Angiographic evaluation of middle cerebral artery reperfusion caused by platelet glycoprotein IIb/IIIa receptor complex antagonist murine 7E3 F(ab’)2 in a model of focal cerebral ischemia in rats

YI YANG, M.D., PH.D., QIU LI, M.D., MARIAN T. NAKADA, PH.D., TAO YANG, M.SC., AND ASHFAQ SHUAIB, M.D., F.R.C.P.(C)

Acute Stroke Program, Division of Neurology, University of Alberta Hospital, Edmonton, Alberta, Canada; and Centocor, Malvern, Pennsylvania

Object. Antagonists of the glycoprotein IIb/IIIa (GPIIb/IIIa) receptor complex are currently used for the treatment of acute coronary syndromes. The platelet GPIIb/IIIa mediates platelet aggregation, and blocking this receptor complex can reduce or prevent arterial thrombosis. To study the recanalization efficacy of a GPIIb/IIIa antagonist in treating cerebral ischemia, we investigated the therapeutic effects of murine 7E3 F(ab’), in a focal embolic cerebral ischemia model in rats.

Methods. Focal cerebral ischemia was produced by introducing an autologous thrombus into the right side of the middle cerebral artery (MCA). Thirty male Wistar rats were randomly divided into three groups of 10 rats each: control, 7E3 F(ab’), administered 1 hour postischemia, and 7E3 F(ab’), administered 3 hours postischemia. Animals in the therapeutic groups received intravenous infusion of 6 mg/kg 7E3 F(ab’), at 1 or 3 hours following cerebral embolization. Brain infarct volume, neurobehavioral scores, duration of bleeding, and findings on angiograms of the MCA (before and after infusion) were assessed in all animals.

Angiographic evaluation revealed full MCA recanalization in three of 10 animals in each 7E3 F(ab’), treatment group. Animals in these groups exhibited a significant reduction in infarct volume when compared with animals in the control group: 1) infarct volume 1 hour postischemia, 22 ± 13.9% (p = 0.005); 2) infarct volume 3 hours postischemia, 22.1 ± 14.8% (p = 0.008); and 3) infarct volume in control animals, 42.4 ± 16%. Postischemia treatment with 7E3 F(ab’), also improved the animal’s neurobehavioral performance. The duration of bleeding significantly increased by more than two times, but there was no associated increase in intracerebral hemorrhage in any group.

Conclusions. On the basis of their findings, the authors conclude that murine 7E3 F(ab’), is a potent and safe anti-platelet agent in this experimental focal embolic cerebral ischemia model. Neuronal lesions were significantly reduced when the treatment was delayed up to 3 hours.

Key Words • glycoprotein IIb/IIIa receptor complex antagonist • cerebral ischemia • thrombolysis • rat

N patients suffering from acute ischemic stroke, early reperfusion induced by rt-PA is effective in improving clinical outcomes. Experimental models of focal cerebral ischemia1,2,19,22 and studies performed in patients with ischemic stroke5,17,23 have shown that neuronal death can be reduced if cerebral blood flow is rapidly restored. Unfortunately, a number of factors, including the short time window for treatment and a serious increase in the risk of cerebral and systemic hemorrhage, limit the use of rt-PA therapy to only a small number of patients. Any improvement in increasing the time window for therapy or decreasing the risk of cerebral hemorrhage would provide immense benefits to patients.
Model of focal cerebral ischemia in rats

ly stimulated thrombosis in the carotid artery in cynomolgus monkeys26 and dogs.25 In addition, there is a single clinical report of the use of such an agent in the prevention of repeated thrombosis of the basilar artery after transluminal angioplasty.31

Based on the pharmacological actions of GPIIb/IIIa receptor antagonists both in inhibiting platelet aggregation and platelet procoagulant activity (thrombin generation and, hence, fibrin formation) and in facilitating spontaneous thrombolysis, we hypothesized that such inhibitors may provide therapeutic benefits in a model of focal embolic cerebral ischemia in which a thrombus is already formed and lodged in an affected cerebral artery.

