Safety and Efficacy of Fasudil Monotherapy and Fasudil-Ozagrel Combination Therapy in Patients With Subarachnoid Hemorrhage: Sub-analysis of the Post-marketing Surveillance Study

Yoshio SUZUKI, Masato SHIBUYA*, Shin-ichi SATOH**, Hirotoshi SUGIYAMA***, Minoru SETO†, and Kintomo TAKAKURA††

Department of Neurosurgery, Nagoya Daini Red Cross Hospital, Nagoya; *Department of Neurosurgery, Chukyo Hospital, Nagoya; **Scientific Affairs & Sales Promotion Department and ***Reliability Assurance Department, Asahi Kasei Pharma Corporation, Tokyo; †Research Center, Asahi Kasei Pharma Corporation, Shizuoka; ††Department of Neurosurgery, Tokyo Women’s Medical University Hospital, Tokyo

Abstract

Sub-analysis of the fasudil post-marketing surveillance study compared the safety and efficacy of fasudil plus ozagrel to fasudil only. A total of 3690 patients received fasudil and 1138 received fasudil plus ozagrel between 1995 and 2000. The occurrence of adverse events, occurrence of low density areas associated with vasospasm on computed tomography, absence of symptomatic vasospasm, and poor clinical outcomes associated with vasospasm were compared between the fasudil and fasudil plus ozagrel groups. The pharmacokinetics of fasudil were assessed in 5 patients with subarachnoid hemorrhage. The drug interaction between fasudil and ozagrel was pharmacologically investigated in vitro and in vivo. The occurrence of adverse events and clinical outcomes were similar between the two groups. The occurrences of symptomatic vasospasm and low density areas were lower in the fasudil group than in the fasudil plus ozagrel group. The average trough value (8-hour value) of the fasudil active metabolite, hydroxyfasudil, was 50 nM. Fasudil showed no pharmacological interaction with ozagrel. The combination of fasudil plus ozagrel was well tolerated, but did not result in better efficacy than fasudil only.

Key words: aneurysmal subarachnoid hemorrhage, cerebral vasospasm, fasudil, ozagrel, post-marketing surveillance study, Rho-kinase

Introduction

Therapy with fasudil, a Rho-kinase inhibitor, is effective for the prevention of cerebral vasospasm and subsequent ischemic injury after surgery for aneurysmal subarachnoid hemorrhage (SAH). In a placebo-controlled double-blind (phase III) trial, fasudil reduced angiographically demonstrable vasospasm, low density regions on computed tomography (CT) associated with vasospasm, symptomatic vasospasm, and the number of patients with poor clinical outcome associated with vasospasm. Fasudil has been approved for the indication of preventing cerebral vasospasm and subsequent ischemic injury in patients undergoing surgery for SAH in Japan. The post-marketing surveillance (PMS) study confirmed the results of the phase III trial and further assessed the tolerability, safety, and efficacy of fasudil in a larger number of patients with SAH. Fasudil is widely used in patients with SAH in Japan. A randomized trial in the People’s Republic of China described the safety and efficacy of fasudil for suppressing cerebral vasospasm after SAH, and fasudil is used in patients undergoing surgery for SAH.

The pathogenesis of delayed cerebral vasospasm and subsequent ischemic injury after SAH is thought to be related to a number of pathological processes, including smooth muscle contraction, endothelial damage, hyperviscosity, and inflammatory reactions. Fasudil is believed to prevent
delayed cerebral vasospasm and subsequent ischemic injury through the inhibition of Rho-kinase which reduces vasoconstriction, occurrence of endothelial damage, hyperviscosity, and inflammatory responses.

Ozagrel is another antispastic drug clinically used in Japan. A randomized double-blind study reported that ozagrel was effective against cerebral vasospasm and cerebral ischemic symptoms after SAH caused by aneurysmal rupture. The mechanism of action for ozagrel is different from that of fasudil. Ozagrel is a thromboxane A2 synthase inhibitor, and ameliorates vascular contraction and platelet aggregation. Combination therapy using fasudil and ozagrel is reported to be effective.

