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# Superiority of platelet integrin GPIIb–IIIa receptor antagonist over aspirin in preventing cyclic flow reductions in the guinea pig middle cerebral artery

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**Abstract**

We established a photothrombotic occlusion model in the guinea pig middle cerebral artery. In this model, the middle cerebral artery was recanalized within 10 to 20 min after thrombotic occlusion, with subsequent cyclic flow reductions. Cyclic flow reductions in the middle cerebral artery are expected to manage cerebral infarction by modulating arterial patency. Therefore, we evaluated the effect of several antiplatelet agents on the frequency of cyclic flow reductions and subsequent cerebral infarction using this model. A platelet integrin GPIIb–IIIa receptor antagonist, ME3277 (sodium hydrogen [4-[(4,5,6,7-tetrahydrothieno[3,2*c*]pyridin-2-yl) carbonylamino] acetyl-*o*-phenylene] dioxydiacetate, 0.3, 1 and 3 mg/kg i.v.) dose-dependently inhibited the ex vivo platelet aggregation induced by ADP (5 μM), collagen (0.8 and 20 μg/ml) and arachidonic acid (100 μM). While the same doses of ME3277 reduced the frequency of the cyclic flow reductions and increased the total patency time of the middle cerebral artery, time to thrombotic occlusion was prolonged only at the highest dose, 3 mg/kg. ME3277 (0.3–3 mg/kg) significantly reduced the infarct volume and improved the neurological deficit at 24 h. In contrast, aspirin (30 mg/kg) did not affect these variables in spite of complete inhibition of platelet aggregation induced by arachidonic acid and collagen (0.8 μg/ml). A thromboxane $A_2$ synthetase inhibitor, sodium ozagrel, significantly increased the total patency time and reduced the infarct volume at 30 mg/kg. Inhibition of prostaglandin $I_2$ generation could explain the effectiveness of sodium ozagrel but not aspirin in this model. These results suggest that platelet integrin GPIIb–IIIa receptor antagonists are more beneficial than aspirin for the treatment of cerebral thrombosis. 

*Keywords:* ME3277; Aspirin; Cyclic flow reductions; Photochemical reaction; Cerebral ischemia; Platelet integrin GPIIb–IIIa receptor

## 1. Introduction

The photothrombotic occlusion model of the middle cerebral artery (Umemura et al., 1993, 1995) is a useful model of focal ischaemia with which to investigate antithrombotic effects, because the arterial occlusion is achieved by a non-mechanical approach unlike in the previous models (Tamura et al., 1981; Longa et al., 1989). The occlusion is produced by a photochemical reaction between rose bengal injected i.v. and transluminal photoirradiation (Watson et al., 1985; Matsuno et al., 1991); this reaction is immediately followed by platelet adhesion, aggregation and formation of occlusive platelet rich thrombi at the site of endothelial injury (Saniabadi et al., 1995). Based on this principle, we have established a guinea pig model of middle cerebral artery occlusion (Kawano et al., 1998). One of the most important features of this model is the appearance of cyclic flow reductions in the middle cerebral artery.

Cyclic flow reductions were first described in the canine coronary artery by Folts et al. (1976). They defined cyclic flow reductions as repetitive cycles of blood flow reduction induced by acute occlusive thrombi, followed by dislodgement of thrombi and restoration of blood flow at the site of vascular injury. Subsequently, similar phenomena have been reported in various species, including coronary artery in pigs (Adachi et al., 1990), carotid artery in monkeys (Williams et al., 1993; Cook et al., 1995) and popliteal artery in humans (Folts et al., 1982). Among these models, cyclic flow reductions in the peripheral

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artery were effectively inhibited by administration of platelet integrin GPIIb–IIIa receptor antagonists as well as aspirin (Roux et al., 1994; Umemura et al., 1996). However, as far as we are aware, the effectiveness of antiplatelets on cyclic flow reductions in the intracranial artery and on subsequent cerebral infarction has not been investigated. Thus, using this model, we evaluated the effects of antiplatelet agents using aspirin, a thromboxane $A_2$ synthetase inhibitor, sodium ozagrel (ozagrel) and ME3277 (sodium hydrogen [4-[(4,5,6,7-tetrahydrothieno-[3,2*c*]pyridin-2-yl) carbonylamino] acetyl-*o*-phenylene] dioxydiacetate) which is known as a selective and potent antagonist of platelet integrin GPIIb–IIIa receptor (IIda et al., 1997).

## 2. Materials and methods

### 2.1. Animal preparation

The experimental protocol was approved by the local Committee on ethics of animal experimentations and extra care was taken to avoid animal suffering. Male Hartley guinea pigs weighing 300–450 g were anaesthetized with 1% isoflurane in 70% $N_2O$ and 30% $O_2$ using a face mask. Arterial blood pressure and heart rate were monitored continuously via a catheter inserted into the femoral artery. Arterial blood gases were analyzed with a gas monitor (model 850, Ciba-corning, Japan). Another catheter was inserted into the jugular vein for injection of rose bengal. Animal body temperature was maintained at 38°C with a heating pad (K-module model K-20, American Pharmaseal).

### 2.2. Middle cerebral artery occlusion

After a left temporal incision, the temporalis muscle was removed using an electric cauterizer. The orbital bone was removed to open a 6-mm-diameter oval window using a dental drill (model PAL-7, Morita, Tokyo). The main trunk of the middle cerebral artery was observed under an operation microscope without cutting the dura matter. Photoirradiation with green light (wavelength, 520–620 nm) was achieved using a xenon lamp (model L-4887, Hamamatsu Photonics, Hamamatsu, Japan) with a heat absorbing filter and a green filter. The photoirradiation was directed by a 3-mm-diameter optic fiber mounted on a micromanipulator. The head of the optic fiber was placed on the middle cerebral artery including the proximal end of the lenticulostriate branch, providing an irradiation dose of 0.636 J/cm$^2$. The blood flow velocity in the middle cerebral artery was measured with a pulse Doppler flow probe (PVD-20, Crystal Biotech) positioned on the middle cerebral artery, 2–3 mm distal to the irradiated segment. When a stable baseline blood flow was established, rose bengal (20 mg/kg body weight) was administered and the green light irradiation was continued for 10 min. ME3277 (0.3, 1 and 3 mg/kg), aspirin (30 mg/kg) and ozagrel (10 and 30 mg/kg) were used for preventing cerebral thrombosis. All drugs were dissolved in 5% glucose solution and injected 2 ml/kg via the vein catheter 10 min before photochemical reaction. The blood flow and mean blood flow in the middle cerebral artery were monitored for 60 min after the photochemical reaction. At the end of the observation period, animals were sterilized with kanamycin spray (Meiji Seika, Tokyo). After closure of the surgical site, animals were allowed to recover from anaesthesia.