The present study was undertaken to determine the antithrombotic and recanalizational efficacies of a potent platelet GPIIb/IIIa monoclonal antibody, murine 7E3 F(ab’)_2, in a clinically relevant cerebral ischemia model in rats. To achieve this aim, intracranial angiography was used to help evaluate the recanalization of the embolized artery after infusion of the GPIIb/IIIa antibody. We also explored the therapeutic window for this effect by administering this agent at two different time points after embolization. The duration of tail bleeding and the incidence of ICH were monitored to assess the drug’s safety profile.

Materials and Methods

All experiments in this study were approved by the Health Sciences Animal Welfare Committee of the University of Alberta and were performed in strict accordance with related guidelines. All animals were deprived of food 12 hours before surgery to normalize the blood glucose level. Any animals that died or demonstrated no neurological deficit within 2 hours after placement of thrombus were excluded from the study. The murine antibody 7E3 immunoglobulin G was produced, purified, digested to F(ab’)_2 with pepsin, and repurified in a manner previously described.6

In Vitro Study of Platelet Aggregation and Antibody Binding

Platelet-rich plasma was prepared from citrate (final concentration 0.32%)-anticoagulated rat blood, and the platelet counts were adjusted to approximately 300,000 platelets/μl. Light transmittance platelet aggregation was performed using an aggregometer. Samples were preincubated with antibody for 10 minutes at 37°C and, then adenosine diphosphate (10 μM final concentration) was added to stimulate platelet aggregation. The aggregation response was continuously monitored for 4 minutes after addition of the agonist, and the maximum aggregation response was recorded. The percentage of inhibition was calculated on the basis of the aggregation response from control samples that contained no antibody. For receptor binding, platelet-rich plasma was incubated with fluorescently labeled 7E3 F(ab’)_2, or abciximab (chimeric 7E3 Fab) for 30 minutes at room temperature. Samples were fixed with buffer, and analyzed with the aid of a flow cytometer to determine the amount of bound fluorescent antibody.

Cerebral Focal Ischemia

Embolic focal cerebral ischemia was induced using the procedure detailed in our previous report.22 Male Wistar rats (350–400 g each) were given a mixture of 3% halothane to induce anesthesia and the anesthesia was maintained by giving them a mixture of 1.5% halothane in 70% N₂/O₃0% O₂ (vol/vol) through a face mask. The rectal temperatures of the animals were monitored and kept at approximately 37°C by using a heating pad and an overhead lamp. Other physiological parameters, including mean arterial blood pressure, O₂ saturation, and pulse, were also monitored throughout the experiment.

In brief, after induction of anesthesia, a longitudinal incision approximately 1.5 cm in length was made in the skin over the middle cervical region to expose the right CCA, right ICA, and right ECA. A modified PE-50 catheter (outer diameters: 0.3 mm at the catheter tip and 0.97 mm at the catheter body) contained 5 μl of 12-hour-old autologous thrombus, which had been incubated at 37°C overnight. The thrombus was introduced into the lumen of the right ICA through a small puncture hole in the right ECA. A 17-mm section of catheter (calculated from the puncture point at the ECA) was gently advanced to the intracranial section of the ICA through the right ECA and the extracranial portion of the ICA. The tip of the catheter at this depth should be 1 to 2 mm away from the origin of the MCA. Finally, the right CCA was temporarily clipped to reduce cerebral blood flow, and the clot in the catheter was gently injected into the ICA. The catheter was withdrawn from the right ECA 10 minutes after injection. After closure of the incision, the animals were allowed to recover and given free access to food.