The present sub-analysis of the fasudil PMS study compared the safety and efficacy of the combination of fasudil plus ozagrel with fasudil only to assess the interactions between fasudil and ozagrel in a large number of patients.

Materials and Methods

I. Sub-analysis of the fasudil PMS study

The open multicenter PMS study for fasudil was conducted in accordance with guidelines laid down by the Ministry of Health and Welfare of Japan between 1995 and 2000. A total of 3690 patients were treated with fasudil only and 1138 patients were treated with fasudil and ozagrel. The data were collected and analyzed by a PMS group.

Fasudil (30 mg) was prescribed for intravenous administration over 30 minutes three times a day. Fasudil treatment was generally started within 24 hours after aneurysmal surgery and this daily regimen continued for 14 consecutive days. Ozagrel (80 mg/day) was given by continuous intravenous infusion for 14 days after aneurysmal surgery.

Adverse event data was collected through physicians reports; any possible association with the study medication was assessed (possible, probable, unknown, or improbable relationship to treatment) by the attending physician. The incidence of intracranial bleeding was confirmed by CT. Adverse events were classified and analyzed by the PMS group.

The endpoints for determining efficacy were defined as: reduction in both incidence and size of low density areas due to vasospasm on postoperative CT; reduction in incidence of symptomatic vasospasm; and reduction in incidence of poor outcome due to delayed neurological deficits from vasospasm. Low density areas on postoperative CT (between days 5 and 42) were classified into five grades: none; mild, including small lacunar-like low density areas less than 1 cm in diameter (corrected for magnification); moderate, including low density areas greater than 1 cm but limited to territories around the branches of major cerebral artery; severe, including low density areas extending to the entire territory of a major cerebral artery; and large, including low density areas expanding over several territories supplied by the major cerebral arteries. Deterioration in level of consciousness, appearance of motor weakness, sensory deficit, and aphasia were recorded as symptomatic vasospasm if there was no other explanation in the postoperative period. Clinical outcome was assessed at 1 month after the hemorrhage according to the Glasgow Outcome Scale (good recovery, moderate disability, severe disability, persistent vegetative state, or death).

II. Pharmacokinetic analysis

The pharmacokinetics of fasudil and its active metabolite hydroxyfasudil were assessed in 5 patients (4 men, 1 woman; mean age 44 years) undergoing surgery for SAH after intravenous infusion of fasudil (30 mg/30 min). Blood samples were taken from the cephalic vein at 30 and 45 minutes, and 2 and 8 hours after the initiation of fasudil administration. Concentrations of fasudil and hydroxyfasudil in plasma were measured by enzyme immunoassay. The area under the plasma concentration-time curve (AUC) from 0 to 2 hours of fasudil (fasudil is quickly metabolized to hydroxyfasudil) or AUC from 0 to 8 hours of hydroxyfasudil was determined using the trapezoidal rule. The maximum plasma concentration or minimum trough plasma concentration (8-hour value) was derived directly from individual measurements. The elimination half-life was calculated by non-linear regression.

III. Drug interaction

Drug interaction between fasudil and ozagrel was investigated using the rat tail-transection bleeding time test and measuring Rho-kinase activities. Male Sprague-Dawley rats, weighing 250 g to 330 g, were anesthetized with pentobarbital sodium (40 mg/kg, i.p.). Bleeding was induced by sectioning the extremity of the tail 2 mm from the tip. The tails were gently blotted with filter paper every 15 seconds and the time in minutes to cessation of bleeding (defined as no rebleeding for 30 seconds) was noted. The observation time was limited to 30 minutes. Care was taken that no pressure was exerted on the tail tips, which could affect hemostasis. Fasudil (1 mg/kg), ozagrel (1 mg/kg), fasudil plus ozagrel, or saline was administered through the left femoral vein 5 minutes before tail transection.

