Antithrombotic Effects of FK419, a Novel Nonpeptide Platelet GPIIb/IIIa Antagonist, in a Guinea Pig Photochemically Induced Middle Cerebral Artery Thrombosis Model: Comparison with Ozagrel and Argatroban

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ABSTRACT
Platelet activation and subsequent aggregation play a key role in the pathogenesis of ischemic brain damage. Recent studies revealed that enhanced platelet activation is also observed after ischemia, suggesting that secondary thrombus formation might participate in the development of cerebral infarction. The binding of platelet glycoprotein GPIIb/IIIa (integrin αIIbβ3) to fibrinogen is the final common pathway in platelet aggregation. Therefore, GPIIb/IIIa antagonists might be useful in acute ischemic stroke as well as in the secondary prevention of ischemic stroke. In the present study, we evaluated the effect of three compounds, FK419 ((S)-2-acetylamino-3-[((R)-(1-[3-(piperidin-4-yl) propionyl] piperidin-3-ylcarbonyl) amino] propionic acid trihydrate), a novel nonpeptide GPIIb/IIIa antagonist, ozagrel, a selective thromboxane A2 synthase inhibitor, and argatroban, a thrombin inhibitor, on middle cerebral artery (MCA) patency and ischemic brain damage using photochemically induced MCA thrombosis model in guinea pigs. FK419, ozagrel, or argatroban was administered 5 min after the termination of photoradiation. FK419 dose-dependently improved MCA patency by decreasing the total occlusion time, time to continuous reperfusion, and the number of cyclic flow reductions, at doses that inhibited ADP-induced platelet aggregation ex vivo. In contrast, ozagrel only improved total occlusion time, and argatroban showed no improvement in MCA patency. FK419 also reduced ischemic brain damage in a dose-dependent fashion, whereas ozagrel and argatroban did not. Finally, FK419 ameliorated neurological deficits, whereas ozagrel and argatroban did not. These results indicate that FK419, a GPIIb/IIIa antagonist, ameliorates ischemic brain damage by improving MCA patency after occlusion and that FK419 is a promising candidate for the treatment of acute ischemic stroke.

Thrombotic cardiovascular disease involves a complex set of interactions of many factors, including platelet aggregation, the coagulation cascade, and vascular-wall constituents. Platelet activation is enhanced after ischemic stroke (van Kooten et al., 1994; Grau et al., 1998; Zeller et al., 1999), leading to secondary thrombus formation and further expansion of the cerebral infarction (Figols et al., 1987; Heye and Cervós-Navarro, 1996). Furthermore, prolonged antiplatelet therapy can successfully prevent secondary vascular events after ischemic stroke (Antiplatelet Trialists’ Collaboration, 1994). Recently, two major trials of aspirin in acute ischemic stroke revealed that early aspirin use produces a small but definite benefit (CAST Collaborative Group, 1997; International Stroke Trial Collaborative Group, 1997; Chen et al., 2000). Thus, antiplatelet therapies show promise in treating both acute and secondary ischemic stroke. The antiplatelet activity of aspirin is limited, however, because it inhibits only a single pathway of platelet activation. In addition, aspirin reduces prostaglandin I2 production in the endothelium, which may lead to decreases in cerebral blood flow (Bednar and Gross, 1999). Although this unfavorable effect is not shown by ozagrel, a selective thromboxane A2 (TXA2) synthase inhibitor, its antiplatelet activity is also limited because it only inhibits a single activation pathway.

After platelet activation is triggered by a variety of agonists, a number of events are induced, such as shape change...
and a conformational change in platelet glycoprotein GPIb/IIIa (integrin α₉β₃), which is abundant on platelets and megakaryocytes. Antagonism of GPIb/IIIa is an attractive antiplatelet strategy because fibrinogen binding to activated GPIb/IIIa is the final common step in platelet aggregation (Pytel et al., 1986), and GPIb/IIIa antagonists suppress platelet aggregation induced by all known agonist stimuli (Cook et al., 1994; Collar et al., 1995). Since a wide variety of platelet activation pathways contribute to thrombus formation, a GPIb/IIIa antagonist should provide more efficacious antithrombotic therapy than commonly available agents.