Evaluation of Recanalization by Using Angiography of the MCA

Within 30 minutes after injection of autologous thrombus into the right MCA, contrast-enhanced angiography was performed to confirm successful clot embolization. This was done by reinserting a catheter through the puncture that had previously been made in the right ECA and injecting 0.2 ml of heparinized (5 U/ml) iohexol into the ipsilateral ICA and its distal territory. The patency of the MCA stem or branches was evaluated in a blinded fashion. Animals in which postoperative angiography revealed a patent right MCA were excluded from this experiment. To evaluate the efficacy of recanalization by postischemic administration of 7E3 F(ab’)_2, a second angiogram was obtained using the same procedure 3 hours after initiation of the 7E3 F(ab’)_2 infusion. The timing of angiography for the control group was set to match that used for postisch- emia 7E3 F(ab’)_2, treatment group. The x-ray exposure settings for angiograms obtained during this study (62 kV, 100 ma, 1/200 second) were kept constant throughout the experiment.

Quantification of the Volume of the Brain Infarct

The details related to quantification of the infarct volume have been reported elsewhere.23 Briefly, 72 hours after MCA embolism, deep anesthesia was induced in the rats by injection of an overdose of pentothal (100 mg/kg). Following this procedure, the animals received an intracardiac perfusion of 100 ml normal saline. Each rat brain was removed from the skull and cooled in ice-cold saline for approximately 5 minutes. For morphometric analysis, 2-mm-thick coronal sections were cut with the aid of a rat brain matrix. A total of eight coronal sections were prepared for estimation of the degree of infarction damage. The coronal sections were stained using 2,3, 5-triphenyltetrazolium chloride. The stained brain sections were then directly placed on a flattened color scanner within 7 days. A clear glass cover was used to provide a black background. Following image acquisition, the images were analyzed in a blinded fashion by using a commercial image-processing software program. Measurements were made by manually outlining the margins of infarcted areas. The total volume of infarct was determined by integration of the distance of the eight chosen sections. In this study, infarct size was expressed as a percentage of the volume of the region examined. The percentage of infarct volume was obtained by calculating the proportion of the corrected infarct size with respect to the total size of the normal hemisphere.

Neurological Deficits

Animals were examined for neurological deficits at 2 and 24 hours after injection of autogenous arterial thrombus and evaluated using a four-tiered grading system: a score of 1 was given if the animal demonstrated forelimb flexion, a score of 2 was given if the animal displayed forelimb flexion and decreased resistance to lateral push, a score of 3 was given if the animal exhibited the signs specified for a score of 2 plus decreased resistance to lateral push and unilateral circling in three successive trials, and a score of 4 was given if the animal demonstrated the signs specified for a score of 3 plus decreased consciousness. Any other neurological behavior was also recorded.
Treatment Protocol

Thirty male Wistar rats were randomly divided into three groups of 10 each: control, murine 7E3 F(ab′/H11032 treatment at 1 hour postischemia, and murine 7E3 F(ab′/H11032 treatment at 3 hours postischemia. Animals in the therapeutic groups received an initial bolus intravenous tail-vein injection (one third dosage) of 7E3 F(ab′/H11032 (total dose 6 mg/kg, total volume 2 ml), which was followed by an intravenous tail-vein infusion lasting 30 minutes at 1 or 3 hours after cerebral embolization. Animals in the control group received the same amount of normal saline (2 ml) through the same bolus and infusion route 3 hours after embolic stroke. The animal experiments were performed by one investigator and the evaluation of recovery from stroke, effects of medications, and the volume of infarct were conducted by a second investigator who was unaware of the specific treatment given to particular animals. The final results were tabulated once all measurements had been made. The investigator responsible for performing angiography and evaluating the rate of recanalization was also unaware of the treatment of each animal.

Duration of Bleeding and Incidence of ICH

Rat-tail transectional bleeding was induced by sectioning the extremity of the tail 3 mm from the tip; the tail was gently blotted with tissue paper every 2 minutes and the time to cessation of bleeding was recorded in minutes. No pressure was exerted on the tail tip because this may have affected hemostasis.

The incidence of ICH was recorded during the 24-hour observation period in each experimental group. Intracranial hemorrhage was assessed by gross inspection of cut sections of the brains.

