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Intraarterial reteplase and intravenous abciximab for treatment of acute ischemic stroke

A preliminary feasibility and safety study in a non-human primate model

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Abstract We performed a preliminary feasibility and safety study using intravenous (IV) administration of a platelet glycoprotein IIb/IIIa inhibitor (abciximab) in conjunction with intraarterial (IA) administration of a thrombolytic agent (reteplase) in a primate model of intracranial thrombosis. We introduced thrombus through superselective catheterization of the intracranial segment of the internal carotid artery in 16 primates. The animals were randomly assigned to receive IA reteplase and IV abciximab ($n=4$), IA reteplase and IV placebo ($n=4$), IA placebo and IV abciximab ($n=4$) or IA and IV placebo ($n=4$). Recanalization was assessed by serial angiography during the 6-h period after initiation of treatment. Postmortem magnetic resonance (MR) imaging was performed to determine the presence of cerebral infarction or intracranial hemorrhage. Partial or complete recanalization at 6 h after initiation of treatment (decrease of two or more points in pre-treatment angiographic occlusion grade) was observed in two animals treated with IA reteplase and IV abciximab, three animals treated with IA reteplase alone and one animal treated with IV abciximab alone. No improvement in perfusion was observed in animals that received IV and IA placebo. Cerebral infarction was demonstrated on postmortem MR imaging in three animals that received IA and IV placebo and in one animal each from the groups that received IA reteplase and IV abciximab or IV abciximab alone. One animal that received IV abciximab alone had a small intracerebral hemorrhage on MR imaging. IA reteplase with or without abciximab appeared to be the most effective regimen for achieving recanalization in our model of intracranial thrombosis. Further studies are required in experimental models to determine the optimal dose, method of administration and efficacy of these medications in acute ischemic stroke.

Keywords Antiplatelet agents · Ischemic stroke · Magnetic resonance imaging · Primates · Thrombolysis

Introduction

Acute ischemic stroke results from occlusion of blood vessels by thrombus consisting of multiple elements including platelet, thrombin, and fibrin mesh [1, 2]. In acute stroke, the dynamic interplay among factors promoting thrombosis versus those promoting thrombolysis is shifted in favor of thrombosis [1]. Although thrombolytic therapy lyses the fibrin mesh component of thrombus, it also leads to increased thrombin expression

and further thrombus deposition and platelet activation [3, 4]. In response to stimulation by thrombin, platelet glycoprotein (GP) IIb/IIIa receptors are activated. GP IIb/IIIa-receptor activation leads to fibrinogen binding and platelet cross-linkage, therefore creating platelet-rich thrombus at the site of lysis [5]. Other consequences of platelet activation that generate thrombus formation include release of plasmogen inhibitor (PAI-1) and vasoconstrictor substances [5]. Thus, platelet activation promotes resistance to thrombolysis and reocclusion [3, 4]. From a theoretical perspective, a powerful antiplatelet agent used in conjunction with a thrombolytic agent might enhance thrombolysis and reduce the risk of reocclusion.

The Thrombolysis in Myocardial Infarction (TIMI) 14 trial evaluated the hypothesis that abciximab, the Fab fragment of the chimeric 7E3 monoclonal antibody fragment directed to the platelet GP IIb/IIIa receptor, is a potent safe addition to thrombolytic regimens for acute myocardial infarction [1]. A total of 888 patients with myocardial infarction presenting within12 h from symptom onset were treated with aspirin and randomized to receive either 100 mg of accelerated-dose alteplase or abciximab (one-time bolus infusion of 0.25 mg/kg and 12-h infusion of 0.125 mcg/kg per min) alone or in combination with streptokinase (500,000 to 1,500,000 IU) or a reduced dose of alteplase (20 to 65 mg) or streptokinase. Higher rates of recanalization were seen in the 50-mg alteplase plus abciximab group than in the alteplase-only group at both 60 min (72 versus 43%) and 90 min (77 versus 62%). Major hemorrhage occurred at a rate of 6% in patients receiving alteplase alone versus 3% with abciximab alone and 1% with 50 mg of alteplase and abciximab. This study demonstrated that abciximab in combination with one-half of the usual alteplase dose facilitates the rate and extent of thrombolysis, thereby producing an early marked increase in complete recanalization.

We developed a new model of intracranial thrombosis and acute ischemic stroke in primates to evaluate the use of intraarterial (IA) thrombolysis in conjunction with intravenous (IV) GP IIb/IIIa blockade. The primary purpose of the study was to acquire preliminary information regarding the feasibility and safety of administration of platelet GP IIb/IIIa inhibitor in conjunction with IA thrombolytic therapy.