The following parameters were measured for evaluation of middle cerebral artery blood flow. (1) Time to thrombotic occlusion; defined as the time taken from rose bengal injection to the primary thrombotic occlusion of the middle cerebral artery. (2) Frequency of cyclic flow reductions during the 60 min observation period. (3) Total patency time during the 60 min observation period, expressed as a percentage of total observation.

### 2.3. Evaluation of infarct volume

After scoring the behavioral test, animals were transcardially perfused with 200 ml of saline solution containing 50 IU/ml heparin followed by 200 ml of the formalin-phosphate buffer (Katayama Chemical, Japan) under isoflurane anaesthesia. Then, the brain was isolated and fixed for at least 24 h in the formalin–phosphate buffer. Each brain was cut into seven coronal slices of 1-mm thickness using a microslicer (DTK-3000W, D.S.K., Kyoto). Three-micrometer thick preparations were obtained from paraffin blocks and stained with hematoxylin-eosin. The damaged area was measured using a computerized image analysis system (VM-30, Olympus, Tokyo). The ischaemic damaged area and whole area of cerebrum were measured in seven coronal sections and calculated as volume. Infarct volume of each region was calculated from the ratio of ischaemic volume divided by the whole volume.

### 2.4. Behavioral testing

Neurological deficits of each animal were evaluated 24 h after the photochemical reaction. In the postural reflex test, the animal was pushed toward the injured side and scored as follows: 0, normal; 1, reduced resistance to lateral push; 2, tumbled down on the injured side. In the righting reflex test, the animal was placed on its back, and scored as follows: 0, a quick rise; 1, a rise within 5 s; 2, no rise within 5 s. In the spontaneous movement test, the animal was placed in a cage and movement activity was observed. Behavior was scored as follows: 0, moved without touching the body; 1, moved with touching; 2, lack of movement with touching. In the wryneck test, the animal was placed in the cage and the torsion angle of its neck

Table 1
Physiological measurements 10 min before drug administration

| Treatment | n | Blood gas | | | Hemodynamics | | |
|---|---|---|---|---|---|---|---|
| | | pH | $pO_2$ (mm Hg) | $pCO_2$ (mm Hg) | MABP (mm Hg) | HR (beats/min) | MBF (kHz) |
| Vehicle | 10 | 7.35 ± 0.01 | 120.2 ± 8.6 | 34.9 ± 1.2 | 39.8 ± 2.1 | 226.1 ± 7.6 | 0.77 ± 0.08 |
| ME3277, 0.3 mg/kg | 10 | 7.38 ± 0.01 | 124.1 ± 8.6 | 36.0 ± 2.0 | 38.9 ± 1.5 | 232.4 ± 5.9 | 0.75 ± 0.09 |
| ME3277, 1 mg/kg | 10 | 7.35 ± 0.01 | 115.2 ± 6.1 | 37.7 ± 1.8 | 41.1 ± 0.9 | 231.4 ± 6.3 | 0.65 ± 0.08 |
| ME3277, 3 mg/kg | 10 | 7.36 ± 0.01 | 124.6 ± 6.9 | 35.7 ± 2.0 | 40.9 ± 1.2 | 233.6 ± 6.4 | 0.76 ± 0.10 |
| Aspirin, 30 mg/kg | 10 | 7.36 ± 0.01 | 118.8 ± 8.4 | 38.5 ± 1.4 | 40.4 ± 1.5 | 230.2 ± 6.4 | 0.67 ± 0.07 |
| Ozagrel Na, 10 mg/kg | 10 | 7.38 ± 0.01 | 124.1 ± 6.9 | 35.6 ± 1.0 | 40.8 ± 2.2 | 230.8 ± 6.5 | 0.85 ± 0.13 |
| Ozagrel Na, 30 mg/kg | 10 | 7.35 ± 0.01 | 135.3 ± 8.3 | 36.1 ± 1.1 | 37.6 ± 2.5 | 214.7 ± 7.5 | 0.77 ± 0.12 |

Values are presented as the mean ± S.E. There were no significant differences among any groups in physiological parameters (ANOVA). MBP, mean blood pressure; HR, heart rate; MBF, mean blood flow in the middle cerebral artery.

was observed. Behavior was scored as follows: 0, normal; 1, torsion angle $< 45°$; 2, torsion angle $> 45°$

### 2.5. *Ex vivo* platelet aggregation and measurement of thromboxane $B_2$ production in platelet rich plasma

Each drug was intravenously administered under the isoflurane anaesthesia to four animals in each group. Thirty minutes after i.v. drug administration, blood samples were collected from the abdominal aorta using a syringe containing 1:9 citrate (final conc. 0.38%). Platelet rich plasma was obtained by centrifugation of the blood samples at $150 \times g$ for 10 min at room temperature. Platelet poor plasma was obtained by recentrifugation of the supernatant at $1200 \times g$ for 10 min. The platelet count was made with a cell counter (MEK-5158P, Nihon Kohden, Japan). The maximal aggregation response was measured according to the method of Born (1962). Inducers of platelet aggregation and their final concentrations in a glass cuvette were as follows: ADP, 5 μM; collagen, 0.8 and 20 μg/ml; arachidonic acid, 100 μM. The light transmission for various inducers was recorded for 7 min after 1 min of preincubation.

The production of thromboxane $A_2$ metabolite, thromboxane $B_2$, was measured according to a previous report (Takiguchi et al., 1995). Immediately after measurement of platelet aggregation for 0.8 μg/ml collagen, an ice cold stopping solution, which contained EDTA 60 mM and indomethacin 100 μM, was added to the aggregated platelet rich plasma. After removal of platelets by centrifugation at $600 \times g$ for 3 min at 4°C, the supernatant was collected for measuring thromboxane $B_2$ production. Thromboxane $B_2$ was measured with the enzyme immunoassay kit (RPN 220, Amersham).