Data Presentation and Statistical Analysis

All data in this study are expressed as the means ± standard deviations. Statistical analysis of more than two groups of animals was performed using one-way analysis of variance and subsequent individual comparisons by using the Scheffé test. The chi-square test was used to compare differences of recanalization rate among the groups. The difference was considered significant when the probability value was less than 0.05.

Sources of Supplies and Equipment

The murine 7E3 F(ab′), was obtained from Centocor (Malvern, PA). The PAP-4 aggregometer was obtained from BioData (Horsham, PA). The FACScan flow cytometer and the PE-50 catheter were obtained from Becton, Dickinson and Co. (Franklin Lakes, NJ). Mean arterial blood pressure, O2 saturation, and pulse were monitored using the MP100 system acquired from Biopac System (Santa Barbara, CA). The iohexal (Omnipaque) was obtained from Nycomed Danmark (Roskilde, Denmark). The Scanjet 4p flatbed color scanner was purchased from Hewlett-Packard Co. (Palo Alto, CA) and the PhotoShop image-processing software (version 4.0) from Adobe Systems, Inc. (San Jose, CA).

Results

Five animals died within 2 hours after suffering embolic stroke and were excluded from the study (two control animals, two in the 7E3 F(ab′), 1-hour treatment group, and one animal in the 7E3 F(ab′), 3-hour treatment group). Five additional animals were excluded from this study because angiograms revealed failures of occlusion of the MCA after surgery. Five of 10 animals in the con-
Model of focal cerebral ischemia in rats

control group and three of 10 animals in each group treated with the GPIIb/IIIa receptor antagonist died during the period from 12 hours after surgery to the final observation (72 hours) in this study. No significant difference in physiological variables was found before or after the introduction of thrombus into the MCA or among different groups. Similarly, the physiological statuses of animals in the 7E3 F(ab\(^{1/2}\)) treatment groups were not different from those in the saline-treated control group. The methods used in this study to produce cerebral thromboembolism were successful in 80% of studied animals. Figure 1 shows the typical location of an injected thrombus in the right MCA and extending partially into the intracranial section of the ipsilateral ICA.

Abciximab binds to rat platelets with a very low affinity, approximately 200-fold lower than it binds to human platelets (Table 1); and correspondingly, this chimeric 7E3 Fab is very weak at inhibiting rat platelet aggregation. In contrast, murine 7E3 F(ab\(^{1/2}\)), binds rat platelets with a reasonable affinity (K\(\text{d}\) = 7 \(\mu\)g/ml), only 35-fold lower than that observed for human platelets (Table 1). The 7E3 F(ab\(^{1/2}\)), inhibits rat platelet aggregation reasonably well with an IC\(_{50}\) of 15 \(\mu\)g/ml. A 4 mg/kg intravenous bolus of 7E3 F(ab\(^{1/2}\)), has been shown to block in vivo thrombus formation in rats. A 6 mg/kg intravenous bolus administered to five rats resulted in 67 \(\pm\) 3\% inhibition of platelet aggregation 24 hours later (unpublished data) and this dose was selected for this study.

Evaluation of Recanalization Using the Angiogram of the MCA

No spontaneous recanalization was observed in animals in the control group, which were given normal saline. Postischemia treatment with 7E3 F(ab\(^{1/2}\)), fully reopened the occluded MCA in three of 10 animals in both treatment groups and partially opened one additional MCA in the 1-hour postischemia treatment group (total recanalization rate \(> 30\%), p < 0.05\) when compared with that in the control group). Figure 2 shows one representative set of angiograms of the MCA from a single animal with recanalization. Animals in the treatment groups in which angiography demonstrated no recanalization also had significantly smaller infarcts than the animals in the control group (data shown in the following section).