Materials and methods

Animal preparation

Rhesus or cynomolgus monkeys weighing 7 to 10 kg each were used in this study, which was approved by the State University of New York at Buffalo Animal Care and Use Committee in accordance with guidelines established by the Animal Welfare Act. Sixteen animals were randomized to one of four treatment groups, which are defined later. The primates were initially sedated with an intramuscular injection of acepromazine (0.2 mg/kg) and atropine sulfate (0.02 mg/kg). General anesthesia was induced by IV administration of tiletamine and zolazepam (2.2 mg/kg). After endotracheal intubation, anesthesia was maintained with 1 to 2% isoflurane inhalation by means of mechanical ventilation. Once anesthetized, the animal was placed in a supine position on an operating table in the angiosuite. A 4-French introducer sheath (Boston Scientific Corporation/Meditech, Watertown, Mass.) was placed in both femoral arteries and both femoral veins under direct exposure following creation of a skin incision and local dissection of the soft tissue in the inguinal region bilaterally. A 4-French catheter (Glidecath, Boston Scientific Corporation/Meditech) was introduced over a 0.035-inch glide wire through the femoral sheath and advanced under fluoroscopy through the abdominal and thoracic segments of the aorta and then into the aortic arch, the right innominate artery and the right common carotid artery. Using magnified road-mapping techniques, the catheter was guided to the origin of the right internal carotid artery. The catheter was advanced approximately 1 to 2 cm into the right internal carotid artery until flow arrest was observed because of proximal occlusion of the internal carotid artery by the catheter. After flow arrest was established, a 2.3-French microcatheter (Prowler Plus, Cordis Endovascular Systems Inc., Miami, Fla.) was advanced over a microguidewire (Transend, Boston Scientific/Meditech) into the intracranial internal carotid artery distal to the origin of the ophthalmic artery (supraclinoid segment). After the microcatheter had been placed in the supraclinoid segment of the internal carotid artery, contrast material was injected through the microcatheter to confirm the position of the microcatheter and patency of the distal intracranial vessels.

Thrombus formation and injection

During femoral artery catheterization, 0.9 ml of arterial blood was withdrawn from the animal. A 200-IU/ml solution of bovine thrombin (Sigma, St. Louis, Mo.) was prepared. The autologous arterial blood (0.9 ml) was mixed with 20 units (0.1 ml) of thrombin and set aside for 15 min to permit clot formation. The mixture was then injected through the 2.3-French microcatheter that had been placed in the supraclinoid segment of the internal carotid artery. The microcatheter was subsequently withdrawn from the guide catheter. After 15 min, an angiogram was performed through the right internal carotid artery. If complete occlusion of the vessel had occurred, the 4-French catheter was with-

drawn into the common carotid artery. If complete occlusion had not occurred (this happened in only three cases), the 2.3-French microcatheter was re-advanced into the supraclinoid segment of the carotid artery. A second thrombus was deposited in the supraclinoid segment in a manner similar to deposition of the first thrombus. The microcatheter was then withdrawn completely, and the 4-French catheter was withdrawn into the common carotid artery. This terminated the temporary flow arrest that lasted for approximately 30 min. If the second deposition of thrombin-blood mixture did not result in complete occlusion of the right internal carotid artery or the proximal middle cerebral artery, the experiment was terminated (in one case). Serial angiograms were performed at 30-min intervals for duration of 2 h. If persistent occlusion was confirmed in the internal carotid or middle cerebral artery after 2 h, the animal was randomized to one of the treatment regimens.

Treatment regimens and randomization

The animals were randomized into the following four treatment regimens: (1) IA reteplase and IV abciximab, (2) IA reteplase and IV placebo, (3) IA placebo and IV abciximab and (4) IA placebo and IV placebo. The active drugs used in this study were reteplase and abciximab. Before administration, one vial of reteplase (Retavase, Centocor Inc., Malvern, Penn.) was diluted with sterile normal saline to prepare a 0.5-unit/ml solution. The total amount injected intraarterially was 1.5 units given as six 0.25-unit boluses (one bolus per min) at 5-min intervals. An equivalent volume of normal saline solution was administered in a similar manner in the groups that were randomized to receive IA placebo. Abciximab (ReoPro, Centocor, Inc.) was diluted in normal saline to prepare a 100-ml solution. Abciximab was intravenously administered as a 0.4 mg/kg bolus, followed by a 0.25 mg/kg/min continuous infusion for a duration of 6 h. An equivalent volume of normal saline was administered in a similar manner in the groups randomized to receive IV placebo. The investigators who administered the treatment (AIQ, AJR) were blinded to treatment regimen allocation. Randomization was performed using computer software (Visual Basic, Microsoft, Redmond, Wash.). The program generated random numbers under pre-specified conditions (i.e., the number of animals that could be allocated to one group).