Human recombinant truncated Rho-kinase α.
Table 1 Baseline characteristics of patients

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Fasudil</th>
<th>Fasudil + ozagrel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total patients</td>
<td>3690</td>
<td>1138</td>
</tr>
<tr>
<td>Age (yrs), mean ± SD</td>
<td>58.6 ± 12.3</td>
<td>58.4 ± 12.1</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>men</td>
<td>1373 (37.2)</td>
<td>391 (34.4)</td>
</tr>
<tr>
<td>women</td>
<td>2317 (62.8)</td>
<td>747 (65.6)</td>
</tr>
<tr>
<td>Hunt &amp; Kosnik grade</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I, Ia</td>
<td>414 (11.2)</td>
<td>129 (11.3)</td>
</tr>
<tr>
<td>II</td>
<td>1421 (38.5)</td>
<td>457 (40.2)</td>
</tr>
<tr>
<td>III</td>
<td>971 (26.3)</td>
<td>329 (28.9)</td>
</tr>
<tr>
<td>IV</td>
<td>674 (18.3)</td>
<td>182 (16.0)</td>
</tr>
<tr>
<td>V</td>
<td>206 (5.6)</td>
<td>41 (3.6)</td>
</tr>
<tr>
<td>missing</td>
<td>4 (0.1)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Fisher grade</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>90 (2.4)</td>
<td>15 (1.3)</td>
</tr>
<tr>
<td>2</td>
<td>788 (21.4)</td>
<td>278 (24.4)</td>
</tr>
<tr>
<td>3</td>
<td>1994 (54.0)</td>
<td>616 (54.1)</td>
</tr>
<tr>
<td>4</td>
<td>811 (22.0)</td>
<td>229 (20.1)</td>
</tr>
<tr>
<td>missing</td>
<td>7 (0.2)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Ticlopidine and/or nizofenone use</td>
<td>316 (8.6)</td>
<td>113 (9.9)</td>
</tr>
</tbody>
</table>

Values represent number of patients, except where otherwise indicated. Values in parentheses represent percentages of total. SD: standard deviation.

Table 2 Adverse events

<table>
<thead>
<tr>
<th>Adverse events</th>
<th>Fasudil</th>
<th>Fasudil + ozagrel</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>3690</td>
<td>1138</td>
</tr>
<tr>
<td>Adverse events</td>
<td>487 (13.2)NS</td>
<td>127 (11.2)</td>
</tr>
<tr>
<td>Intracranial bleeding</td>
<td>71 (1.9)NS</td>
<td>17 (1.5)</td>
</tr>
<tr>
<td>Hypotension</td>
<td>14 (0.4)NS</td>
<td>6 (0.5)</td>
</tr>
</tbody>
</table>

Values represent number of patients. Values in parentheses represent percentages of total. NS indicates no significant difference from fasudil + ozagrel group.

IV. Statistical analysis

Characteristics at base line were compared using Student’s t-test, the $\chi^2$ test, and Wilcoxon’s test. Adverse events and efficacy were compared using the $\chi^2$ test. The rat tail bleeding time test and kinase assay were analyzed using two-way analysis of variance. Tests were two sided, with a significance level of $p < 0.05$.

Results

I. Sub-analysis of the PMS study

Comparison of the demographic and background data of patients, including Hunt and Kosnik and Fisher scores on admission, found no statistically significant differences between patients receiving fasudil and patients receiving fasudil plus ozagrel (Table 1). The proportion of patients with ticlopidine (antiplatelet) and/or nizofenone (brain-protecting drug) treatment was not different between the two groups (Table 1).

Adverse events were observed in a total of 487 (13.2%) of 3690 patients in the fasudil group, and in 127 (11.2%) of 1138 patients in the fasudil plus ozagrel group. There was no significant difference between the two groups (Table 1). The incidences of intracranial bleeding and hypotension were 1.9% (71/3690) and 0.4% (14/3690), respectively, in the fasudil group, and 1.5% (17/1138) and 0.5% (6/1138), respectively, in the fasudil plus ozagrel group. There were no significant differences in the incidences of intracranial bleeding and hypotension between the two groups (Table 2).

The two treatments were largely comparable in frequency of adverse events classified according to organ system (Table 3). However, the frequency of reported hepatic and hepatobiliary disorders was significantly higher in the fasudil group than in the fasudil plus ozagrel group (Table 3).