Evaluation of Infarct Volume

The percentage of infarct volume in the two groups of treated animals compared with that in control animals is illustrated in Fig. 3. A significant reduction in infarct volume was demonstrated in animals in the 7E3 F(ab\(^{1/2}\)), treatment groups compared with those in the control group (reduction 1 hour postischemia 22 \(\pm\) 13.9\%, \(p = 0.005\); reduction 3 hours postischemia 22.1 \(\pm\) 14.8\%, \(p = 0.008\)). More severe damage was observed in the vehicle-treated animals, in which cerebral infarction involved 42.4 \(\pm\) 16\% of brain tissue on the affected side. The benefit afforded by 7E3 F(ab\(^{1/2}\)), in reducing the volume of the infarct when administered 3 hours postischemia was similar to that observed when the drug was given 1 hour postischemia (\(p = 0.49\)). We also observed that in animals treated with 7E3 F(ab\(^{1/2}\)), at 1 hour—but not at 3 hours—postischemia and in which the MCA did not recanalize (as evident on angiography), a significant decrease in brain infarction occurred, compared with control animals (infarct reduction 1 hour postischemia, 27.6 \(\pm\) 12.9\% compared with that in controls, 42.4 \(\pm\) 16\%, \(p = 0.036\); infarct reduction 3 hours postischemia, 29.4 \(\pm\) 12.6\% compared with that in controls, \(p = 0.06\)).

Neurological Deficits

As shown in Fig. 4, animals treated with saline exhibited no significant improvement in neurobehavioral score between 2 and 24 hours following cerebral embolization.
groups, respectively). Postischemia treatment with 7E3 F(ab’), resulted in a significant reduction of neuronal damage after focal cerebral ischemia in both medicated groups. A 3-hour delay between embolization and treatment with 7E3 F(ab’), resulted in equal neuroprotection when compared with treatment 1 hour postischemia.

(3.8 ± 0.5 at 2 hours compared with 3.8 ± 0.4 at 24 hours, p = 0.4). In contrast, postischemia treatments with 7E3 F(ab’), significantly improved neurological outcome at 24 hours after focal cerebral ischemia, compared with the results of the neurological assessment at 2 hours after cerebral ischemia (1-hour postischemia treatment 4 ± 0 compared with 2.8 ± 0.6, respectively, p < 0.001; 3-hour postischemia treatment 3.9 ± 0.3 compared with 2.9 ± 0.3, respectively, p < 0.001). However, we found no significant difference in neurobehavioral improvement between the two 7E3 F(ab’), treatment groups at 24 hours following cerebral embolization.

Duration of Tail Bleeding and Incidence of ICH

The tail cross-section bleeding time was 9.3 ± 1.9 minutes for the control group, which received normal saline. In contrast, the bleeding time significantly increased by 2.5 times in animals treated with 7E3 F(ab’), when compared with animals in the control group (animals treated 1 hour postischemia 24.3 ± 2.4 minutes, p < 0.001; animals treated 3 hours postischemia 24.4 ± 2.5 minutes, p < 0.001).

The incidence of ICH did not significantly increase in the treatment groups when compared with control animals (two of 10 rats in the control group; three of 10 and two of 10 in the 1-hour and 3-hour postischemia treatment groups, respectively).

Discussion

The GPIIb/IIIa receptor plays a crucial role in platelet aggregation and platelet thrombus formation. Inhibition of GPIIb/IIIa with antibodies, snake-venom peptides containing the arginine-glycine-aspartic acid sequence, or peptides and peptidomimetics based on that sequence, result in abolition of platelet aggregation and platelet thrombus formation that can lead to arterial occlusion. In accordance with these data, we demonstrated that systemic administration of murine 7E3 F(ab’), 1 or 3 hours after induction of thrombus formation significantly attenuated brain infarction in a focal cerebral ischemia model. In this study, the first to our knowledge, we examined the recanalization efficacy and safety of using murine 7E3 F(ab’), in a clinically relevant and reproducible MCA embolization model. Our results demonstrate that treatment with murine 7E3 F(ab’), at 1 or 3 hours after onset of ischemia can significantly attenuate the size of a brain infarct, improves neurological outcome, and, importantly, does not significantly increase the risk of brain hemorrhage.