Administration of treatment regimens

A 2-French microcatheter (Prowler 14, Cordis Endovascular Systems, Inc, Miami) was introduced over a microguidewire through the 4-French catheter into the right internal carotid artery 2 h after thrombus placement. The microcatheter was placed proximal to the occlusion site. Penetration of the thrombus by the microcatheter or the microwire was avoided. Immediately after IA treatment was initiated, IV treatment regimen was given as a bolus followed by a continuous infusion for 6 h. After completion of IA reteplase or IA placebo administration, the microcatheter was removed. The 4-French catheter was maintained in the right common carotid artery for diagnostic angiography.

Assessment of recanalization

Cerebral angiograms were performed through a right common carotid artery contrast injection at the following time points: (1) before the treatment regimen was initiated, (2) after each bolus of IA treatment regimen was administered (six angiographic images were obtained) and (3) hourly for 6 h after completion of IA treatment. The angiograms were collected for grading at a later time. Grading was performed by an investigator (AIQ), who was blinded to the treatment used. For the grading, vessels were categorized into the following orders according to area of distribution and corresponding deficits:

1. First-order vessel—primary vessel for all vessels, e.g., internal carotid artery
2. Second-order vessel—main divisions of first-order artery, e.g., middle cerebral artery and anterior cerebral artery
3. Third-order vessel—main division of second-order artery, e.g., M_2 (middle cerebral artery) and A_2 (anterior cerebral artery)
4. Fourth-order vessel—all other intracranial vessels that cannot be classified as first, second or third-order vessels

Based on this categorization, grading was performed according to the following scheme (Fig. 1): grade 0: normal circulation; grade 1: filling defect, stenosis or irregularity without flow obstruction; grade 2: occlusion of one or more fourth-order arteries, e.g., M_3 branch of the middle cerebral artery; grade 3: occlusion of one or more third-order arteries, e.g., M_2 branch of the middle cerebral artery; grade 4: occlusion of one or more second-order arteries, e.g., M_1 branch of middle cerebral artery. There is some filling of a second-order artery with filling of initial branches (third-order or fourth-order vessels), e.g., proximal middle cerebral artery with filling of lenticulostriate branches; grade 5: occlusion of all second-order arteries in the affected distribution, e.g., middle cerebral artery and anterior cerebral artery. There is no filling of any branch (third or fourth-order vessel) originating from second-order vessels. Grade 6: occlusion of the entire first-order artery in the affected

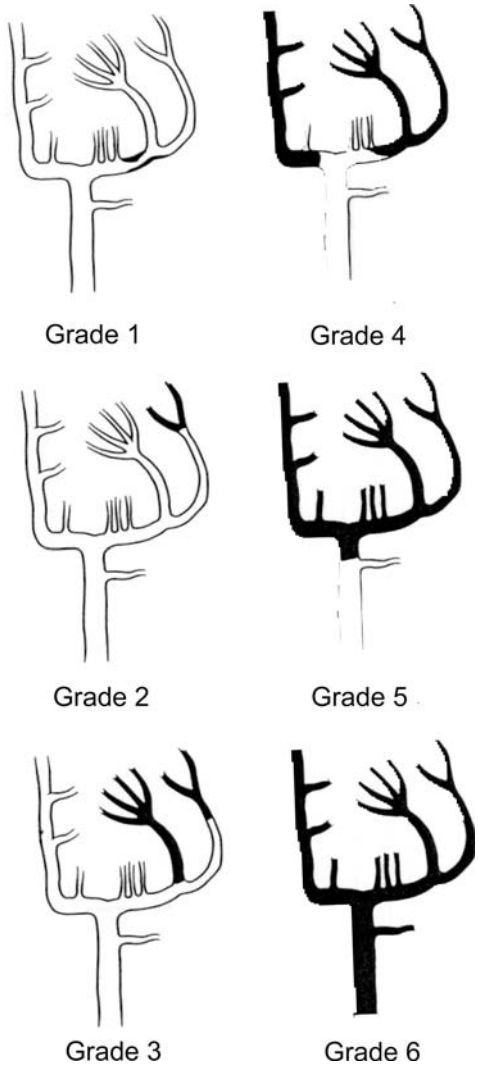

Fig. 1 Different angiographic occlusion grades according to site of thrombus and distal vessels visualized. The *black area* represents vessels with no flow and the *white area* represents patent vessels

distribution, i.e., internal carotid artery. There is no filling of any branch (third- or fourth-order vessels) originating from a first-order vessel.