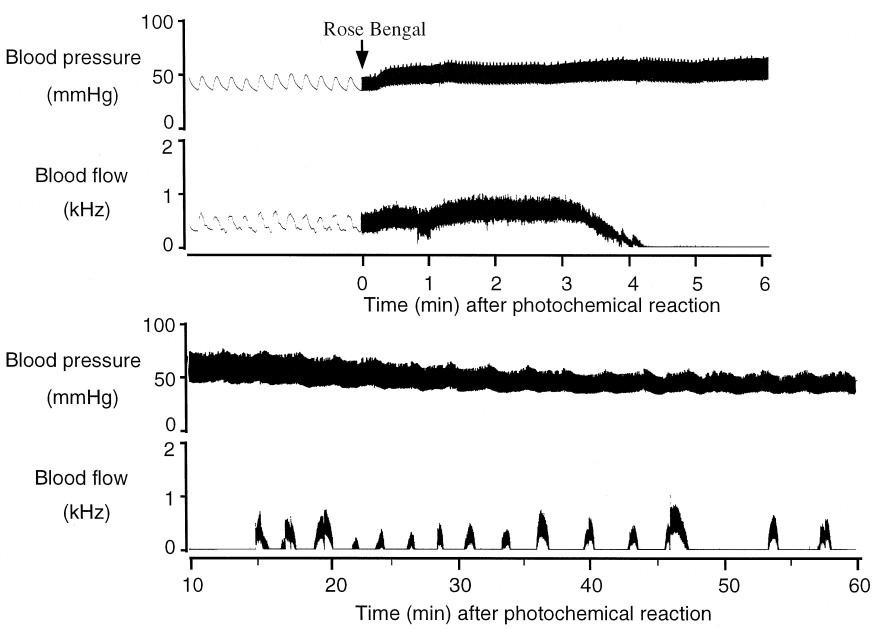


Fig. 1. Simultaneous recording of typical changes in blood pressure and blood flow in the middle cerebral artery.

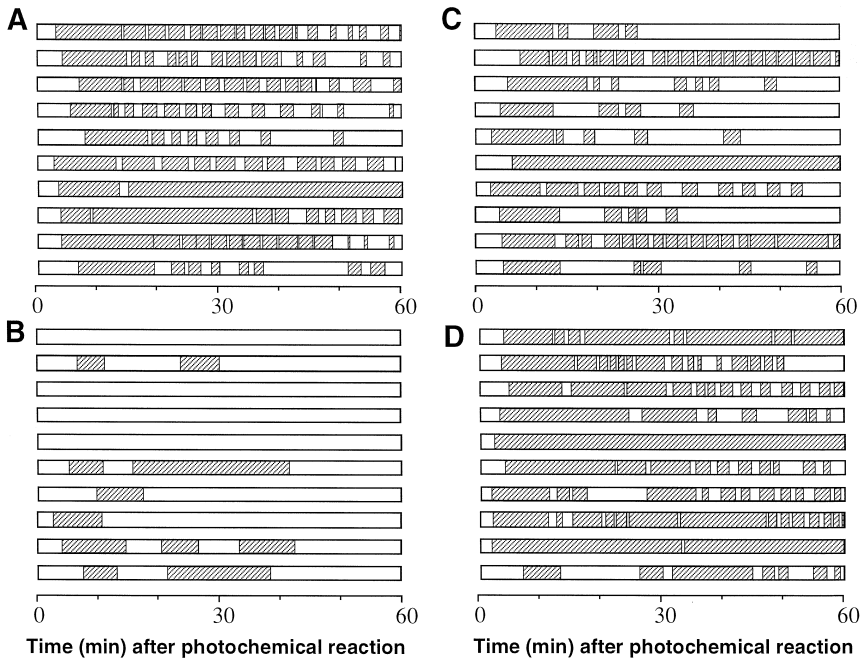


Fig. 2. Schematic presentation of the patency status of the middle cerebral arteries of guinea pigs following photochemical reaction. In each column, open and hatched areas represent the presence and absence of blood flow in the middle cerebral artery, respectively. Data for individual guinea pigs are presented in each group ($n = 10$): (A) vehicle, (B) 3 mg/kg ME3277, (C) 30 mg/kg ozagrel and (D) 30 mg/kg aspirin.

### 2.6. Drugs

ME3277 was synthesized by Meiji Seika Kaisha. Sodium ozagrel was purchased from Ono Pharmaceutical, Osaka, Japan. Aspirin was purchased from Wako, Osaka. ADP and collagen were obtained from MC Medical, Tokyo. Sodium arachidonic acid was purchased from Sigma.

### 2.7. Statistical analysis

All data are expressed as mean ± S.E. or median values. Student's *t*-test or Wilcoxon's test was used for comparisons between two groups. For comparison among more than two groups, one-way analysis of variance (ANOVA) with Dunnett's post hoc test was used for parameteric analysis. Kruskal–Wallis test with Steel's post hoc test was used for nonparametric analysis. A $P$ value $< 0.05$ was considered to be a significant difference.

## 3. Results

### 3.1. Evaluation of blood flow in the middle cerebral artery

In this model, sham animals (i.e., naive guinea-pigs; those having received rose bengal without exposure to green light, and those exposed to green light without

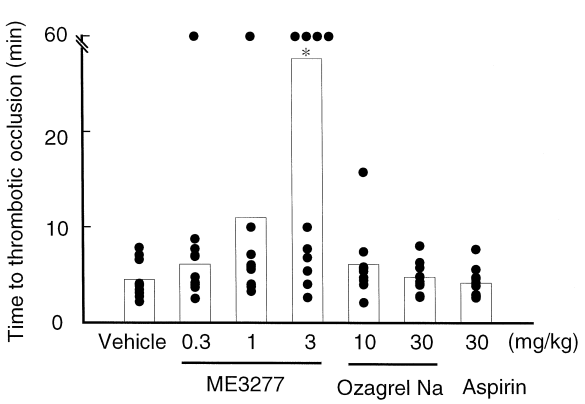


Fig. 3. Effect of antiplatelet agents on time to thrombotic occlusion of guinea pig middle cerebral artery. All data are expressed as median values ($n = 10$). $^{*}P < 0.05$ vs. the vehicle group (Steel's test).