Thrombin is a central bioregulator of coagulation and is therefore a key target in the therapeutic prevention of thromboembolic disorder. Thrombin catalyzes the conversion of fibrinogen to fibrin and activates fibrin-stabilizing factor XIII and clotting factors that are necessary for prothrombin transformation. Thrombin is also a potent activator of a variety of cell events, including platelet aggregation, secretion and formation of TXA₂, and contraction of smooth muscle. Thrombin plays an important role in arterial thrombosis, and specific thrombin inhibitors, hirudin and argatroban, prevent reocclusion of canine coronary artery (Fitzgerald and Fitzgerald, 1989) and reduce the formation of microthrombi and ischemic lesions (Kawai et al., 1996). Argatroban is approved in the United States and Canada for both prophylaxis and treatment of thrombosis in patients with heparin-induced thrombocytopenia, and in Japan and Korea for treatment of various thrombotic disorders.

Photochemically induced thrombosis models are widely used to examine the effects of antithrombotic agents (Umemura et al., 1993; Nishiyama et al., 1994; Kaku et al., 1998). In these models, a photochemical reaction between rose bengal and transilluminated light irradiation leads to endothelial injury followed by platelet adhesion, aggregation, and formation of an occlusive platelet-rich thrombus at the irradiated site (Saniahiyadi et al., 1995). Particularly in the thrombotic middle cerebral artery (MCA) occlusion model in guinea pigs, spontaneous reperfusion and repeated cyclic flow reductions (CFRs), which are caused by the periodic generation of occlusive platelet thrombi, are observed after occlusion (Kawano et al., 1998). Furthermore, the extent of brain damage is related to CFRe and total patency time (Kawano et al., 1998, 1999).

Recently, FK419 ((S)-2-acetylamino-3-[(R)-1-[3-(piperidin-4-yl)propionyl]piperidin-3-ylcarbonyl]amino)propionic acid trihydrate; Fig. 1), a novel nonpeptide GPIb/IIIa antagonist, has been discovered in our laboratories. FK419 is a selective GPIb/IIIa antagonist that inhibits platelet aggregation in human, dogs, and guinea pigs, with weak activity in prolongation of bleeding time in dogs. In this report, we compared the efficacy of FK419, ozagrel, and argatroban in the guinea pig MCA thrombosis model.

**Materials and Methods**

**Reagents.** FK419 (anhydrate or trihydrate forms), argatroban and ozagrel (anhydrate form or hydrochloride salt) were synthesized by Fujisawa Pharmaceutical Co., Ltd. (Osaka, Japan). FK419 was dissolved and diluted in saline for in vitro studies. For in vivo studies, FK419 was used as an anhydride and also dissolved in saline. An equal molar ratio of NaOH was added when ozagrel was dissolved in saline. Argatroban was dissolved in 5% glucose containing 1.1 or 1.0 M equivalent of HCl. Drugs or saline were injected (i.v. bolus of 1.5 ml/kg) followed by infusion at 5 ml/kg/h. The ratios of bolus and infusion dosages of testing drugs were used to yield the constant blood concentration faster. Rose bengal was obtained from Wako Pure Chemicals (Osaka, Japan). All other chemicals and solvents used were commercial products and of analytical grade.

**Animals.** Male Hartley guinea pigs (342.2–626.2 g; SLC, Shizuoka, Japan) were used. The animals were housed at least 1 week before the experiments. They were maintained on 12-h light/dark cycle at a controlled temperature (23°C ± 1°C) and humidity (55 ± 5%). All animal experimental procedures were performed under the guidelines of the Animal Experiment Committee of Fujisawa Pharmaceutical Co., Ltd.

**Platelet Aggregation Studies in Vitro.** Blood samples were taken from male Hartley guinea pigs. Blood was collected into 1:10 sodium citrate (3.8%, pH 7.4) and kept in a polypropylene tube. Platelet-rich plasma (PRP) was prepared by rapid centrifugation (centrifuge model 5100 and Swing-rotor RS720; Kubota Corp., Tokyo, Japan) of whole blood at 1200 rpm for 10 min. Platelet-poor plasma (PPP) was prepared from the remaining blood by centrifugation at 3000 rpm for 10 min. The platelet aggregation assay was performed using an NBS Hema Trace 801 T-4A, 8-channel aggregometer (Niko Bioscience, Tokyo, Japan). Light transmittance of PPP was calibrated as 100%. PRP was incubated with test compound for 2 min in the aggregometer at 37°C. Platelet aggregation agonists (ADP 1 µM or collagen 1 µg/ml)) were added, and the change in relative light transmittance was monitored as an aggregation curve for 7 min (ADP) or 10 min (collagen) at 37°C. Aggregation percentage was measured at maximum response during the observation period. Percentage inhibition of aggregation in drug-treated samples was determined relative to aggregation in the control sample.