Platelet aggregation measurements

Platelet aggregometry was performed using the ADP-based Accumetric Rapid Platelet Function Assay instrument (Accumetrics, San Diego, Calif.). Platelet aggregation in citrate-anticoagulated whole blood was measured in response to agonist ADP at the following time points: (1) at the start of the experiment when the 4-French introducer sheath was placed (2) immediately before initiation of IA treatment; (3) at completion of IA treatment; (4) 6 h after administration of IA treatment.

Removal of brain

Six hours after the initiation of therapy, the animals were humanely killed by means of an IV overdose of pentobarbital (100 ml/kg). Selective catheterization of the contralateral internal carotid artery was performed, and the brain was perfused with 500 cc of normal saline solution at 100 to 150 mm of pressure for 30 min. The brain was then perfused with 10% paraformaldehyde (500 ml) for 30 min, removed by means of craniotomy and preserved in 10% paraformaldehyde solution.

Postmortem magnetic resonance imaging

Each brain was imaged using T_1-weighted, T_2-weighted and proton-density sequences on a 1.5-T MR unit. The slice thickness of each image was 3 mm. The images were interpreted by a neuroradiologist (RAA) who was blinded to treatment allocation. Cerebral infarctions on coronal image sections were classified as major or minor. Major cerebral infarction was defined as an infarction that was visualized on more than three sections (>9 mm) or involved the caudate nucleus, lentiform nucleus and cortex on any slice. Smaller infarctions that were seen on MR imaging were considered minor infarctions. The 6-h survival period after administration of the treatment regimen was selected as it represented the time frame by which ischemic injury is unequivocally visualized on T_2-weighted MR sequences [6, 7].

Statistical analysis

Sample size calculations were performed to determine the number of primates in each group required to demonstrate a statistically significant difference ($P < 0.05$) using the chi-square test. If we assume that none of the primates in the control group will have recanalization, and 75% of the primates will have recanalization in the treatment group, a sample size of eight animals is required in each group. If we assume that 50% of the primates in a treated group will have recanalization, a sample size of 12 animals will be required in each group to demonstrate adequately a significant difference. Because of the insufficient number of primates in each group, statistical tests have not been performed to avoid providing inadequate results.

Results

Overall results

The 16 primates were randomized to four treatment groups consisting of four animals each. The site of occlusion, treatment received, pretreatment recanaliza-

tion grade, final recanalization grade and results of MR imaging are summarized in Table 1. Partial or complete recanalization at 6 h after initiation of treatment (defined as a decrease of two or more points in pre-treatment angiographic grade) was observed in two animals treated with IA reteplase and IV abciximab, three animals treated with IA reteplase alone and one animal treated with IV abciximab alone. No recanalization was observed in any of the animals that received IV and IA placebo. Cerebral infarction was demonstrated on postmortem MR imaging in three animals that received both IA and IV placebo. One animal each from the groups that received IA reteplase and IV abciximab or IV abciximab alone had cerebral infarction. No infarctions occurred in the group that received only reteplase. Two animals in the IA and IV placebo group had major infarctions, and one animal in the IA placebo and IV abciximab group had a major infarction. Two other infarctions were classified as minor. One animal that received IV abciximab alone had a small intracerebral hemorrhage on MR imaging. Intracranial hemorrhage was not visualized in the other animals.

Changes in recanalization

Changes in angiographic grade after therapy over time for each group are provided in Fig. 2. Maximum improvement in angiographic grade was seen in the group that received IA reteplase with IV placebo. Less prominent improvement was observed in the group that received IA reteplase followed by IV abciximab. No significant improvement in angiographic grade was observed in the groups that received placebo alone or abciximab alone. Maximum recanalization effect was observed at 2 h following treatment in the group that received IA reteplase and IV placebo (Fig. 3). The recanalization effect was sustained during the 6-h observation period in the IA reteplase and IV abciximab group. In the group that received IA reteplase and IV placebo, recanalization diminished over the observation period and approached that observed in the IA reteplase and IV abciximab group at the conclusion of the experiment.

