Tissue Factor Contributes to Microvascular Defects After Focal Cerebral Ischemia

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Background and Purpose: Microvascular perfusion defects occur after occlusion and reperfusion of the middle cerebral artery in examples of focal cerebral ischemia. In addition to cellular (eg, polymorphonuclear leukocyte) contributors to the focal "no-reflow" phenomenon, activation of coagulation may also play a role. We have tested a potential role of tissue factor–mediated coagulation in the microvascular perfusion defects seen after focal cerebral ischemia–reperfusion in a baboon model of reversible middle cerebral artery occlusion with the marine anti–tissue factor monoclonal antibody TF9-6B4. Tissue factor is the principal resident procoagulant substance in cerebral tissues and has a distinct perivascular distribution.

Methods: Microvascular patency in the basal ganglia after 3-hour middle cerebral artery occlusion and 1-hour reperfusion was quantified by computerized video imaging of carbon-tracer perfused tissues. Animals were randomized to receive intravenous TF9-6B4 (10 mg/kg) 10 minutes before middle cerebral artery occlusion (n=6) or no treatment (n=6) in an open study.

Results: In the control animals, a significant decrease in patency was confirmed in microvessels less than 30 μm in diameter. Infusion of TF9-6B4 before middle cerebral artery occlusion produced a stable maximal level of circulating antibody within 10 minutes, which lasted the duration of ischemia and reperfusion. An increase in reflow in microvessels of all size classes occurred after TF9-6B4 infusion, which was significant in those 7.5 to 30 μm (P=.038) and 30 to 50 μm (P=.013) in diameter.

Conclusions: These results indicate that tissue factor–mediated events may also contribute to no-reflow in noncapillary microvessels after focal cerebral ischemia. (Stroke 1993;24:847–854)

KEY WORDS • cerebral ischemia • microcirculation • procoagulant • baboons

Incomplete perfusion of the microvasculature after transient focal or global cerebral ischemia and reperfusion constitutes the "no-reflow" phenomenon.1 Polymorphonuclear (PMN) leukocytes2-7 and platelets,8 in addition to other endothelium- and subendothelium-related mechanisms,9-11 have been implicated in the formation of these perfusion defects. In addition to cellular contributors to the microvascular perfusion defects after focal cerebral ischemia–reperfusion, coagulation system activation may play a role. This was initially suggested by the ability of the combination of heparin and ticlopidine to significantly reduce postischemia–reperfusion microvascular occlusion formation and platelet deposition in a nonhuman primate model.12

The potent endogenous procoagulant, tissue factor (TF), found prominently in cerebral tissues13 and on perivascular cells,13-15 may be an additional contributor to the development of microvascular occlusions. TF is a 30-kD glycosylated transmembrane protein expressed by epithelia and selected other cells, including vascular adventitial fibroblasts. It is the high-affinity receptor for factors VII and VIIa and is the cofactor necessary for the catalytic function of factor VIIa.16 Binding of factor VII or VIIa to TF is Ca2+ dependent, and the TF–factor VIIa complex has been shown to be the major initiator of the coagulation cascade.17,18 In addition, factors X and IX are activated by the TF–factor VIIa complex, thereby catalyzing thrombin generation and fibrin formation.17,19,20 TF is the principal procoagulant within brain tissue, where it has a prominent perivascular distribution around noncapillary cerebral microvessels (>10 μm in diameter), especially in gray matter.13,15 Although information is scant, electron microscopy has demonstrated fibrin in microvessels associated with degranulated platelets and PMN leukocytes but not in capillaries, after middle cerebral artery (MCA) occlusion and reperfusion.2 The exposure of TF to the plasma column during vascular ischemia may contribute to intravascular coagulation and post–ischemia–reperfusion perfusion defects.

To test the hypothesis that TF-mediated fibrin formation may contribute to the no-reflow phenomenon in focal cerebral ischemia, a well-defined, endotoxin-free, murine, anti–human TF monoclonal antibody (MoAb),
TF9-6B4, which strongly inhibits baboon TF, has been used in the baboon model of reversible MCA occlusion. TF9-6B4 has been shown to inhibit TF function in humans by competitively binding factors VII and VIIa to the cognate binding site on native TF, thus abrogating initiation of coagulation.

