status and clinical outcome, we used only the Motor System of JSS and mRS in the present study. To determine the efficacy of fasudil in acute stroke, it may be useful to examine in clinical trials the effects of fasudil on the sensory, visual and cognitive deficits, as well as its effects using other scales, such as the Barthel index.

Treatment with fasudil within 48 h of onset improved the outcome of acute ischemic stroke. This study found fasudil to be a useful and safe drug for the patients with acute ischemic stroke. The ideal therapeutic window for fasudil seems to be at least 48 h. Fasudil is particularly effective at targeting secondary injurious mechanisms after onset of stroke; such as, inflammatory reactions and hemodynamic dysfunctions. Fasudil provides a novel and efficacious approach for treatment of acute stroke.

Acknowledgements

The authors thank Mark Smith for pertinent comments.

Appendix A

A.1. Committee members

Hideo Tohgi, MD, Iwate Medical University; Kyuya Kogure, MD, Kogure Hospital; Kintomo Takakura, MD, Tokyo Women’s Medical University; Eiichi Ohtomo, MD, Yokufukai Geriatric Hospital; Shunsaku Hirai, MD, Gunma University; Akiro Terashi, MD, Nippon Medical School; Fumio Gotoh, MD, Keio University; Jun Yoshida, MD, Nagoya University; Masato Shibuya, MD, Chukyo Hospital; Eiichi Ito, MD, National East Nagoya Hospital; Masatoshi Fujishima, MD, Kyushu University; Mitsuyoshi Nakashima, MD, Hamamatsu University School of Medicine; Nobuo Aoki, MD, Tokyo Medical and Dental University.

A.2. Participating centers

This trial was performed with the cooperation of the doctors and staff of the following neurological and neurosurgical institutions and hospitals in Japan: Asahikawa Red Cross Hospital, Nayoro City General Hospital, Hakodate Red Cross Hospital, Abashiri Neurosurgical Hospital, Towada City Hospital, Iwate Medical University Hospital, Research Institute for Brain and Blood Vessels Akita, Mito National Hospital, Mihara Memorial Hospital, National Saitama Hospital, Saitama Cardiovascular Respiratory Center, Tokyo Women’s Medical University Hospital, Tokai University Hospital, Yokohama Stroke and Brain Center, Saku Central Hospital, Shizuoka Saiseikai General Hospital, Shizuoka Kosei Hospital, Chukyo Hospital, Toyota Medical Corporation Kariya General Hospital, Komaki City Hospital, Kamo Hospital, Toyohashi Municipal Hospital, National Mie Chuo Hospital, Osaka Police Hospital, Minami Wakayama National Hospital, Chikamori Hospital, Kyushu Rosai Hospital, Saiseikai Yahata Hospital, and National Kyushu Medical Center.