Changes in platelet aggregation

Changes in platelet aggregation before and after administration of the treatment regimens are depicted in Fig. 4. There was an increase in platelet aggregation in all animals after thrombus was placed in the internal carotid artery. Marked reduction in platelet aggregation was apparent in the IV abciximab and placebo group at 30 min after the initiation of treatment. The group that received IV abciximab in addition to IA reteplase had a prominent reduction in platelet aggregation at 30 min, 90 min and 6 h. We observed less prominent inhibition of platelet aggregation in animals that received IA reteplase and IV abciximab than in those receiving IV abciximab and IA placebo. A more than 80% reduction in platelet aggregation was observed in the IA reteplase and IV abciximab group. The group that received IA reteplase alone demonstrated an increase in platelet activation after administration of the treatment regimen that was seen on the assay obtained 6 h after initiation

Table 1 Thrombus location, arterial recanalization and postmortem MR imaging findings. *IA* Intraarterial, *IV* intravenous, *ICA* internal carotid artery, *MCA* middle cerebral artery, *MR* magnetic resonance

Group	Occlusion site	Initial grade	Final grade	Infarction	Intracranial hemorrhage
IA placebo and IV placebo					
1	Distal ICA	5	6	Present	None
2	Proximal ICA	6	6	None	None
3	Proximal MCA	3	2	Present	None
4	Proximal ICA	6	5	Present	None
IA placebo and IV abciximab					
1	Distal ICA	5	2	None	None
2	Proximal ICA	6	6	None	Present
3	Proximal ICA	6	6	None	None
4	Distal ICA	5	5	Present	None
IA reteplase and IV placebo					
1	Proximal ICA	6	5	None	None
2	Distal ICA	5	3	None	None
3	Proximal ICA	6	0	None	None
4	Proximal ICA	6	3	None	None
IA reteplase and IV abciximab					
1	Proximal MCA	3	3	None	None
2	Proximal ICA	6	2	Present	None
3	Distal ICA	5	6	None	None
4	Proximal ICA	6	2	None	None

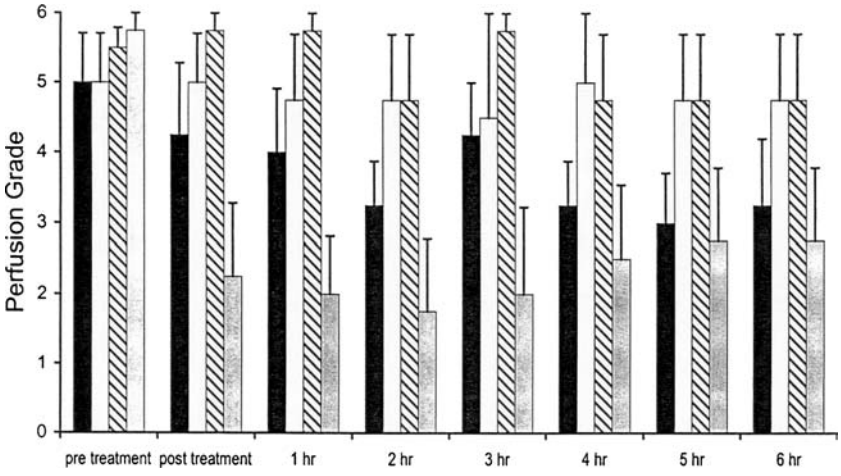

Fig. 2 Changes in angiographic occlusion grade before and after initiation of treatment in each group. *Black bar* represents IA reteplase and IV abciximab group; *white bar* represents IA and IV placebo group; *hatched bar* represents IA placebo and IV abciximab group; *gray bar* represents IA reteplase and IV placebo group. *Standard error bars* are provided with each value

of the treatment regimen. There was no change in platelet aggregation in the group that received IA and IV placebo.

Discussion

Summary of findings

In this animal model of intracranial thrombosis in which a small number of animals were studied, IA reteplase alone or in combination with IV abciximab appeared to be the most effective strategy for recanalization. The maximum recanalization effect associated with IA reteplase alone appeared to occur at 2 h after administration. The administration of IV abciximab alone or in combination with IA reteplase appeared to be more effective than a placebo for prevention of cerebral infarction; IV abciximab alone appeared to have a favorable effect on protection against cerebral infarction, as seen on postmortem MR imaging, despite no clear effect on angiographic recanalization. No intracranial hemorrhages were observed on postmortem MR imaging in animals that received IA reteplase alone or in combination with IV abciximab. A prominent reduction in platelet aggregation was observed in both groups that received IV abciximab. An increase in platelet aggregation was observed after administration of IA reteplase and was sustained for up to 6 h after administration.