The occurrences of mild, moderate, severe, or large low density areas due to vasospasm on CT and symptomatic vasospasm were lower in the fasudil group than in the fasudil plus ozagrel group (p < 0.01) (Table 4). The proportion of patients with the

(1–543) and Rho-kinase $\beta$ (1–727) as N-terminal His-tagged proteins were expressed in Sf9 cells with a baculovirus system (Invitrogen Corporation, Carlsbad, Calif., U.S.A.) and purified on a HiTrap chelating column charged with Ni$^{2+}$ (GE Healthcare UK Ltd., Chalfont St Giles, Buckinghamshire, England). The inhibitory effects of fasudil, hydroxyfasudil, and ozagrel on Rho-kinase activities were examined by kinase assay based on an enzyme immunoassay system. Microtiter plates were coated with a myosin binding subunit (MBS) of myosin phosphatase (500 ng/ml, 50 $\mu$l/well). The phosphorylation reaction was started by adding 85 $\mu$l/well of a reaction mixture (10 ng/ml Rho-kinase $\alpha$ or Rho-kinase $\beta$/100 mM NaCl/5 mM dithiothreitol/5 mM MgSO$_4$/30 $\mu$M adenosine triphosphate for Rho-kinase $\alpha$ or 10 $\mu$M adenosine triphosphate for Rho-kinase $\beta$ assay/50 mM Tris-HCl [pH 7.5]) in the absence or presence of fasudil, hydroxyfasudil, or ozagrel. After incubating for 10 minutes at 30°C, the reaction was stopped by adding 85 $\mu$l of 2% phosphoric acid. The phosphorylated MBS was detected with the anti phospho-MBS antibody. MBS phosphorylation produced by the addition of a reaction mixture was defined as 100%.

IV. Statistical analysis

Characteristics at base line were compared using Student’s t-test, the $\chi^2$ test, and Wilcoxon’s test. Adverse events and efficacy were compared using the $\chi^2$ test. The rat tail bleeding time test and kinase assay were analyzed using two-way analysis of variance. Tests were two sided, with a significance level of $p < 0.05$.

Results

I. Sub-analysis of the PMS study

Comparison of the demographic and background data of patients, including Hunt and Kosnik and Fisher scores on admission, found no statistically significant differences between patients receiving fasudil and patients receiving fasudil plus ozagrel (Table 1). The proportion of patients with ticlopidine (antiplatelet) and/or nizofenone (brain-protecting drug) treatment was not different between the two groups (Table 1).

Adverse events were observed in a total of 487 (13.2%) of 3690 patients in the fasudil group, and in 127 (11.2%) of 1138 patients in the fasudil plus ozagrel group. There was no significant difference between the two groups (Table 1). The incidences of intracranial bleeding and hypotension were 1.9% (71/3690) and 0.4% (14/3690), respectively, in the fasudil group, and 1.5% (17/1138) and 0.5% (6/1138), respectively, in the fasudil plus ozagrel group. There were no significant differences in the incidences of intracranial bleeding and hypotension between the two groups (Table 2).

The two treatments were largely comparable in frequency of adverse events classified according to organ system (Table 3). However, the frequency of reported hepatic and hepatobiliary disorders was significantly higher in the fasudil group than in the fasudil plus ozagrel group (Table 3).

The occurrences of mild, moderate, severe, or large low density areas due to vasospasm on CT and symptomatic vasospasm were lower in the fasudil group than in the fasudil plus ozagrel group (p < 0.01) (Table 4). The proportion of patients with the
Table 3 Patients with adverse events classified according to organ system (excluding intracranial bleeding and hypotension)