**Coagulation Time in Vitro.** Coagulation times of PPP were measured using an automatic coagulometer (CA-6000; Sysmex and ozagrel (anhydrate form or hydrochloride salt) were synthesized by Fujisawa Pharmaceutical Co., Ltd. (Osaka, Japan). FK419 was used as an anhydride and also dissolved in saline. An equal molar ratio of NaOH was added when ozagrel was dissolved in saline. Argatroban was dissolved in 5% glucose containing 1.1 or 1.0 M equivalent of HCl. Drugs or saline were injected (i.v. bolus of 1.5 ml/kg) followed by infusion at 5 ml/kg/h. The ratios of bolus and infusion dosages of testing drugs were used to yield the constant blood concentration faster. Rose bengal was obtained from Wako Pure Chemicals (Osaka, Japan). All other chemicals and solvents used were commercial products and of analytical grade.

**Platelet Aggregation Studies ex Vivo.** Male Hartley guinea pigs were anesthetized with isoflurane (2% for induction, 1% for maintenance) in a mixture of air and 30% O₂. A catheter was inserted into the right jugular vein for the administration of drugs. After recovery from anesthesia, FK419 or ozagrel was administered to four or five animals in each group. Saline was given to control animals at the same volume. Blood samples were collected into 3.8% sodium citrate from the abdominal aorta 5 min, 1 h, or 3 h after dosing of FK419 or 1 h after dosing of ozagrel. PRP and PPP were prepared and ADP- or collagen-induced platelet aggregation was measured using an NBS Hema Trace 801.

**Measurement of Thromboxane B₂ (TXB₂) Production ex Vivo.** The ability of ozagrel to inhibit ex vivo production of TXB₂, a stable degradation product of TXA₂, was evaluated after collagen-induced platelet aggregation or whole blood coagulation. Immediately after collagen-induced platelet aggregation, response was terminated by the addition of a 10th volume of ice-cold stop solution (1.9% EDTA-2Na, 0.07% indomethacin, 0.76% NaCl, and 5% ethanol, pH 7.4). The assayed supernatant was obtained by centrifugation at 6000g for 5 min at 4°C and stored at −20°C. For whole blood coagulation, blood samples collected into a polypropylene tube were immediately incubated at 37°C for 1 h. Serum was then separated by centrifugation at 6000g for 5 min at 4°C and stored at −20°C.
Concentration of TXB₂ was measured by an enzyme immunoassay kit (RPN220; Amersham Biosciences UK, Ltd., Little Chalfont, Buckinghamshire, UK) after extraction of TXB₂ (Amrep C2; Amersham Biosciences UK, Ltd.).

**Coagulation Time ex Vivo.** Sixty minutes after initiation of argatroban administration, animals were anesthetized with ether, and their blood was drawn from the abdominal aorta into a plastic tube containing 3.8% sodium citrate. PPP was prepared and coagulation times were measured as described above.

**Photochemically Induced MCA Occlusion Model.** The left MCA was photochemically occluded according to the method of Kawano et al. (1998). Briefly, animals were anesthetized with isoflurane (2% for induction, 1% for maintenance) in a mixture of air and 30% O₂. A catheter for the administration of drugs or rose bengal was inserted (2% for induction, 1% for maintenance) in a mixture of air and MCA was photochemically occluded according to the method of son et al. (1986). Briefly, circling, forelimb paralysis, hindlimb paresis, and resistance to lateral push were scored as follows: 0 (normal), 1 (slight to moderate deficit), and 2 (severe deficit). The sum of each score is represented as the total neurological score.

For analysis of ischemic brain damage, animals were sacrificed 24 h after photoinhibition to analyze blood gases, hematocrit, total hemoglobin, and brain temperature. Temporal muscle temperature was monitored as an index of brain temperature.