Selection and characteristics of the primate model

Primates were selected for this study because of similarities in the primate and human intracranial circulation. The anatomy and distribution of the internal carotid artery in primates allows selective catheterization to be performed. Platelet response to thrombin and thrombin-related stimulation is similar in primates and humans [8, 9]. Furthermore, abciximab exhibits cross reactivity with GP IIb/IIIa and $\alpha v\beta 3$ receptors in primates [10]. A mixture of arterial blood and thrombin was injected in a healthy blood vessel to introduce a thrombus. Therefore, the model best represents the characteristics of an embolic stroke. The model may not represent those situations in which thrombus is superimposed on underlying fissured atherosclerotic plaque where platelet response may be more pronounced due to exposure of the subendothelium. Previous models of ischemic stroke have used a thrombin-blood mixture to introduce thrombosis within the blood vessels [11, 12]. We determined the concentration of the thrombin-blood mixture and the time required to develop a semisolid clot in vitro in a previous experiment [13]. A temporary flow arrest was instituted to avoid interference from antereograde arterial flow during deposition of thrombus and prevent excessive fragmentation and embolization. In the present study, the animals (primates) were studied in the acute period (observed for 8 h) after thrombus introduction. Thrombolysis was initiated 2 h later to accomplish drug delivery within 3 h from onset of thrombosis. The time frame was based on the experience of clinical trials evaluating thrombolytic use in patients with ischemic stroke [14].

Selection of therapeutic regimen

Abciximab was administered intravenously as a 0.4 mg/kg single-bolus dose; this was followed by a 0.25 μg/kg/min infusion for a period of 6 h. This dosing regimen has been shown to result in almost complete inhibition of platelet aggregation in primates (10). The dose of reteplase was extrapolated from clinical studies that have documented the maximum risk-benefit ratio with 20 units of reteplase administered intravenously [15, 16, 17]. Estimating that 20 units is the total IV dose for a human weighing 70 kg, the IV dose for a 10-kg primate