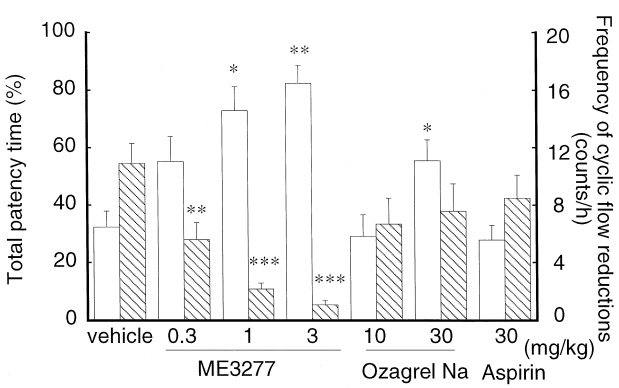


Fig. 4. Effects of antiplatelet agents on the frequency of cyclic flow reductions (hatched column) and the total patency time (open column) for 60 min. Each value represents the mean ± S.E. ($n = 10$). $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ vs. vehicle (Dunnett's test, $P < 0.001$).

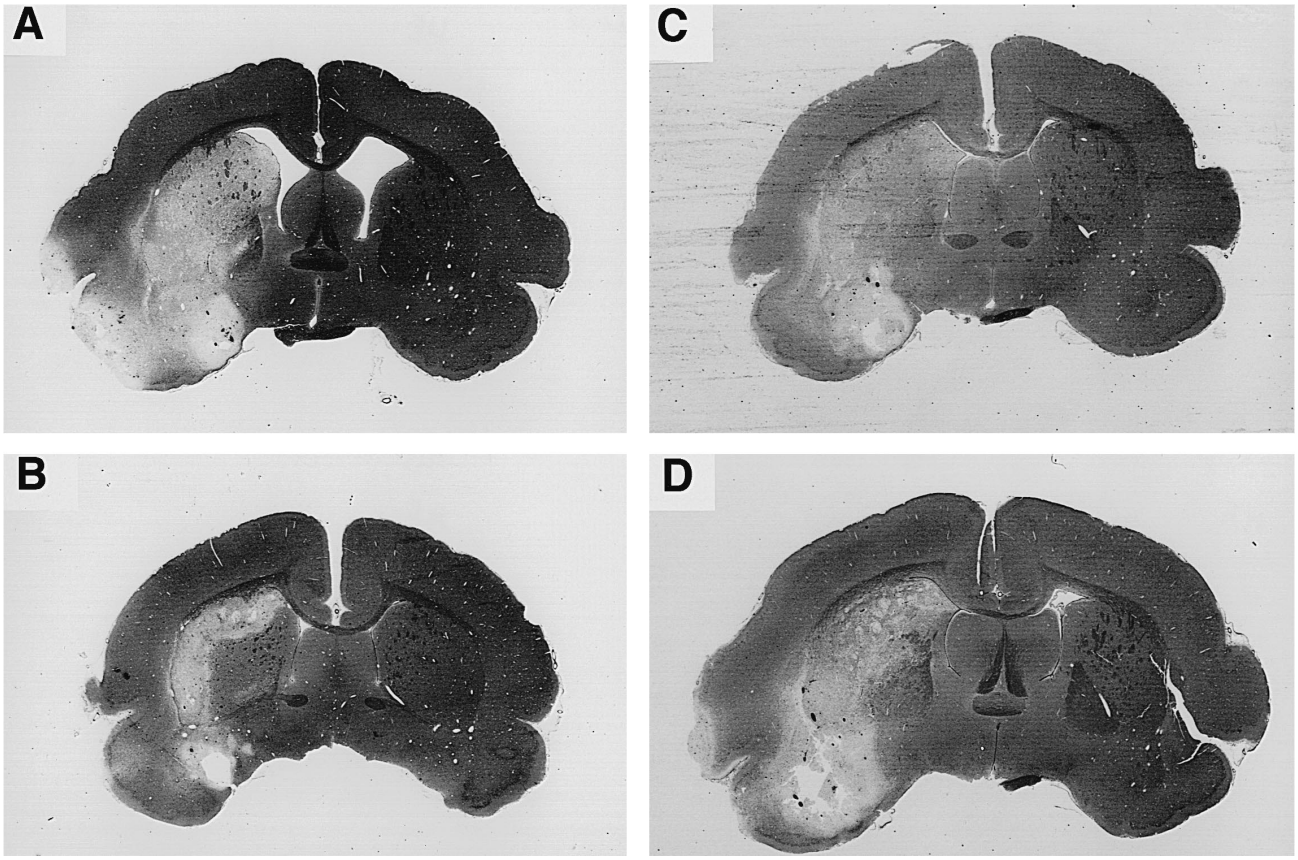


Fig. 5. Effect of antiplatelets on the brain damage 24 h after photochemical reaction of the middle cerebral artery. Coronal brain sections stained with hematoxylin-eosin show the extent of hemispheric infarction in each group ($n = 10$): (A) vehicle, (B) 3 mg/kg ME3277, (C) 30 mg/kg ozagrel and (D) 30 mg/kg aspirin.

receiving rose bengal) did not exhibit vessel occlusion, any neurological deficit or cerebral infarction (data not shown). The physiological variables of animals showed no significant differences among groups at 10 min before photochemical reaction (Table 1). The mean blood pressure was slightly increased by rose bengal injection but there were no significant differences among groups (data not shown). Fig. 1 shows the typical recording of middle cerebral artery patency and blood pressure during 60 min in vehicle-treated animals. The middle cerebral artery blood flow was periodically occluded, then reperfused. Surgical microscopy revealed that this phenomenon was due to platelet rich thrombus formation at the irradiated site. The pattern of the blood flow in each group is illustrated in Fig. 2.

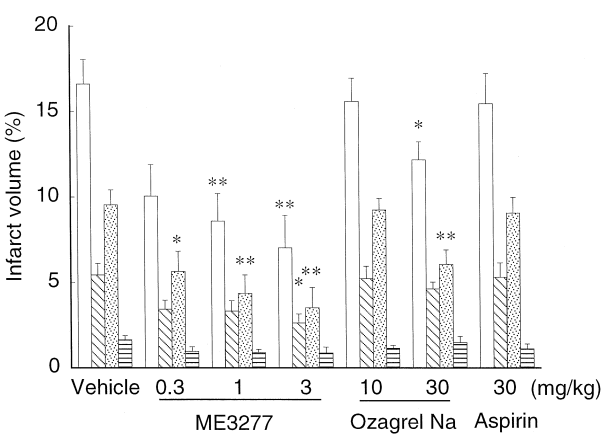


Fig. 6. Effects of antiplatelets on the total infarct volume (open column) as well as the infarct volume in the cerebral cortex (hatched column), striatum (dotted column) and amygdala (striped column). Each value represents the mean ± S.E. ($n = 10$). $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. vehicle (Dunnett's test, $P < 0.001$).