**Statistical Analysis.** Values are expressed as the mean ± S.E.M. In biochemical experiments, IC₅₀ values were calculated. All data from in vivo studies except for neurological deficits were evaluated by one-way analysis of variance (ANOVA) followed by Dunnett’s multiple comparison test. Kruskal-Wallis test followed by Dunnett’s multiple comparison test were used for neurological deficits. A P value less than 0.05 was considered significant.

**Results**

**Inhibition of Platelet Aggregation in Vitro.** FK419 dose-dependently inhibited ADP- and collagen-induced platelet aggregation in vitro. IC₅₀ values for inhibition of ADP and collagen were 380 ± 28 and 920 ± 32 nM, respectively. In

**Table 1**

**Inhibitory effect of FK419 on ADP-induced platelet aggregation ex vivo in guinea pigs**

<table>
<thead>
<tr>
<th>Dose (mg/kg + mg/h for 3 h)</th>
<th>Platelet Aggregation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 min</td>
</tr>
<tr>
<td>Control</td>
<td>64.9 ± 4.4 (0.0)</td>
</tr>
<tr>
<td>0.03 + 0.1</td>
<td>37.1 ± 4.8** (42.8)</td>
</tr>
<tr>
<td>0.06 + 0.2</td>
<td>10.9 ± 4.2** (83.2)</td>
</tr>
<tr>
<td>0.12 + 0.4</td>
<td>0.0 ± 0.0** (100.0)</td>
</tr>
</tbody>
</table>

* P < 0.05; ** P < 0.01 versus control (one-way ANOVA followed by Dunnett’s multiple comparison test).

**Table 2**

**Inhibitory effects of ozagrel on platelet aggregation and TXB₂ production ex vivo in guinea pigs**

<table>
<thead>
<tr>
<th>Dose (mg/kg + mg/h for 1 h)</th>
<th>Platelet Aggregation</th>
<th>TXB₂ Production</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADP</td>
<td>Collagen</td>
<td>Collagen-Induced Platelet Aggregation</td>
</tr>
<tr>
<td>%</td>
<td>ng/ml</td>
<td>%</td>
</tr>
<tr>
<td>Control</td>
<td>58 ± 3</td>
<td>78 ± 1</td>
</tr>
<tr>
<td>3 + 10</td>
<td>59 ± 4</td>
<td>78 ± 1</td>
</tr>
<tr>
<td></td>
<td>(−1.4)</td>
<td>(−3.4)</td>
</tr>
<tr>
<td>30 + 100</td>
<td>62 ± 1</td>
<td>79 ± 1</td>
</tr>
<tr>
<td></td>
<td>(−7.2)</td>
<td>(−25.0)</td>
</tr>
</tbody>
</table>

**Notes:** Significance was determined by one-way ANOVA followed by Dunnett’s multiple comparison test. There were no significant differences in platelet aggregation among the groups.
Prolongation of Coagulation Time in Vitro. Argatroban concentration-dependently prolonged PT and APTT with 2-fold prolongation concentrations of 810 ± 44 and 1300 ± 60 nM, respectively. In contrast, FK419 or ozagrel had no effect on PT or APTT at concentrations up to 100 μM.

Platelet Aggregation, Production of TXB₂ and Coagulation Time Ex Vivo. FK419 dose-dependently and consistently inhibited ADP-induced platelet aggregation at each time point and at all doses tested (Table 1). Platelet aggregation was inhibited around 50% at 0.03 mg/kg + 0.1 mg/kg/h. Almost complete inhibition was achieved at a dose of 0.12 mg/kg + 0.4 mg/kg/h. In another experiment, FK419 at doses of 0.009 mg/kg + 0.03 mg/kg showed approximately only 10% inhibition of ADP- and collagen-induced platelet aggregation (data not shown). Ozagrel did not inhibit platelet aggregation induced by ADP or collagen but did significantly reduce TXB₂ production at a dose of 30 mg/kg bolus + 100 mg/kg/h infusion for 1 h (Table 2). Argatroban significantly prolonged APTT and PT (about 8-fold at 18 mg/kg + 60 mg/kg/h).