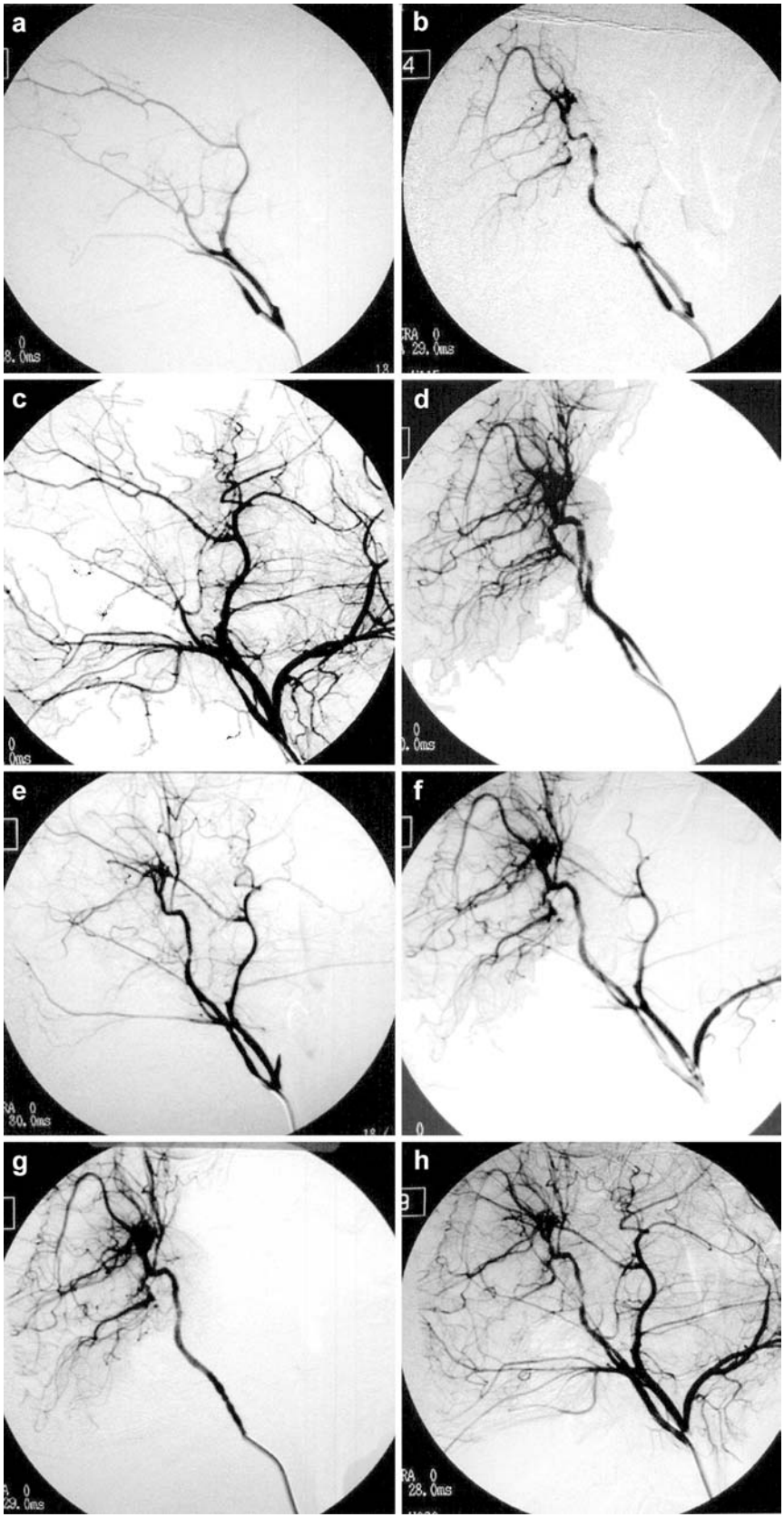

Fig. 3 Cerebral angiographic images (*lateral view*) from an animal in the IA reteplase and IV placebo treatment group. **a** Complete occlusion of the right internal carotid artery at its distal cervical segment immediately after thrombus introduction. There is no flow in the right internal carotid artery at its intracranial segment or in the middle or anterior cerebral artery. **b** Improved flow in the internal carotid artery and intracranial circulation immediately after administration of IA reteplase. Attenuated flow in the intracranial internal carotid and anterior cerebral arteries is suggestive of partial thrombosis or vasospasm. **c** One hour after IA reteplase, there is no filling in the internal carotid artery (reocclusion) at its intracranial segment. **d** Two hours after IA reteplase, there is filling in the anterior cerebral and middle cerebral arteries. **e** Three hours after IA reteplase, there is poor flow in anterior and middle cerebral artery (reocclusion). **f** Four hours after IA reteplase, there is complete filling of anterior and middle cerebral arteries. **g** Five hours after IA reteplase, there is complete filling in the middle cerebral artery branches. **h** Six hours after IA reteplase, there is attenuation of flow in the intracranial segment of the internal carotid artery with reduced flow in the middle cerebral artery branches

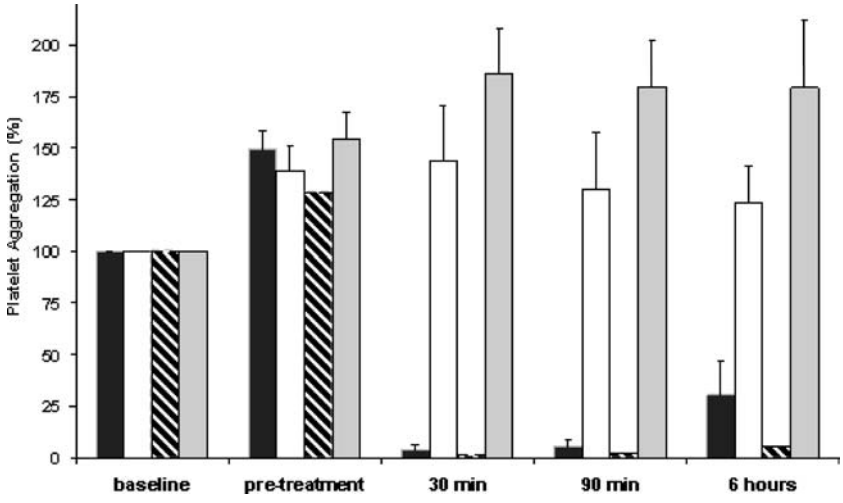

Fig. 4 Changes in platelet aggregation before and after initiation of treatment in each group. *Black bar* represents IA reteplase and IV abciximab group; *white bar* represents IA and IV placebo group; *hatched bar* represents IA placebo and IV abciximab group; *gray bar* represents IA reteplase and IV placebo group. *Standard error bars* are provided with each value

would be 2.9 units. In our previous clinical studies, we used half of the total IV dose for IA administration [18, 19]. For this study, we used 1.5 units (half of the IV dose). Dose adjustments for reteplase according to body weight were not made because the drug was administered intraarterially, and therefore the volume of drug exposed to clot was not dependent on systemic volume of distribution (as is the case with an intravenously administered medication). Furthermore, previous studies have concluded that dose adjustment of reteplase according to patient weight is not necessary [20].

Intraarterial reteplase and intravenous abciximab for acute ischemic stroke

In attempts to optimize the characteristics of fibrinolytics, a third-generation recombinant tissue plasminogen activator, reteplase, which is a structurally modified form of alteplase, was introduced [15, 16, 18, 21, 22, 23]. A prospective study was performed at our center to evaluate the safety and recanalization efficacy of 8 U of IA reteplase for treating ischemic stroke in 16 patients who were considered poor candidates for IV recombinant tissue plasminogen activator (alteplase) therapy, despite presentation within the time window for intervention [18]. Complete or almost-complete recanalization (TIMI grade 3 or 4) was achieved in the arteries in 14 (88%) patients, with partial recanalization (TIMI grade 2) or no response (TIMI grade 1) in one patient each. Neurological improvement was observed in 7 (44%) patients at 24 h. The high rate of recanalization observed in our primate study corresponds with the results achieved in the clinical study.