Materials and Methods

Twelve adolescent male baboons (Papio anubis/cynocephalus) weighing 7.7 to 14.0 kg were used for the present studies. All animals lacked evidence of disease during a quarantine period of 1 month before entry into this study. The procedures used throughout this study were approved by the institutional Animal Research Committee and were performed in accordance with standards set by the National Research Council (Guide for the Care and Use of Laboratory Animals), the National Institutes of Health Policy on Humane Care and Use of Laboratory Animals, and the US Department of Agriculture Animal Welfare Act. In compliance with these standards, every effort was taken to ensure that the subjects were free of pain or discomfort.

Preparation of the nonhuman primate model of right MCA occlusion and reperfusion and surgical implantation of the MCA occlusion device (Mentor Corporation, Goleta, Calif) have been described in detail. Halothane anesthesia was administered as 3% to 5% induction followed by 1.5% to 2.0% maintenance. After surgical recovery, all animals were allowed a 7-day interval before entry into the experimental protocol. All animals entered into the study were clinically free of infection or apparent inflammation and had normal neurological function (score, 100).

In this randomized open study, six animals received 10 mg/kg TF9-6B4 by intravenous infusion 10 minutes before MCA occlusion, and six animals received no intervention and served as a control group. All subjects were awake. The right MCA was occluded for 180 minutes by inflation of the extrinsic MCA balloon under local anesthesia. During this period momentary and variable agitation was observed in some animals approximately 5 to 10 minutes after occlusion, as the neurological deficit set in. The balloon was then deflated to allow reperfusion of the MCA territory.

Each experiment was terminated 60 minutes after MCA balloon deflation by perfusion-fixation with carbon tracer at high mean arterial pressure. Perfusion-fixation by left ventricular puncture was conducted under thiopental sodium (15 mg/kg infusion) anesthesia and mechanical ventilation as previously described. Isosmotic perfusion flush solution consisted of 25 g/L bovine serum albumin (BSA; Sigma Chemical Co, St Louis, Mo), 2000 IU/L heparin, and 6.7 μM sodium nitroprusside (Fisher Scientific, Fair Lawn, NJ) in Plasmalyte (Baxter Healthcare, Deerfield, Ill) adjusted to 340 mOsm/L with NaCl, pH 7.4, and 4°C to allow washout of all blood elements under antithrombotic conditions. The carbon suspension/fixedate solution consisted of India ink (Pelikan Fount India, Pelikan AG, Hannover, FRG) diluted 1:1 (vol/vol) in Plasmalyte/paraformaldehyde (2%)/glutaraldehyde (0.5%) adjusted to 340 mOsm/L and chilled to 4°C. Before dilution, the India ink was centrifuged at 500g for 10 minutes to eliminate large carbon aggregates. The perfusion flush solution was delivered at 180 to 210 mm Hg (700 to 800 mL/min flow) for 4.0 minutes and was followed immediately by tracer perfusion-fixation at constant pressure for 17.0 minutes. A perfusion circuit was obtained by incising the right atrium to allow egress of the perfusate.

The perfusion-fixed brain was immersed in alcohol-formaldehyde-acid (AFA) solution for 7 days, and the 2-mm coronal sections were immersed in AFA solution for an additional week. Tissue blocks (1.0×1.0×0.2 cm) from stereanatomically identical sites of the left and right basal ganglia and from the temporal lobe in the left (nonischemic) side were embedded in glycol methacrylate (Polysciences, Inc, Warrington, Pa), sectioned at 10-μm thickness, and stained with basic fuchsin and methylene blue.

The relative number and minimum transverse diameters of patient carbon-filled microvessels 10 μm thick at 30-μm intervals in sections from the normal and post-ischemia-reperfusion territories were determined automatically with a computerized video imaging system using a Hamamatsu C2400-07 Newvicon NTSC video camera (Hamamatsu Photonics, Hamamatsu, Japan) staged vertically on an Axioskop light microscope with an image processing unit (Kontron and Carl Zeiss, Munich, FRG). Ninety nonoverlapping images at ×200 optical magnification in a 9×10 field matrix (25 mm²) from each section were processed automatically and in a blinded fashion as previously described. An identical number of fields were evaluated from each of the paired basal ganglia such that 2000 vessels in the left control basal ganglia were analyzed in each subject. Reproducibility and reliability data have been reported previously. The ratio of all microvessels in the left (normal) basal ganglia to the left temporal cortex (layers I to VI) were 0.59±0.27 (n=6) and 0.60±0.23 (n=6) for control and treated animals, respectively, which compares favorably with previous observations.

Relative microvascular patency was expressed as “percent reflow,” the ratio of the number of carbon-containing microvessels in the ischemic to control basal ganglia normalized per 1.0 cm² and expressed per 100 vessels. Accepted definitions of microvascular size were used for data analysis of discrete vessel size classes.