There is other evidence that GPIIb/IIIa receptor antagonists not only prevent thrombus formation, but also can recanalize occluded arteries in cases of coronary artery ischemia. Abciximab, a chimeric monoclonal antibody Fab fragment that blocks GPIIb/IIIa, has also been shown to recanalize occluded coronary vessels alone, without the need for concomitant fibrinolytic therapy in patients.14-16,27 In addition to these reports, investigators from the prospective and randomized TIMI 14 trial1 reported a 32% incidence of TIMI 3 flow at 90 minutes in patients receiving abciximab alone, a rate similar to that achieved using streptokinase alone.10 This apparent thrombolytic effect may be due to the displacement of fibrinogen from GPIIb/IIIa receptor by abciximab, or could be related to the ability of abciximab to prevent plasminogen activator inhibitor–1 secretion from platelet granules.1 Another potential explanation is that thrombi are dynamic, continuously forming and dissolving depending on the balance between thrombotic and lytic factors. Addition of abciximab may prevent the accrual of new platelets to the thrombus, thus tipping the balance in favor of endogenous lysis. There are limited animal models with which to test abciximab because of its limited species crossreactivity. Abciximab itself is not effective in dogs because of its low affinity for dog platelets, but the bivalent F(ab’), form of 7E3, the parent antibody of abciximab, is effective and has been used in a variety of dog thrombus models.11 Recently, the utility of 7E3 F(ab’), in rats has been reported; this agent appears to have pharmacodynamic properties similar to those exhibited by abciximab in humans12 and was, therefore, utilized in the present study in rats.

In the current experiment, early recanalization did not produce a significantly better outcome when compared with that found in animals in which the MCA did not reopen at 3 hours postischemia. The clear explanation for the lack of significant difference between the two groups is unknown, but the following evidence from recent studies may provide some hints. Choudhri and coworkers recently reported that administration of the GPIIb/IIIa receptor antagonist GPI 562 immediately after reperfusion has been shown to reduce cerebral infarct volumes by 70% in a murine model of reversible focal cerebral ischemia. Because the drug was administered after reestablishment of blood flow, the highly significant therapeutic response may be due to the effects exerted by this GPIIb/IIIa receptor antagonist in the microvasculature, where secondary thrombosis in small vessels may persist despite reestablishment of blood flow. Supportive data for small-vessel thrombosis was offered by documented enhanced accumulations of fibrin and platelets on the affect-
ed side of the cerebral hemisphere after recanalization of the MCA.7 Fibrin accumulates in the microvessels in a time-dependent manner following focal cerebral ischemia and reperfusion.24 Authors of a recent clinical pathological study showed that, following a fatal ischemic stroke, there is increased coagulation (formation of microthrombi) in the ischemic hemisphere during the stage of fresh infarction. Later, both the ischemic and contralateral hemispheres exhibit abnormally enhanced coagulation.15 Another observation that supports small-vessel thrombi formation during the postinfarction period is the reported increase in brain infarction found after ischemic stroke in patients.25 This postischemic microvascular thrombosis may result in postischemic hyperperfusion and ongoing neuronal damage. In another study,26 pretreatment with a GPIIb/IIIa receptor antagonist led to a reduction in platelet aggregation and thrombosis introduced by anodal electrolytic stimulation of the intimal surface of the ICA in dogs. Therefore, inhibition of the GPIIb/IIIa receptor could decrease the degree of ischemic damage by inhibiting fibrin and platelet accumulation. The previous findings may provide a possible explanation for the observation in our current study that some animals treated with murine 7E3 F(ab')2, were found to have no MCA recanalization, although they did appear to receive therapeutic benefits. Postischemia treatment with murine 7E3 F(ab')2 at 1 or 3 hours may decrease the progression of postischemia fibrin formation in microvessels in the ischemic area.