We observed cerebral infarction in only one animal that received IV abciximab, despite no clear benefit on recanalization. A possible benefit of IV abciximab is supported by the results of a randomized, double-blind, placebo-controlled, dose-escalation trial evaluating the use of IV abciximab in 74 patients presenting within 24 h after ischemic stroke onset [24]. Four escalating doses of abciximab were studied. At 3 months, there was a trend toward less residual disability among patients who were treated with abciximab than those who received placebo. This beneficial effect may be a result of rapid restoration of blood flow and reduced tissue injury and necrosis [25]. Second, abciximab may prevent microvascular obstruction caused by the formation of platelet emboli or distal microthrombi [26]. Finally, it is possible that the interaction of abciximab with receptors other than the GP IIb/IIIa receptor, such as the vitronectin receptor [27, 28] or leukocyte integrin Mac-1 [29], may prevent leukocyte-mediated reperfusion injury.

Recanalization and platelet aggregation

We observed a dynamic process of recanalization following administration of the treatment regimen in all groups. The peak response was observed approximately 2 h after IA reteplase administration. Clinical studies routinely assess recanalization immediately after administration of IA thrombolytics [18, 19, 30]. Our study indicates that assessment of recanalization during IA administration of thrombolytics may under- or overestimate the effect of treatment. The changes in recanalization documented by serial angiography during the 6-h period suggest an active process of ongoing thrombosis and thrombolysis for hours after initiation of treatment. We observed an increase in platelet aggregation following reteplase administration. The augmented platelet aggregation may be caused by the release of thrombin from the effectively lysed clot and explain in part the dynamic changes in recanalization status. The present study provides answers to important questions concerning the degree of platelet inhibition

that can be obtained by antagonizing platelet GP IIb/IIIa with abciximab in the setting of thrombolysis. Observations in previous studies suggest that enhanced platelet activation, as observed in conjunction with acute coronary syndromes [3, 4, 31, 32], leads to a requirement for a higher dose of platelet GP IIb/IIIa antagonist [31]. In another study, a higher concentration of abciximab was needed to inhibit the aggregation of platelets stimulated ex vivo with thrombin receptor-agonist peptide compared with ADP [33]. This observation led to speculation that a higher dose of abciximab might be needed in a setting characterized by elevated indexes of thrombin activity, such as in the case of an acute myocardial infarction [32]. We observed increased platelet activation after thrombus introduction and less prominent inhibition of platelet aggregation in animals receiving IA reteplase and IV abciximab compared with those receiving IV abciximab alone. However, a more than 80% reduction in platelet aggregation was observed in animals receiving both study drugs.

Issues related to data interpretation

The study is limited by the small number of animals in each group. Given the variability within each treatment group and among each treatment groups, the number of subjects in each group is insufficient to detect statistically or clinically significant differences. The present study serves as a basis for a larger experimental study, designed with sufficient statistical power to address the issues of comparative recanalization, infarct size and clinical benefit. We used a mixture of thrombin and autologous blood to generate the thrombus. The method of thrombus introduction may not replicate the characteristics of spontaneous thrombus or thrombus superimposed on fissured plaque. We acknowledge that there may be individual differences between the severity of arterial occlusion in each subject. We expect that randomization and administration of medication in a blinded fashion may reduce the bias introduced by this variation.

We used post-mortem MR imaging to identify and characterize cerebral infarction and intracerebral hemorrhage. Previous investigators have observed that post-mortem MRI is sensitive and reliable in identifying intracerebral lesions [34, 35]. Boyko et al. [34] performed MR imaging on formalin fixed specimens from 71 patients with cerebrovascular diseases. They observed that the sensitivity of MR imaging for detection of infarction exceeded pathologic evaluation of 1 cm brain sections. We used a new angiographic scheme to characterize the extent of recanalization. The scheme was developed to provide a more precise evaluation of recanalization by providing multiple grades of response. The scheme also adjusted for the initial location of the occlusion that may influence the size of infarction. Therefore, the scheme was used instead of the TIMI scheme, which has been used in some studies evaluating response to thrombolysis [36]. The scheme used in the present study would require further evaluation in future studies for interobserver variability and relationship to outcome measures.

Conclusions

Our study provided preliminary information supporting that IA reteplase with or without abciximab appeared to be an effective regimen for achieving recanalization in our model of intracranial thrombosis. Further studies are required in experimental models to determine the optimal dose, method of administration and efficacy of these medications in acute ischemic stroke.

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