Neurological function was assessed according to the weighted (100-point) scale suggested by Spetzler and colleagues. TF9-6B4 was purified from cell culture by protein A affinity chromatography in the following manner. The culture supernatant was diluted 1:1 with binding buffer (0.1 M glycine and 3 M NaCl, pH 8.9) and passed over a protein A (IPA 300, Repligen, Boston, Mass) column to bind murine immunoglobulin (Ig). Antibody was eluted with 50 mM acetate and 100 mM NaCl (pH 4.0). Elution was monitored by UV absorbance. The protein A column eluate was loaded onto a G-25 sepharose (Pharmacia, Stockholm, Sweden) column equilibrated with 50/50 phosphate-buffered saline (PBS) buffer (50 mM phosphate and 50 mM NaCl, pH 7.0) to exchange buffers. After buffer exchange, the antibody was filter concentrated to 5 to 6 mg/mL using a Membrex Benchmark rotary biopurification system. MoAb in 50/50 buffer was then passed over DEAE Sepharose FF (Pharmacia) in nonbonding mode to reduce lipopoly saccharide and DNA contamination. The final product was sterilized by filtration through a 0.2-μm filter. The TF9-6B4 product was more than 90% pure by sodium
dodecyl sulfate–polyacrylamide gel electrophoresis analysis and had endotoxin levels less than 2 EU/mL by standard Limulus amebocyte lysate chromogenic assay (Whittaker, Walkersville, Md). Material from a single lot was generously supplied by the R.W. Johnson Pharmaceutical Research Institute (La Jolla, Calif).

Peripheral blood samples for TF-9-6B4 (murine IgG) level determinations were obtained by venipuncture and drawn into heparin sodium (100 IU/mL) at various times: before TF9-6B4 infusion and MCA occlusion and at 10, 60, 120, 180, and 240 minutes after MCA occlusion. Plasmas from each sample were frozen and stored at −70°C until assay.

Baboon plasma levels of murine IgG, were measured using a capture enzyme-linked immunosorbent assay. Microtiter plates (Costar, Cambridge, Mass) were coated with 8.4 μg of goat anti-mouse IgG, IgM, and IgA (Organon Technika, Durham, NC) in 100 μL PBS buffer (Ortho Diagnostic Systems, Raritan, NJ) overnight at 4°C. Goat anti-mouse antibody was removed and the plate was blocked with 5% newborn calf serum (Irvine Scientific, Irvine, Calif) in PBS for 1 hour at 37°C. Blocking solution was removed, and 100 μL of test plasma or purified TF-9-6B4 reference antibody, appropriately diluted in T-wash (50 mM tris(hydroxymethyl) aminomethane hydrochloride, 150 mM NaCl, 2.5% newborn calf serum, 2 mg/mL BSA, 0.5% polyoxyethylene-sorbitan monolaurate 20, pH 7.6), was added to the microtiter wells. Microtiter plates were incubated for 1 hour at 37°C. The test sample was removed, and the plates were washed six times with PBS plus 0.5% polyoxyethylene-sorbitan monolaurate 20. One hundred microtiter of T-wash containing 250 μg horseradish peroxidase–conjugated goat anti-mouse IgG1 (Nordic Immunology, Tilburg, the Netherlands) was added to each well and incubated for 1 hour at 37°C. Conjugated antibody was removed, and the microtiter plate was washed six times with PBS plus 0.5% polyoxyethylene-sorbitan monolaurate 20. 0-Phenylendiaminedihydrochloride substrate (Sigma) was prepared according to the manufacturer’s instructions, and 100 μL was added to each well. After a 30-minute incubation at room temperature, the reaction was stopped by addition of 50 μL per well 4N sulfuric acid to each well. The ODc was read, and the serum levels of murine IgG were calculated by comparison of ODe obtained from test samples with an ODe standard curve prepared from purified TF9-6B4.

Complete blood counts were performed on a System 9000 cell counter (Baker Instrument, Allentown, Pa). All data are presented as the mean or mean±SD. Student’s t test (one-tailed) was used for analysis of the cohort data. Significance was set at P<.05 for all comparisons and for comparisons of reflow patency, because it is unlikely that TF inhibition would promote occlusion formation.

Results

No subjects were excluded from data analysis on grounds of inadequate or inappropriate carbon-tracer perfusion.