Antithrombotic Effect of FK419, Ozagrel, and Argatroban. MCA blood flow decreased to zero approximately 5 min after photoradiation in all groups (Fig. 2). We observed spontaneous reperfusion after the primary occlusion and subsequent periods of reperfusion and reocclusion (CFRs; Fig. 2), regardless of the treatment group. After repeated CFRs, MCAs were completely reperfused. FK419 dose-dependently reduced the number of CFRs, with significance at 0.03 mg/kg + 0.1 mg/kg/h (Fig. 3). FK419 also significantly shortened total occlusion time and the time to continuous reperfusion at the same dose. Although not significant, FK419 treatment also decreased the time to first reperfusion. Although ozagrel significantly shortened total occlusion time at all doses tested, there were no significant effects on number of CFRs, time to continuous reperfusion, and time to first reperfusion (Fig. 4). Similarly, no improvement in MCA patency was observed with argatroban treatment (Fig. 5).

Effects of FK419, Ozagrel, and Argatroban on Ischemic Brain Damage. There were no significant changes in physiological parameters measured at any time points in FK419-treated animals (Table 3). TTC staining of brain sections revealed that vehicle-treated animals sustained consistent cortical and subcortical lesions (Fig. 6A). FK419 reduced the lesioned area in a dose-dependent fashion (Figs. 6B and 7A), with significance in total area (cerebral cortex + striatum) and cerebral cortex at 0.03 mg/kg + 0.1 mg/kg/h and higher. Percentage reductions in ischemic brain damage in total and cerebral cortex were as follows: 0.009 mg/kg + 0.03 mg/kg/h, 19 and 18%; 0.03 mg/kg + 0.1 mg/kg/h, 32 and 36%; 0.06 mg/kg + 0.2 mg/kg/h, 47 and 42%. In contrast, ozagrel did not significantly reduce brain damage, and argatroban actually tended to aggravate brain damage (Fig. 7, B and C). FK419 treatment (0.03 mg/kg + 0.1 mg/kg/h and above) tended to improve total neurologic score (Fig. 8). Significant improvement of forelimb paralysis was observed at 0.06 mg/kg bolus + 0.2 mg/kg/h infusion for 3 h.

Discussion

The final step in platelet aggregate formation is mediated by GPIIb/IIIa. We have recently discovered FK419, a novel nonpeptide GPIIb/IIIa antagonist, which is a broad inhibitor of platelet aggregation, regardless of the agonist used to induce aggregation, with weak activity in prolongation of bleeding time in dogs (Mihara et al., unpublished observations). Furthermore, FK419 does not affect agonist-directed intracellular calcium mobilization in platelets (Honda et al., unpublished observations), suggesting that it does not directly alter platelet activation but elicits antiaggregatory effects via GPIIb/IIIa antagonism. The ability of FK419 to inhibit converging platelet aggregation pathways is a major advantage compared with other clinically available anti-

![Fig. 2. Typical recording and quantitative analysis of MCA blood flow.](https://example.com/fig2)

![Fig. 3. Antithrombotic effect of FK419. A, number of CFRs; B, total occlusion time; C, time to continuous reperfusion; and D, time to first reperfusion were evaluated using MCA blood flow. FK419 was administered 5 min after the end of photoradiation for 3 h. Each column represents the mean ± S.E.M. of 10 to 11 animals. *, P < 0.05; **, P < 0.01 versus control (one-way ANOVA followed by Dunnett’s multiple comparison test).](https://example.com/fig3)
Fig. 4. Antithrombotic effect of ozagrel. A, number of CFRs; B, total occlusion time; C, time to continuous reperfusion; and D, time to first reperfusion were evaluated from MCA blood flow. Ozagrel was administered 5 min after the end of photoirradiation for 3 h. Each column represents the mean ± S.E.M. of 9 to 10 animals. *, P < 0.05 versus control (one-way ANOVA followed by Dunnett’s multiple comparison test).

Fig. 5. Antithrombotic effect of argatroban. A, number of CFRs; B, total occlusion time; C, time to continuous reperfusion; and D, time to first reperfusion were evaluated from MCA blood flow. Argatroban was administered at a dose of 18 mg/kg + 60 mg/kg/h for 3 h from 5 min after the end of photoirradiation. Each column represents the mean ± S.E.M. of 15 animals.