<table>
<thead>
<tr>
<th></th>
<th>Fasudil</th>
<th>Fasudil + ozagrel</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>3690</td>
<td>1138</td>
</tr>
<tr>
<td>Hemorrhage (excluding intracranial bleeding)</td>
<td>10 (0.3)NS</td>
<td>5 (0.4)</td>
</tr>
<tr>
<td>Cardiovascular system disorders</td>
<td>19 (0.5)NS</td>
<td>7 (0.6)</td>
</tr>
<tr>
<td>Blood and lymphatic system disorders</td>
<td>56 (1.5)NS</td>
<td>15 (1.3)</td>
</tr>
<tr>
<td>Hepatic and hepatobiliary disorders</td>
<td>338 (9.2)*</td>
<td>80 (7.0)</td>
</tr>
<tr>
<td>Urinary system disorders</td>
<td>25 (0.7)NS</td>
<td>4 (0.4)</td>
</tr>
<tr>
<td>Hypersensitivity</td>
<td>13 (0.4)NS</td>
<td>7 (0.6)</td>
</tr>
<tr>
<td>Gastro-intestinal system disorders</td>
<td>3 (0.1)NS</td>
<td>3 (0.3)</td>
</tr>
<tr>
<td>Others</td>
<td>16 (0.4)NS</td>
<td>8 (0.7)</td>
</tr>
</tbody>
</table>

Values represent number of patients. Values in parentheses represent percentages of total. *p<0.05 vs. fasudil + ozagrel group. NS indicates no significant difference from fasudil + ozagrel group.

Table 4 Results of the endpoints for determining efficacy

<table>
<thead>
<tr>
<th></th>
<th>Fasudil</th>
<th>Fasudil + ozagrel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low density area (none)</td>
<td>72.7%**</td>
<td>67.6% (567/839)</td>
</tr>
<tr>
<td>Symptomatic vasospasm (no vasospasm)</td>
<td>74.3%**</td>
<td>68.0% (664/976)</td>
</tr>
<tr>
<td>Clinical outcome (good recovery)</td>
<td>79.9%NS</td>
<td>76.7% (589/768)</td>
</tr>
</tbody>
</table>

Values are percentages of the total within each group. Values in parentheses are number of patients. **p<0.01 vs. fasudil + ozagrel group. NS indicates no significant difference from fasudil + ozagrel group.

Table 5 Pharmacokinetic parameters for fasudil and hydroxyfasudil in 5 patients with subarachnoid hemorrhage after intravenous infusion of fasudil (30 mg/30 min)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Fasudil</th>
<th>Hydroxyfasudil</th>
</tr>
</thead>
<tbody>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (ng/ml)</td>
<td>127.4 ± 21.8</td>
<td>96.8 ± 9.0</td>
</tr>
<tr>
<td>AUC (ng h/ml)</td>
<td>88.0 ± 15.1</td>
<td>399.8 ± 34.3</td>
</tr>
<tr>
<td>T&lt;sub&gt;1/2&lt;/sub&gt; (h)</td>
<td>0.4 ± 0.1</td>
<td>3.3 ± 0.6</td>
</tr>
<tr>
<td>Trough value (ng/ml)</td>
<td>16.6 ± 1.0</td>
<td></td>
</tr>
</tbody>
</table>

Pharmacokinetic parameters are expressed as the mean ± standard error of 5 patients with subarachnoid hemorrhage. AUC: area under the plasma concentration-time curve, C<sub>max</sub>: maximum plasma concentration, T<sub>1/2</sub>: elimination half-life.

Table 6 Effects of ozagrel and fasudil on the tail transection bleeding time in rats

<table>
<thead>
<tr>
<th>Compound</th>
<th>Bleeding time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>7.9 ± 0.6</td>
</tr>
<tr>
<td>Ozagrel (1 mg/kg)</td>
<td>14.2 ± 1.1**</td>
</tr>
<tr>
<td>Fasudil (1 mg/kg)</td>
<td>8.1 ± 0.7</td>
</tr>
<tr>
<td>Ozagrel + fasudil</td>
<td>12.0 ± 0.8</td>
</tr>
</tbody>
</table>

Values are mean ± standard error of 15 experiments. **shows significant (p<0.01) main effect. There was no interaction between ozagrel and fasudil that contributed to prolongation of bleeding time.

poor clinical outcomes (moderate disability or worse) was similar between the fasudil and fasudil plus ozagrel groups (Table 4).