Baseline and post–MCA occlusion hematocrit levels, total leukocyte counts, platelet counts, and neurological scores between the TF9-6B4–treated group and the untreated group were not statistically different (Table 1). Both groups displayed an abrupt, significant increase in total leukocyte count at the time of MCA occlusion. This has been previously observed. A nonsignificant rise in platelet count was seen in both the TF9-6B4–treated and untreated cohorts during ischemia and reperfusion.

Plasma murine MoAb levels were significantly elevated over baseline within 10 minutes of infusion, reached a peak level within 1 hour, and remained elevated throughout the ischemia-reperfusion period (Fig 1).

After 180 minutes of MCA occlusion and 60 minutes of reperfusion, the untreated group displayed a significant reduction in the number of patent lenticulostrial microvessels with minimum diameters of 4.0 to 7.5 μm (P=.003) and 7.5 to 30.0 μm (P=.018) in the ischemic compared with the nonischemic basal ganglia (Fig 2). These vessels are consistent with capillaries and with postcapillary venules and precapillary arterioles, respectively. In contrast, the anticipated reduction in number of patent microvessels less than 30.0 μm in diameter in the ischemic territory was not observed in the TF9-6B4–treated cohort (P>.20) (Fig 3).

Infusion of TF9-6B4 just before MCA occlusion resulted in a significant increase in microvascular reflow (Figs 4 and 5). Improvement in reflow reached statistical significance in the microvessel classes of 7.5- to 30-μm diameter (postcapillary venules and precapillary arterioles) (P=.038) and of 30.0- to 50.0-μm diameter (connecting venules and small arterioles) (P=.013). A nonsignificant increase (P=.118) in reflow after TF9-6B4 was seen in capillaries (4.0 to 7.5 μm in diameter). The normalized patency difference (percent reflow) in microvessels in the size class of greater than 50 μm in diameter was not significant because of the small number of vessels (16±12 cm² for control subjects and 37±19 cm² for treated subjects) contained in this size class and the variable percent reflow noted among subjects. The relatively small numbers of vessels in the 30.0- to 50.0-μm-diameter and the greater than 50.0-μm-diameter ranges may have influenced the results.

Parenchymal hemorrhage was not a feature of the TF9-6B4–treated group. Hemorrhagic infarction, seen in four of the six treated subjects, was not different from the two events seen in the untreated group.

Motor function measured by neurological assessment score declined within 5 to 15 minutes of MCA occlusion in all animals and remained unchanged for the duration of each experiment (Table 1). The neurological score in animals treated with TF9-6B4 did not improve in the untreated group at any time during the ischemia and reperfusion period.

To evaluate any contribution of microvascular dilatation to the reflow improvement observed in the TF9-6B4 group, the normalized proportion of microvessels in each size class was determined at 1-μm intervals. The mean proportion of microvessels at each interval was nearly identical between the treated and untreated groups.

Discussion

Little attention has been focused on the role of fibrin formation, or other consequences of local thrombin generation, in microvascular obstruction after focal cerebral ischemia-reperfusion. These experiments are
TABLE 1. Comparison of Laboratory Values and Neurological Score in TF9-6B4-Treated and Untreated Groups

<table>
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<th>N</th>
<th>Baseline</th>
<th>60 Min after MCA occlusion</th>
<th>60 Min after reperfusion*</th>
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<td>WBC ($\times 10^3/\mu$L)</td>
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<td>Untreated</td>
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<tr>
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<td>Platelet ($\times 10^3/\mu$L)</td>
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<td>555±45</td>
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MCA, middle cerebral artery; WBC, white blood cell.
*After 180 minutes of MCA occlusion.

the first indication that inhibition of the potent endogenous procoagulant, TF, by a purified MoAb against the high-affinity factor VII/VIIα binding site on TF may increase microvascular reflow after focal cerebral ischemia and reperfusion. The infusion of TF9-6B4 before occlusion of the MCA is an important feature of these experiments. Infusion of TF9-6B4 resulted in a significant increase in microvascular patency in 7.5- to 50.0-μm-diameter vessels, consistent with postcapillary venules/precapillary arterioles and connecting venules/small arterioles. Increased reflow in the greater than 7.5-μm-diameter microvascular classes is consistent with the presence of TF around microvessels greater than 10.0 μm in diameter in the primate cerebral cortex and basal ganglia. The 25% increase in mean reflow observed in the capillary group may reflect increased flow in some precapillary arterioles. The significant increased reflow in the 30.0- to 50.0-μm-diameter vessel cohort and that of the greater than 50.0-μm-diameter cohort involved comparisons of small vessel numbers (Figs 2 and 3). An analysis of normalized microvascular

FIG 1. Line graph shows mean plasma concentrations of murine anti–tissue factor (TF) monoclonal antibody (MoAb) after infusion and middle cerebral artery occlusion. All levels are significantly elevated compared with baseline (P<.001).