TABLE 3
Physiological variables before and after FK419 treatment in photochemically induced MCA occlusion model
FK419 was administered 5 min after the end of photoirradiation. Values in parentheses present bolus (mg/kg) and 3-h infusion (mg/kg/h) dosages. Values are expressed as the mean ± S.E.M. of 5 animals.

<table>
<thead>
<tr>
<th>Item</th>
<th>Timing</th>
<th>Control</th>
<th>FK419 (0.03 ± 0.1)</th>
<th>FK419 (0.06 ± 0.2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>%Change in mean blood pressure</td>
<td>Before</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>1 h</td>
<td>5.5 ± 4.7</td>
<td>6.4 ± 7.6</td>
<td>9.5 ± 5.7</td>
</tr>
<tr>
<td></td>
<td>3 h</td>
<td>8.8 ± 5.0</td>
<td>−0.5 ± 2.2</td>
<td>7.3 ± 12.4</td>
</tr>
<tr>
<td>%Change in heart rate</td>
<td>Before</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>1 h</td>
<td>1.7 ± 4.5</td>
<td>3.3 ± 3.5</td>
<td>4.0 ± 2.4</td>
</tr>
<tr>
<td></td>
<td>3 h</td>
<td>6.7 ± 5.6</td>
<td>3.2 ± 3.5</td>
<td>6.5 ± 5.8</td>
</tr>
<tr>
<td>pH</td>
<td>Before</td>
<td>7.317 ± 0.017</td>
<td>7.326 ± 0.015</td>
<td>7.334 ± 0.021</td>
</tr>
<tr>
<td></td>
<td>1 h</td>
<td>7.357 ± 0.006</td>
<td>7.357 ± 0.008</td>
<td>7.346 ± 0.019</td>
</tr>
<tr>
<td></td>
<td>3 h</td>
<td>7.391 ± 0.027</td>
<td>7.383 ± 0.006</td>
<td>7.397 ± 0.014</td>
</tr>
<tr>
<td>pCO2 (mm Hg)</td>
<td>Before</td>
<td>44.7 ± 2.5</td>
<td>46.9 ± 2.3</td>
<td>48.1 ± 1.6</td>
</tr>
<tr>
<td></td>
<td>1 h</td>
<td>44.2 ± 2.5</td>
<td>46.5 ± 2.0</td>
<td>44.9 ± 1.6</td>
</tr>
<tr>
<td></td>
<td>3 h</td>
<td>45.0 ± 3.9</td>
<td>49.2 ± 2.7</td>
<td>46.1 ± 1.3</td>
</tr>
<tr>
<td>pO2 (mm Hg)</td>
<td>Before</td>
<td>122.5 ± 13.9</td>
<td>129.4 ± 10.9</td>
<td>124.8 ± 12.3</td>
</tr>
<tr>
<td></td>
<td>1 h</td>
<td>135.3 ± 9.5</td>
<td>129.2 ± 5.7</td>
<td>144.0 ± 15.7</td>
</tr>
<tr>
<td></td>
<td>3 h</td>
<td>116.5 ± 21.4</td>
<td>119.1 ± 14.0</td>
<td>121.5 ± 14.3</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>Before</td>
<td>40.4 ± 0.7</td>
<td>41.1 ± 0.9</td>
<td>41.5 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>1 h</td>
<td>37.4 ± 0.9</td>
<td>38.1 ± 0.8</td>
<td>40.5 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>3 h</td>
<td>34.8 ± 0.7</td>
<td>35.0 ± 0.4</td>
<td>35.8 ± 0.7</td>
</tr>
<tr>
<td>Total hemoglobin (g/dl)</td>
<td>Before</td>
<td>13.1 ± 0.2</td>
<td>13.4 ± 0.3</td>
<td>13.5 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>1 h</td>
<td>12.2 ± 0.3</td>
<td>12.4 ± 0.2</td>
<td>13.2 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>3 h</td>
<td>11.3 ± 0.3</td>
<td>11.3 ± 0.1</td>
<td>11.6 ± 0.3</td>
</tr>
<tr>
<td>Blood glucose (mg/dl)</td>
<td>Before</td>
<td>183 ± 20</td>
<td>187 ± 21</td>
<td>197 ± 28</td>
</tr>
<tr>
<td></td>
<td>1 h</td>
<td>144 ± 10</td>
<td>144 ± 7</td>
<td>151 ± 6</td>
</tr>
<tr>
<td></td>
<td>3 h</td>
<td>151 ± 15</td>
<td>151 ± 7</td>
<td>146 ± 6</td>
</tr>
<tr>
<td>Temporal muscle temperature (°C)</td>
<td>Before</td>
<td>36.2 ± 0.1</td>
<td>36.4 ± 0.2</td>
<td>36.3 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>1 h</td>
<td>36.3 ± 0.1</td>
<td>36.5 ± 0.1</td>
<td>36.1 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>3 h</td>
<td>36.7 ± 0.1</td>
<td>36.8 ± 0.1</td>
<td>36.6 ± 0.2</td>
</tr>
</tbody>
</table>