II. Pharmacokinetic parameters

The pharmacokinetic parameters for fasudil and hydroxyfasudil are listed in Table 5. Hydroxyfasudil was detected following intravenous infusion of fasudil with maximum plasma concentration of approximately 80% of the parent dose. The elimination half-life of hydroxyfasudil was longer than that of fasudil. The AUC value of hydroxyfasudil was approximately 4.5 times higher than that of fasudil. The trough value (8-hour value) of hydroxyfasudil was 16.6 ng/ml (50 nM).

III. Drug interaction

Ozagrel (1 mg/kg, i.v.) induced significant prolongation of bleeding time in rats, but fasudil (1 mg/kg, i.v.) did not. There was no interaction between ozagrel and fasudil that contributed to prolongation of bleeding time (Table 6).

Fasudil (0.2 μM) inhibited Rho-kinase α and β by 39-51%. Hydroxyfasudil (0.2 μM) inhibited Rho-kinase α by 47%, and hydroxyfasudil (0.1 μM) inhibited Rho-kinase β by 52%. Ozagrel (100 μM) did not affect Rho-kinase activity. There were no interactions between either fasudil and ozagrel, or hydroxyfasudil and ozagrel that affected Rho-kinase activities (Table 7).

Discussion

I. Adverse events

Adverse events were observed in 487 (13.2%) of 3690 patients in the fasudil group. The PMS study found adverse events in 3.8% (56/1462) of the fasudil-treated patients who met the eligibility criteria of the phase III trial and 5.7% (8/140) of the placebo-treated patients in the phase III trial. This higher occurrence of adverse events in the present study is accounted for by the presence of blood biochemistry...
abnormalities, which was not considered in the adverse events from the phase III trial and the previously reported sub-analysis of the PMS study. The occurrence of adverse events in the present study excluding blood biochemistry abnormalities was 5.2% (193/3690) in the fasudil group, with no significant difference compared with the occurrence of adverse events in the placebo-treated patients in the phase III trial. The occurrence of adverse events in patients receiving fasudil plus ozagrel was 11.2%. The present study found that both fasudil plus ozagrel combination therapy and fasudil monotherapy were well tolerated, with no significant difference between the two groups.

The two treatments were largely comparable in frequency of adverse events classified according to the organ system but hepatic and hepatobiliary disorders were significantly more common in the fasudil group. However, the frequency of hepatic and hepatobiliary disorders in patients without blood biochemistry abnormality was 1.0% (35/3690) in the fasudil group and 1.0% (11/1138) in the fasudil plus ozagrel group, with no significant difference. The PMS study also reported comparable frequency of hepatic and hepatobiliary disorders after fasudil treatment and placebo treatment in the phase III trial. The majority of patients had mild blood biochemistry abnormality in the fasudil group, and they tended to recover.

Increased risk of intracranial bleeding or hypotension is absent or extremely low with fasudil monotherapy. Fasudil is a potent vasodilator and ozagrel has suppressive effects on platelet aggregation and vasoconstriction, so the combination therapy of fasudil plus ozagrel could cause intracranial bleeding and hypotension in patients with SAH. The incidence of intracranial bleeding was 1.9% (71/3690) in the fasudil group and 1.5% (17/1138) in the fasudil plus ozagrel group, with no significant difference. These results demonstrate that the risk of intracranial bleeding is extremely low for the combination therapy of fasudil plus ozagrel. However, ozagrel only tended to increase bleeding in animal models.

Institution of combination therapy may have to wait until confirmation of complete stop of bleeding in patients undergoing surgery for SAH. The incidence of hypotension in patients treated with fasudil only and fasudil plus ozagrel was 0.4% (14/3690) and 0.5% (6/1138), respectively, with no significant difference. These results indicate that the risk of hypotension with combination therapy is extremely low. However, higher doses of fasudil may lower blood pressure, especially in dehydrated patients. Daily water balance and normovolemic state must be maintained in patients.