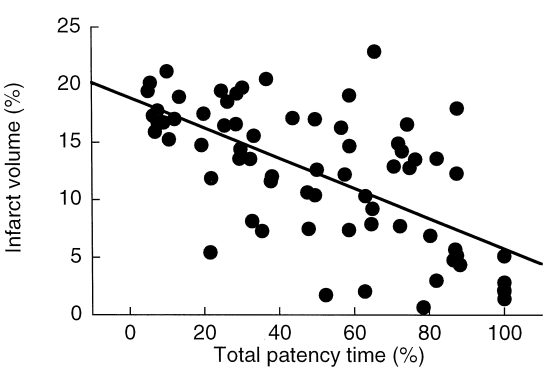


Fig. 7. Correlation between total reflow time in the middle cerebral artery and total infarct volume at 24 h ($r = -0.65$, $n = 70$, $P < 0.001$).

Table 2
Effects of antiplatelet agents on behavior in guinea pig 24 h after thrombotic occlusion

| Treatment | *n* | Postural reflex | Righting reflex | Spontaneous moving | Wryneck |
|---|---|---|---|---|---|
| Vehicle | 10 | 2.0 (0.25–2.0) | 1.0 (0.0–1.75) | 1.0 (0.25–2.0) | 1.0 (0.25–1.0) |
| ME3277, 0.3 mg/kg | 10 | 0.0 (0.0–1.0) | 0.0 (0.0–0.0)[a] | 0.0 (0.0–1.0) | 0.0 (0.0–1.0) |
| ME3277, 1.0 mg/kg | 10 | 0.0 (0.0–0.75)[a] | 0.0 (0.0–0.0)[a] | 0.0 (0.0–1.0) | 0.0 (0.0–0.0) |
| ME3277, 3.0 mg/kg | 10 | 0.0 (0.0–0.0)[a] | 0.0 (0.0–0.0)[a] | 0.0 (0.0–0.0)[a] | 0.0 (0.0–0.0)[a] |
| Aspirin, 30 mg/kg | 10 | 1.0 (0.25–1.75) | 0.0 (0.0–0.75) | 1.0 (1.0–1.75) | 0.0 (0.0–1.0) |
| Ozagrel Na, 10 mg/kg | 10 | 0.5 (0.0–1.0) | 0.0 (0.0–0.75) | 1.0 (0.0–2.0) | 0.5 (0.0–1.0) |
| Ozagrel Na, 30 mg/kg | 10 | 1.0 (0.0–1.0) | 1.0 (1.0–1.0) | 1.0 (1.0–1.0) | 0.0 (0.0–1.0) |

[a] $P < 0.05$ vs. vehicle (Steel's test).
Values are presented as the median and interquartile range.

Administration of 3 mg/kg ME3277 significantly prolonged ($P < 0.05$) the median value for time to thrombotic occlusion (4.5 min in vehicle to 27.7 min, Fig. 3).

Administration of ME3277 significantly decreased the frequency of cyclic flow reductions from $10.9 \pm 1.4$/h (vehicle) to $5.6 \pm 1.2$/h ($P < 0.01$), $2.2 \pm 0.4$/h ($P < 0.001$) and $1.1 \pm 0.3$/h ($P < 0.001$) at 0.3, 1 and 3 mg/kg, respectively (Fig. 4). Consequently, ME3277 increased total patency time from $32.4 \pm 5.6\%$ (vehicle) to $55.3 \pm 8.7\%$, $72.9 \pm 8.3\%$ ($P < 0.05$) and $82.5 \pm 6.2\%$ ($P < 0.01$) at 0.3, 1 and 3 mg/kg, respectively (Fig. 4). Although 30 mg/kg ozagrel did not inhibit the frequency of cyclic flow reductions, it significantly increased the total patency time to $55.6 \pm 7.3\%$ ($P < 0.05$). Aspirin and 10 mg/kg ozagrel did not increase either total patency time or frequency of cyclic flow reductions.

### 3.2. Infarct volume at 24 h after the photochemical reaction

Histological staining of brain sections with hematoxylin-eosin revealed that vehicle-treated animals consistently sustained lesions in cortical and subcortical areas including the striatum and the amygdala (Fig. 5). The total infarct volume at 24 h after the photochemical reaction was $187.4 \pm 17.4$ mm$^3$ in the vehicle group. The percentile infarct volume is shown in Fig. 6. ME3277 dose-dependently improved total infarct volume from $16.6 \pm 1.4\%$ to $10.1 \pm 1.9\%$, $8.59 \pm 1.6\%$ ($P < 0.05$) and $7.03 \pm 1.9\%$ ($P < 0.01$) at 0.3, 1 and 3 mg/kg, respectively. ME3277 also significantly reduced infarct volume in the cortex as well as basal ganglia. In four animals of the 3 mg/kg ME3277-treated group that did not show occlusion for 60 min, the infarct volume was small (< 3%). Administration of ozagrel at 30 mg/kg significantly improved infarct volume in the total area ($12.2 \pm 1.1\%$, $P < 0.05$) and cortex area. None of the drugs affected the infarct volume in the amygdala area. Neither 10 mg/kg ozagrel nor aspirin improved infarct volume in any areas. Total infarct volume was well correlated with the total patency time ($r = -0.650$, $n = 70$, $P < 0.001$, Fig. 7).

### 3.3. The effects on functional deficit

Table 2 summarizes the results for neurological symptoms. Administration of ME3277 improved neurological deterioration. Postural reflex was significantly improved at doses of 1 and 3 mg/kg, and the righting reflex was improved at all doses. Spontaneous moving and wryneck were significantly improved at a dose of 3 mg/kg. In