The presence of microvascular occlusion has not been clearly established as the main factor responsible for the progression of neuronal injury after MCA occlusion. Recently, Li and colleagues27 reported a different observation in a focal ischemia model in which rats were subjected to a 2-hour MCA occlusion by using a filament method. In their study, secondary microcirculatory compromise was not observed after a 2- to 8-hour reperfusion. This lack of occlusion in the microvasculature is surprising and may reflect the type of model used for the study. In our study a different model was used to produce cerebral ischemia. Injection of a clot in the distal ICA produced a more prolonged occlusion of the MCA, which was evident on angiograms. This may also allow for the formation of fibrin clots in smaller vessels. Our results indicate that postischemia treatment with 7E3 F(ab')2, may produce benefits by dissolving the preformed thrombus in some animals and preventing secondary platelet aggregation in most animals. In the present study we did not evaluate the effects of the 7E3 agent on the patency of small blood vessels.

Thrombolysis is an attractive but potentially dangerous therapy for cerebral ischemia.1,4,11,23 Risk factors for hemorrhage include time to thrombolysis, the presence of hypertension or coagulation abnormalities, and the dose of the lytic agent.16 The safety and efficacy of the treatment is critically dependent on the timing of intervention and on the dose of thrombolytic agent used.16 Choudhri, et al.,7 reported that, although the GPIIb/IIIa antagonist they used caused a dose-dependent increase in the duration of tail vein bleeding, the incidence of ICH was not significantly increased at therapeutic doses, except at the highest doses tested. This finding is in accordance with our observations. We observed no increase in the incidence of ICH in the treated groups, although the duration of bleeding was prolonged by more than two times that of the control group. In our study, we did not test dose effectiveness as it relates to the development of ICH. However, the fact that we did not find a rise in the incidence of ICH, when the GPIIb/IIIa antagonist was given at 1 or 3 hours after MCA embolization, may indicate that murine 7E3 F(ab')2, has a relatively wide range of safety.

Cerebral angiography offers the best method to detect arterial occlusion in acute ischemic stroke. It has been used successfully in evaluating the efficacy of thrombolysis in patients suffering from massive MCA strokes.4 In this study, cerebral angiograms revealed reopening of the MCA in one third of the animals treated with 7E3 F(ab')2, which is similar to the recanalization rate achieved using abciximab alone17 or rt-PA alone at a dose of 10 mg/kg in a similar embolic model.4 The recanalization rate was lower than the reopening rate (53.8%) in another focal ischemia model in which MCA thrombosis was produced by a regional photochemical reaction followed by rt-PA infusion 30 minutes after the MCA occlusion.18 The small difference in recanalization rates between the two models may be related to different models used to produce focal ischemia in the two studies. Combination therapy in which a GPIIb/IIIa antagonist is coupled with a low-dose fibrinolytic agent is likely the most effective and safe method to treat thrombotic occlusions; future studies will address this approach in this model.

Conclusions

We determined the efficacy and safety of using murine 7E3 F(ab')2, a platelet GPIIb/IIIa receptor antagonist, in a model of focal cerebral ischemia in rats. In this study 7E3 F(ab')2, reduced brain infarction and improved neurological outcome. Cerebral angiography revealed recanalization in one third of the animals. Therapeutic effects were similar in animals with and without recanalization in the group that received treatment at 1 hour, but not in those animals that received treatment at 3 hours, following ischemic insult. We also observed that administration of 7E3

![Fig. 4. Bar graph demonstrating neurological deficit scores in the three different groups of rats. Animals receiving vehicle showed no significant difference in neurological behavior between the 2-hour and 24-hour observations. In contrast, animals receiving 7E3 F(ab')2, 1 hour or 3 hours postischemia demonstrated significant improvement in behavioral scores at 24 hours postischemia compared with 2 hours postischemia. *p < 0.05 when compared between 2- and 24-hour scores; NS = no significant difference.](image-url)
F(ab′), at 3 hours after cerebral ischemia did not increase the risk of ICH.

References

15. Huang J, Rebello SS, Faul JD, et al: Correlation between the in vivo efficacy of GPIIb/IIIa receptor antagonists (m7E3, MK- 383 and DMP-728) and ex vivo platelet inhibition. Pharmacolog- y 58:88–95, 1999

Y. Yang, et al.