### II. Efficacy

The combination therapy using fasudil and ozagrel is significantly more effective in reducing the incidence of low density areas on CT and in reducing poor clinical outcomes associated with vasospasm than ozagrel only. These results suggest that a combination therapy consisting of fasudil and ozagrel has superior efficacy for treating SAH patients. However, the present results indicated that the combination of fasudil plus ozagrel did not result in better efficacy than fasudil only.

Combination therapy of fasudil and ozagrel may be superior to ozagrel but not fasudil because the mechanism of action for ozagrel is different from fasudil. Ozagrel is a thromboxane A₂ synthase inhibitor, and ameliorates vascular contraction and platelet aggregation. Fasudil is a Rho-kinase inhibitor and does not have direct anticoagulant and antiplatelet effects, but upregulates endothelial nitric oxide synthase activity in endothelial cells and prevents the occurrence of endothelial injury, such as platelet aggregation. Fasudil relaxes the cerebral arterial strips contracted by various vasoconstrictive substances including thromboxane A₂. The clinical effectiveness of fasudil in patients with SAH may be due to improvement of hemodynamic function by preventing chronic vasospasm and hyperviscosity, and the prevention of inflammatory responses via inhibition of

---

**Table 7  Inhibitory effects of fasudil, hydroxyfasudil, or ozagrel on Rho-kinase α or β activity**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Rho-kinase α activity (%)</th>
<th>Rho-kinase β activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100.0 ± 1.7</td>
<td>100.0 ± 1.6</td>
</tr>
<tr>
<td>Fasudil</td>
<td>61.0 ± 0.6**</td>
<td>49.0 ± 0.8**</td>
</tr>
<tr>
<td>Ozagrel</td>
<td>98.3 ± 1.5</td>
<td>99.3 ± 1.0</td>
</tr>
<tr>
<td>Fasudil + ozagrel</td>
<td>61.5 ± 0.3</td>
<td>48.5 ± 0.4</td>
</tr>
<tr>
<td>Control</td>
<td>100.0 ± 1.3</td>
<td>100.0 ± 1.2</td>
</tr>
<tr>
<td>Hydroxyfasudil</td>
<td>52.7 ± 0.5**</td>
<td>47.9 ± 0.4**</td>
</tr>
<tr>
<td>Ozagrel</td>
<td>99.0 ± 1.8</td>
<td>99.5 ± 1.7</td>
</tr>
<tr>
<td>Hydroxyfasudil + ozagrel</td>
<td>53.9 ± 1.2</td>
<td>48.2 ± 0.6</td>
</tr>
</tbody>
</table>

Inhibitory effects of fasudil (0.2 μM), hydroxyfasudil (0.2 μM), and ozagrel (100 μM) on Rho-kinase α activity, and of fasudil (0.2 μM), hydroxyfasudil (0.1 μM), and ozagrel (100 μM) on Rho-kinase β activity were examined. Myosin binding subunit phosphorylation produced by the addition of a reaction mixture (control) was defined as 100%. Values are mean ± standard error of 4 experiments. **shows significant (p<0.01) main effect. There were no interactions between either fasudil and ozagrel, or hydroxyfasudil and ozagrel that affected Rho-kinase activities.

Neurol Med Chir (Tokyo) 48, June, 2008
neutrophil and monocyte infiltration, and prevention of production of O$_2^-$ in neutrophils and vessels.

The exact reason why the occurrence of symptomatic vasospasm and low density areas were lower in the fasudil group than in the fasudil plus ozagrel group is unclear. One possible explanation is that this study was limited by a lack of randomization as the investigators were not required to randomize treatment of their patients. Therefore, there may be some selection bias against the treatment method. A further evaluation using a randomized control trial that compares the efficacy of fasudil plus ozagrel against fasudil only may help to more concretely define the efficacy of the combination therapy.

The present results seem to suggest that fasudil has no pharmacological interaction with ozagrel. One of the methods used in this study to investigate drug interaction potential was measuring rat tail-transection bleeding time; however, some species differences, such as responses to several agonists by platelets and the platelet content in plasma, may exist. There may no clearly defined correlation between prolongation of bleeding time in rats and clinical outcome. Additional research may be required to determine drug interaction between fasudil and ozagrel; for example, inhibitory effects on human platelet aggregation.