Table 3
Effect of antiplatelet agents on ex vivo platelet aggregation and thromboxane $B_2$ production in guinea pig platelet rich plasma

| Treatment | *n* | Platelet aggregation (%) | | | | Thromboxane $B_2$ production (ng/ml) |
|---|---|---|---|---|---|---|
| | | ADP (5 μM) | Collagen (0.8 μg/ml) | Collagen (20 μg/ml) | Arachidonic acid (100 μM) | |
| Vehicle | 4 | 79.3 ± 2.9 | 76.3 ± 6.5 | 83.8 ± 2.6 | 81.5 ± 1.8 | 27.63 ± 1.91 |
| ME3277, 0.3 mg/kg | 4 | 36.5 ± 19.7[a] | 4.5 ± 0.3[b] | 53.5 ± 16.2 | 32.3 ± 19.9[b] | 2.66 ± 0.77[b] |
| ME3277, 1 mg/kg | 4 | 8.8 ± 2.9[b] | 3.8 ± 0.9[b] | 25.8 ± 7.2[b] | 7.3 ± 1.1[b] | 6.09 ± 3.47[b] |
| ME3277, 3 mg/kg | 4 | 3.3 ± 1.4[b] | 6.3 ± 1.0[b] | 17.8 ± 3.4[b] | 8.8 ± 0.5[b] | 3.56 ± 1.97[b] |
| Aspirin, 30 mg/kg | 4 | 83.5 ± 1.0 | 3.3 ± 1.1[d] | 89.8 ± 2.6 | 9.8 ± 0.9[c] | 1.83 ± 0.71[d] |
| Ozagrel Na, 10 mg/kg | 4 | 76.0 ± 7.2 | 82.0 ± 2.7 | 79.0 ± 7.8 | 76.8 ± 7.7 | 12.62 ± 1.73[b] |
| Ozagrel Na, 30 mg/kg | 4 | 78.8 ± 5.5 | 84.8 ± 2.0 | 80.0 ± 6.2 | 75.3 ± 10.2 | 18.34 ± 4.10 |

Significantly different from vehicle: [a] $P < 0.05$; [b] $P < 0.01$ (Dunnett's test); [c] $P < 0.05$; [d] $P < 0.01$ (Student's *t*-test).
Values are presented as the mean ± S.E. Each blood sample was collected at 30 min after drug injection under isoflurane anaesthesia. Thromboxane $B_2$ was collected from platelet rich plasma aggregated with 0.8 μg/ml collagen.

contrast, none of the parameters were affected by ozagrel or aspirin. Four of the vehicle-treated animals and one of the 10 mg/kg ozagrel-treated animals exhibited stereotypy (i.e., animals continuously swung their head vertically).

### *3.4. Effects on platelet aggregation and thromboxane $B_2$ production*

Table 3 shows the inhibitory effects of antiplatelet agents on the ex vivo aggregation and thromboxane $B_2$ (a metabolite of thromboxane $A_2$) production. Aspirin at 30 mg/kg completely inhibited the platelet aggregation induced by 0.8 μg/ml collagen and 100 μM arachidonic acid. In contrast, aspirin at the same dose did not affect ADP- nor 20 μg/ml collagen-induced platelet aggregation. Ozagrel did not inhibit platelet aggregation with any inducers. ME3277 inhibited the platelet aggregation with all inducers. Although administration of ozagrel 10 mg/kg significantly inhibited thromboxane $B_2$ production, the higher dose (30 mg/kg) had no further effect. Administration of aspirin or ME3277 significantly and strikingly inhibited the thromboxane $B_2$ production.

## 4. Discussion

We have established the guinea pig model of thrombus formation in the middle cerebral artery and demonstrated cyclic flow reductions after recanalization of the artery (Kawano et al., 1998). In this study, we found that GPIIb–IIIa antagonist effectively improved cerebral infarction as well as neurological deficit by prevented cyclic flow reductions while aspirin did not affect these parameters. The mean systemic blood pressure in guinea pig was relatively low even in the awake condition (approximately 50 mm Hg) as reported previously (Brown et al., 1989). We excluded the possibility that hypotension affected cyclic flow reductions or the effectiveness of antiplatelet agents in this model, because the autoregulation of cerebral blood flow was well maintained at 25–70 mm Hg of mean arterial blood pressure under isoflurane anaesthesia (data not shown).

GPIIb–IIIa receptor antagonist, ME3277, inhibited the frequency of cyclic flow reductions and increased total patency time at doses which inhibit ex vivo platelet aggregation. This indicates that cyclic flow reductions generated in the intracranial artery are sensitive to platelet integrin GPIIb–IIIa receptor antagonist as shown in previous reports on the peripheral artery (Roux et al., 1994; Umemura et al., 1996). In contrast, the time to thrombotic occlusion (i.e., primary occlusion) in the middle cerebral artery was relatively resistant to ME3277, especially at lower doses (Fig. 3). This discrepancy might be explained by the difference in mechanisms between the primary occlusion and reocclusion during cyclic flow reductions. During the photochemical reaction, platelets adhered to the exposed subendothelium and released various factors including thromboxane $A_2$ that strongly facilitated platelet cohesion on the adhered platelets and vasoconstriction, which cooperatively occluded the blood vessel. In this situation, thrombotic occlusion is considerably resistant to platelet integrin GPIIb–IIIa receptor antagonist since platelet integrin GPIIb–IIIa receptor antagonists do not inhibit platelet adhesion (Andre et al., 1996; Kamat et al., 1997) and thromboxane $A_2$ production (Byrne et al., 1997; Carroll et al., 1997). Once occluded artery is recanalized, a few layers of adhesive platelets are retained on the exposed subendothelium, which could alleviate the subsequent activation of platelet cohesion and vasoconstriction.

Pretreatment of aspirin did not affect cyclic flow reductions nor total patency time, which did not correspond to earlier studies with peripheral arteries (Folts, 1991; Umemura et al., 1996). Because aspirin inhibits the formation of prostaglandin $I_2$ as well as thromboxane $A_2$, by inhibiting cyclooxygenase (Vane, 1978), the lack of efficacy in the middle cerebral artery might be explained by inhibition of prostaglandin $I_2$ formation. This possibility is supported by the efficacy of ozagrel which increased prostaglandin $I_2$ production with inhibition of thromboxane $A_2$ production (Kuzuya et al., 1986). The lack of ex vivo platelet aggregation inhibition with ozagrel is explained by prostaglandin endoperoxide accumulation due to inhibition of thromboxane $A_2$ synthesis since prostaglandin endoperoxide can directly stimulate platelet thromboxane $A_2$ receptor on platelets (Hanasaki and Arita, 1988). In vivo, accumulated prostaglandin endoperoxide is rapidly converted to prostaglandin $D_2$ or prostaglandin $I_2$ (Hamberg and Fredholm, 1976; Marcus et al., 1980; Orchard et al., 1983), which inhibit platelet aggregation. Thus, ozagrel increased the total patency time as well as improved infarct volume, despite that it did not affect the neurologic deficit.