Thrombotic compounds, such as aspirin and ozagrel, that only inhibit a single pathway and thus show limited efficacy.

We chose to use a phototherapeutic occlusion model to determine the in vivo efficacy of FK419. This model provides a robust and reproducible platform in which the extent of brain damage reportedly correlates well with number of CFRs and total MCA occlusion time (Kawano et al., 1998, 1999). Consistent with these reports, we observed spontaneous reperfusion and continuous CFRs after MCA occlusion. CFRs are putatively caused by periodic acute occlusive platelet thrombi, and similar phenomena have been reported in various species (Nishiyama et al., 1994; Kaku et al., 1998; Kawano et al., 2000), including humans (Foils et al., 1982).

In the current studies, the parameters of MCA blood flow were somewhat variable in three experiments, especially the time to first reperfusion and number of CFRs. Individual analysis revealed that total occlusion time was relatively well correlated with brain damage. However, the correlation was not high enough to draw definitive conclusions for predicting brain damage. Therefore, other parameters might also contribute to the brain damage. Further studies are needed to clarify the relationship of MCA blood flow and the final outcome of brain infarction in this model. Despite these ca-
veats, as the total occlusion time and brain damage were relatively consistent in three experiments, it is reasonable to assume that comparison of the efficacies of three drugs is justified.

FK419 dose-dependently inhibited ADP-induced platelet aggregation ex vivo in guinea pigs, and we set three doses of almost no inhibition, about 50%, and 80% inhibition of platelet aggregation for evaluating its efficacy in stroke model. It was found that FK419 dose-dependently reduced the number of CFRs and improved MCA patency. Consistent with this improvement in MCA blood flow, FK419 decreased ischemic brain damage in a dose-dependent manner. These results indicate that FK419 might ameliorate the development of ischemic brain damage (not just delay the progression) by preventing secondary thrombus formation after occlusion, whereas the measurements of brain damage at later time points are needed for better understanding of the mode of action of FK419.

In addition to preventing CFRs, FK419 tended to decrease the time to first reperfusion, suggesting that FK419 might possess thrombolytic activity, in addition to its antiaggregatory activity, and that this thrombolytic effect against a primary thrombus, in part, contributed to its improvement on MCA patency. Thrombolytic activity of GPIIb/IIIa antagonists have been reported in coronal and femoral arteries (Mousa et al., 1994; Gold et al., 1997; Domanovits et al., 1998), the mechanism of which is supposed to be related to platelet disaggregation (Mousa et al., 1994) and inhibition of plasminogen activator inhibitor-1 secretion (Tsao et al., 1997). Although further studies are needed to clarify the thrombolytic effect of FK419, such activity may provide additional benefits in the treatment of acute ischemic stroke.

To elucidate the potential of FK419 as an agent for acute ischemic stroke, we compared the efficacy of FK419 with ozagrel, a clinically used TXA2 inhibitor, and argatroban, a thrombin inhibitor, in the same MCA thrombosis model. In contrast to FK419, ozagrel did not inhibit platelet aggregation ex vivo, consistent with previous results (Kawano et al., 1999). Lack of inhibition of platelet aggregation ex vivo with
FK419 may offer even better therapeutic benefits for recombinant tissue-plasminogen activator-resistant thrombi. Thus, although further studies are needed to define the therapeutic potential of FK419, we propose that FK419, a novel GPIb/IIIa antagonist, shows promise as a therapy for acute ischemic stroke.

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References


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