The lowest concentration of fasudil and hydroxyfasudil at which inhibition of Rho-kinase occurs is 25–50 nM. Intravenous infusion of 30 mg fasudil over 30 minutes provides a minimum trough plasma concentration of 50 nM for hydroxyfasudil, well above the concentration needed to inhibit Rho-kinase. This suggests that the clinical effectiveness of fasudil in patients with SAH is associated with maintaining the total effective plasma concentration of fasudil plus hydroxyfasudil over the prescribed 14-day treatment period.

III. Conclusions

The present sub-analysis of the PMS study shows that combining fasudil plus ozagrel in patients undergoing surgery for SAH was well tolerated, but did not result in a better effect than that achieved by administering fasudil only.

Acknowledgment

We would like to thank all the doctors, staff, and participating patients and families who have helped with this study. The authors wish to thank Ms. Naomi Kagami for the statistical analysis, and Mr. Mark Smith for pertinent comments.

References


Commentary

This is an interesting report of comparison of cohorts in a post-marketing surveillance (PMS) study of fasudil, a recently approved drug in Japan for prevention of vasospasm and its neurologic sequelae after subarachnoid hemorrhage. The two cohorts included 3690 patients who received fasudil alone (a rho-kinase inhibitor which inhibits vasoconstriction, endothelial damage, hyperviscosity and inflammatory response), and 1138 patients who received fasudil and another novel agent, ozagrel (a thromboxane-A2 synthase inhibitor which inhibits vasoconstriction and platelet adhesion). This was an open label study, with no attempt at randomization or blinding. The use of ozagrel, and for that matter other adjunctive therapies, along with fasudil, was totally left to the investigator’s clinical choice.

The primary aim of such PMS studies is to identify unexpected side effects and drug interactions in larger cohorts followed for longer periods than the original trials. These results indeed confirm apparent safety of this therapy. There did not appear to be a significant difference in hypotension, bleeding, and various other adverse events between fasudil monotherapy and combined therapy with ozagrel.

The investigators documented apparently lower prevalence of symptomatic vasospasm and low density areas on CT in the monotherapy group, but no difference in clinical outcomes. They discuss potential mechanistic implications and advantages of monotherapy versus combined regimen. We must caution about such clinical interpretations and conclusions, in the absence of randomized treatment assignment, blinding and other bias control. Equally important, the manuscript proofs which I reviewed do not acknowledge the source of financial support for this PMS, which is presumably the pharmaceutical company marketing fasudil, and if this is the same company which markets ozagrel. We are not told what was the a priori publication policy related to this and other analyses, and whether this was controlled by the sponsoring company. There are surely potential biases and secondary gains inherent to reporting some post hoc analyses and not others.

The hypothesis of potential superiority, or even equivalency of fasudil monotherapy cannot be accepted without specific testing in a separate trial. Ques-
tions remain whether there is sufficient clinical equipoise and pharmaceutical industry interest (scientific, financial, marketing, etc.) in such hypothesis. We also wonder why more aggressive attempts are not being taken to approve these drugs for use in the United States if the data on safety and efficacy is as robust as it seems, and what benefits American patients are being denied without access to these emerging therapies.

Issam A. AWAD, M.D., M.Sc., F.A.C.S., M.A. (Hon.)
Evanston Northwestern Healthcare
Department of Neurological Surgery
Northwestern University
Feinberg School of Medicine
Evanston, Illinois, U.S.A.

The issue of testing drugs in the therapy of patients with subarachnoid hemorrhage addresses a longstanding problem for which there has been much talk but no solution. Although not likely to produce the quality of data that would come from formal trials, an alternative approach is to develop a well structured program of post-marketing surveillance for drugs that are used in preventing cerebral vasospasm after subarachnoid hemorrhage. This could look at both efficacy and side effects. The post-marketing surveillance study would be less expensive than the alternative formal trials, and it also can resolve many of the issues involved.

Yazhuo ZHANG, M.D.
Deputy Director
Beijing Neurosurgical Institute
Beijing, P.R.C.