Another explanation for the inefficiency of aspirin might be aspirin-resistant platelet aggregation in the guinea pig. Under high shear stress conditions, platelet integrin GPIIb–IIIa receptor directly bound to another integrin GPIIb–IIIa receptor on platelet via a Von Willbrand factor, and this type of aggregation is known as shear induced platelet aggregation (Goto et al., 1998). Shear induced platelet aggregation is also known to be an important mechanism of cerebral infarction (Uchiyama et al., 1994), and is inhibited by platelet integrin GPIIb–IIIa receptor antagonists but not aspirin (Uchiyama et al., 1983; Konstantopoulos et al., 1995; Turner et al., 1995; Maalej and Folts, 1996; Barstad et al., 1996).

In the current model, total patency time of middle cerebral artery for 60 min after the photochemical reaction (i.e., total patency time) could be an important factor determining infarct volume at 24 h as indicated in Fig. 7. The small infarct volume in four animals of the 3 mg/kg ME3277 group that did not show any sign of occlusion during the 60 min supports this idea. The observation

period of 60 min was enough since cyclic flow reductions continued for 60–70 min after the photochemical reaction as shown in a previous report (Kawano et al., 1998). The frequency of cyclic flow reduction and time to thrombotic occlusion are weaker contributors to cerebral infarction than total patency time. The dose of ME3277 that prevents cerebral infarction well corresponds to the dose affecting total patency time but not the time to thrombotic occlusion. A high dose of ozagrel did not reduce the frequency of cyclic flow reductions while it increased total patency time. This is explained by the fact that a high dose of ozagrel increased total patency time by prolonging the duration of perfusion in each cycle, by unknown mechanisms.

In humans, cyclic flow reductions may be present in the acute phase of stroke, since rethrombosis after thrombolysis has been observed in the cerebral artery (Nakayama et al., 1998; Von Kummer et al., 1995; Wallace et al., 1997). Therefore, inhibition of cyclic flow reductions by platelet integrin GPIIb–IIIa receptor antagonists is expected to prevent development of infarction in humans.

In conclusion, we demonstrated that cyclic flow reductions occurred in the guinea pig middle cerebral artery after thrombus formation. Cyclic flow reductions modulate total patency time after the acute phase of arterial occlusion, and determine infarct volume and neurological function at 24 h. The cyclic flow reductions generated in the intracranial artery are sensitive to platelet integrin GPIIb–IIIa receptor antagonist but not aspirin. These findings suggest that platelet integrin GPIIb–IIIa receptor antagonist is a more beneficial antithrombotic agent than aspirin for the treatment of cerebral thrombosis.

## References


Adachi, H., Fujimori, T., Shoji, T., 1990. Inhibitory effects of a novel antiplatelet aggregating agent, E-5510, on cyclic flow variations in electrically stimulated coronary arteries of the pig. J. Cardiovasc. Pharmacol. 16, 733–741.

Andre, P., Arbeille, B., Drouet, V., Hainaud, P., Bal dit Sollier, C., Caen, J.P., Drouet, L.O., 1996. Optimal antagonism of GPIIb/IIIa favors platelet adhesion by inhibiting thrombus growth. An ex vivo capillary perfusion chamber study in the guinea pig. Arterioscler. Thromb., Vasc. 16, 56–63.

Barstad, R.M., Orvim, U., Hamers, M.J., Tjonnfjord, G.E., Brosstad, F.R., Sakariassen, K.S., 1996. Reduced effect of aspirin on thrombus formation at high shear and disturbed laminar blood flow. Thromb. Haemost. 75, 827–832.

Born, G.V.R., 1962. Aggregation of blood platelets by adenosine diphosphate and its reversal. Nature 194, 927–929.

Brown, J.N., Thorne, P.R., Nuttall, A.L., 1989. Blood pressure and other physiological responses in awake and anesthetized guinea pigs. Lab. Anim. Sci. 39, 142–148.

Byrne, A., Moran, N., Maher, M., Walsh, N., Crean, P., Fitzgerald, D.J., 1997. Continued thromboxane $A_2$ formation despite administration of a platelet glycoprotein IIb/IIIa antagonist in patients undergoing coronary angioplasty. Arterioscler. Thromb., Vasc. 17, 3224–3229.

Carroll, R.C., Wang, X.F., Lanza, F., Steiner, B., Kouns, W.C., 1997. Blocking platelet aggregation inhibits thromboxane $A_2$ formation by low dose agonists but does not inhibit phosphorylation and activation of cytosolic phospholipase $A_2$. Thromb. Res. 88, 109–125.

Cook, J.J., Sitko, G.R., Bednar, B., Condra, C., Mellott, M.J., Feng, D.M., Nutt, R.F., Shafer, J.A., Gould, R.J., Connolly, T.M., 1995. An antibody against the exosite of the cloned thrombin receptor inhibits experimental arterial thrombosis in the African green monkey. Circulation 91, 2961–2971.

Folts, J., 1991. An in vivo model of experimental arterial stenosis, intimal damage, and periodic thrombosis. Circulation 83, IV3–IV14.

Folts, J.D., Crowell, E.B. Jr., Rowe, G.G., 1976. Platelet aggregation in partially obstructed vessels and its elimination with aspirin. Circulation 54, 365–370.

Folts, J.D., Detmer, D.E., Nadler, R., 1982. Possible platelet thrombi formation in dog and human femoral artery. Tex. Heart Inst. J. 9, 19–26.

Goto, S., Ikeda, Y., Saldivar, E., Saldivar, M., Saldivar, R.Z., 1998. Distinct mechanisms of platelet aggregation as a consequence of different shearing flow conditions. J. Clin. Invest. 101, 479–486.

Hamberg, M., Fredholm, B.B., 1976. Isomerization of prostaglandin $H_2$ into prostaglandin $D_2$ in the presence of serum albumin. Biochim. Biophys. Acta 431, 183–189.

Hanasaki, K., Arita, H., 1988. Characterization of thromboxane $A_2$/prostaglandin $H_2$ ($TXA_2$/$PGH_2$) receptors of rat platelets and their interaction with $TXA_2$/$PGH_2$ receptor antagonists. Biochem. Pharmacol. 37, 3923–3929.

IIda, H., Hui, C., Sugano, T., Kumagai, M., Hachisu, M., Omoto, S., 1997. Antithrombotic effects of a novel GPIIb/IIIa antagonist, ME3277, in several experimental models (in abstract). Thromb. Haemost. 78, 666.

Kamat, S.G., Turner, N.A., Konstantopoulos, K., Hellums, J.D., McIntire, L.V., Kleiman, N.S., Moake, J.L., 1997. Effects of Integrelin on platelet function in flow models of arterial thrombosis. J. Cardiovasc. Pharmacol. 29, 156–163.

Kawano, K., Ikeda, Y., Kondo, K., Umemura, K., 1998. Increased cerebral infarction by cyclic flow reductions: studies in the guinea pig MCA thrombosis model. Am. J. Phys. 275, R1578–R1583.

Konstantopoulos, K., Kamat, S.G., Schafer, A.I., Banez, E.I., Jordan, R., Kleiman, N.S., Hellums, J.D., 1995. Shear-induced platelet aggregation is inhibited by in vivo infusion of an anti-glycoprotein IIb/IIIa antibody fragment, c7E3 Fab, in patients undergoing coronary angioplasty. Circulation 91, 1427–1431.

Kuzuya, T., Hoshida, S., Yamagishi, M., Ohmori, M., Inoue, M., Kamada, T., Tada, M., 1986. Effect of OKY-046, a thromboxane $A_2$ synthetase inhibitor, on arachidonate-induced platelet aggregation: possible role of ''prostaglandin $H_2$ steal'' mechanism. Jpn. Circ. J. 50, 1071–1078.

Longa, E.Z., Weinstein, P.R., Carlson, S., Cummins, R., 1989. Reversible middle cerebral artery occlusion without craniectomy in rats. Stroke 20, 84–91.

Maalej, N., Folts, J.D., 1996. Increased shear stress overcomes the antithrombotic platelet inhibitory effect of aspirin in stenosed dog coronary arteries. Circulation 93, 1201–1205.

Marcus, A.J., Weksler, B.B., Jaffe, E.A., Broekman, M.J., 1980. Synthesis of prostacyclin from platelet-derived endoperoxides by cultured human endothelial cells. J. Clin. Invest. 66, 979–986.

Matsuno, H., Uematsu, T., Nagashima, S., Nakashima, M., 1991. Photochemically induced thrombosis model in rat femoral artery and evaluation of effects of heparin and tissue-type plasminogen activator with use of this model. J. Pharmacol. Methods 25, 303–317.

Nakayama, T., Tanaka, K., Kaneko, M., Yokoyama, T., Uemura, K., 1998. Thrombolysis and angioplasty for acute occlusion of intracranial vertebrobasilar arteries. Report of three cases. J. Neurosurg. 88, 919–922.

Orchard, M.A., Blair, I.A., Dollery, C.T., Lewis, P.J., 1983. Blood can synthesize prostacyclin. Lancet 2, 565.

Roux, S., Carteaux, J.P., Hess, P., Falivene, L., Clozel, J.P., 1994.

Experimental carotid thrombosis in the guinea pig. Thromb. Haemost. 71, 252–256.

Saniabadi, A.R., Umemura, K., Matsumoto, N., Sakuma, S., Nakashima, M., 1995. Vessel wall injury and arterial thrombosis induced by a photochemical reaction. Thromb. Haemost. 73, 868–872.

Takiguchi, Y., Asai, F., Wada, K., Nakashima, M., 1995. Comparison of antithrombotic effects of GPIIb–IIIa receptor antagonist and $TXA_2$ receptor antagonist in the guinea-pig thrombosis model: possible role of $TXA_2$ in reocclusion after thrombolysis. Thromb. Haemost. 73, 683–688.

Tamura, A., Graham, D.I., McCulloch, J., Teasdale, G.M., 1981. Focal cerebral ischaemia in the rat: 1. Description of technique and early neuropathological consequences following middle cerebral artery occlusion. J. Cereb. Blood Flow Metab. 1, 53–60.

Turner, N.A., Moake, J.L., Kamat, S.G., Schafer, A.I., Kleiman, N.S., Jordan, R., McIntire, L.V., 1995. Comparative real-time effects on platelet adhesion and aggregation under flowing conditions of in vivo aspirin, heparin, and monoclonal antibody fragment against glycoprotein IIb–IIIa. Circulation 91, 1354–1362.

Uchiyama, S., Takeuchi, M., Osawa, M., Kobayashi, I., Maruyama, S., Aosaki, M., Hirosawa, K., 1983. Platelet function tests in thrombotic cerebrovascular disorders. Stroke 14, 511–517.

Uchiyama, S., Yamazaki, M., Maruyama, S., Handa, M., Ikeda, Y., Fukuyama, M., Itagaki, I., 1994. Shear-induced platelet aggregation in cerebral ischemia. Stroke 25, 1547–1551.

Umemura, K., Wada, K., Uematsu, T., Nakashima, M., 1993. Evaluation of the combination of a tissue-type plasminogen activator, SUN9216, and a thromboxane $A_2$ receptor antagonist, vapiprost, in a rat middle cerebral artery thrombosis model. Stroke 24, 1077–1081.

Umemura, K., Kawai, H., Ishihara, H., Nakashima, M., 1995. Inhibitory effect of clopidogrel, vapiprost and argatroban on the middle cerebral artery thrombosis in the rat. Jpn. J. Pharmacol. 67, 253–258.

Umemura, K., Nishiyama, H., Kikuchi, S., Kondo, K., Nakashima, M., 1996. Inhibitory effect of a novel orally active GP IIb/IIIa inhibitor, SC-54684A on intimal thickening in the guinea pig femoral artery. Thromb. Haemost. 76, 799–806.

Vane, J.R., 1978. Inhibitors of prostaglandin, prostacyclin, and thromboxane synthesis. Adv. Prostaglandin Thromb. Res. 4, 27–44.

Von Kummer, R., Holle, R., Rosin, L., Forsting, M., Hacke, W., 1995. Does arterial recanalization improve outcome in carotid territory stroke?. Stroke 26, 581–587.

Wallace, R.C., Furlan, A.J., Moliterno, D.J., Stevens, G.H., Masaryk, T.J., Perl, J.n., 1997. Basilar artery rethrombosis: successful treatment with platelet glycoprotein IIB/IIIA receptor inhibitor. Am. J. Neuroradiol. 18, 1257–1260.

Watson, B.D., Dietrich, W.D., Busto, R., Wachtel, M.S., Ginsberg, M.D., 1985. Induction of reproducible brain infarction by photochemically initiated thrombosis. Ann. Neurol. 17, 497–504.

Williams, J.K., Bellinger, D.A., Nichols, T.C., Griggs, T.R., Bumol, T.F., Fouts, R.L., Clarkson, T.B., 1993. Occlusive arterial thrombosis in cynomolgus monkeys with varying plasma concentrations of lipoprotein(a). Arterioscler. Thromb., Vasc. 13, 548–554.