FIG 2. Bar graph shows mean number of patent microvessels per square centimeter in nonischemic (cross-hatched) and ischemia-reperfusion (IR) (open) basal ganglia of untreated animals. A significant reduction in vessel patency is seen in classes 4.0 to 7.5 μm (P=.003) and 7.5 to 30 μm (P=.018) in diameter. The difference in classes 30 to 50 μm and 50 to 100 μm in diameter is not significant.
diameter distribution in the nonischemic MCA territories argues strongly against vasodilation as a contributor to perfusion enhancement by TF-6B4.

These studies address the hypothesis that microvascular perfusion defects observed after temporary occlusion of the MCA may result from thrombosis within the microvasculature, either as a consequence of ischemic vascular injury, reperfusion, or both. The findings here support this hypothesis and indicate that fibrin formation is initiated at the earliest step in coagulation activation by TF. Recently, PMN leukocyte obstruction of capillaries and adherence to postcapillary venule endothelium has been implicated in impaired reflow after MCA reperfusion in this model. Blocking of PMN leukocyte adherence just before MCA reperfusion by a function-inhibiting MoAb of the leukocyte integrin CD11b/CD18 has been shown to improve reflow in the cerebral microvasculature. Those observations, and other more indirect studies that suggest that granulocytes contribute to post-MCA reperfusion defects, are not inconsistent with a role for coagulation activation in postreperfusion microvascular perfusion defects in this territory. We have confirmed the presence of fibrin after ischemia-reperfusion in leukocyte and platelet aggregates and along the luminal or abluminal endothelial surface of occluded capillaries and postcapillary venules. Fibrin formation in post-ischemia-reperfusion perfusion defects has also been suggested by a significant reduction of postreperfusion occlusions after preischemia heparin and ticlopidine in the baboon model and a similar effect after heparin exposure in a 4-hour MCA occlusion-reperfusion macaque model. Separately, preischemia infusion of a novel antithrombin in a rodent forebrain ischemia model was shown to decrease microvascular occlusion formation after reperfusion. Whether those results relate to the clinical observations suggesting increased thrombin generation and increased fibrinogen turnover in patients with recently completed cerebral infarctions is not yet established.

That TF may contribute to post-ischemia-reperfusion cerebral microvascular occlusion is consistent with its presence in microvessels greater than 10.0 μm in diameter in the primate central nervous system. TF is an integral membrane glycoprotein that is ubiquitous outside the vascular system; it is predominantly found on fibroblasts in the adventitial layers of the vessel with only minimal amounts in the media. Although the intima has little TF antigen, exposed arterial subendothelium presents TF activity in vitro after injury. In the nonhuman primate, TF is not associated with cerebral capillaries, which is consistent with their lack of an adventitial layer. It is expected that preischemia inhibition of vascular-related TF activity would improve reflow in 7.5- to 30-μm-diameter microvessels, but not in capillaries, if fibrin formation is important to no-reflow. Other mechanisms for capillary obstruction, eg, by PMN leukocyte adhesion (which can be elicited by thrombin), could account for the relative lack of reflow at the capillary level (Figs 4 and 5).

Increased vascular permeability after ischemia-reperfusion may expose perivascular TF to the plasma compartment with subsequent intravascular fibrin formation and occlusion. After cerebral arterial occlusion, hypoxic vessels become rapidly permeable to small ions. At 2 to 3 hours posts ischemia the blood-brain barrier becomes permeable to certain plasma proteins (eg, albumin) allowing their contact with the high suben-

**Figure 3.** Bar graph shows mean number of patent microvessels per square centimeter in nonischemic (cross-hatched) and ischemia-reperfusion (I/R) (open) basal ganglia of TF-6B4-treated animals. Differences between the number of microvessels in each diameter class were not significant (P > .20 each).

**Figure 4.** Graph shows mean percent reflow by microvessel diameter in untreated (●, n=6) and TF-6B4-treated (○, n=6) subjects.

**Figure 5.** Bar graph shows mean percent reflow in each diameter class of untreated and TF-6B4-treated groups. Increase in percent reflow in classes 7.5 to 30 μm (P=.038) and 30 to 50 μm (P=.013) in diameter was significant. Differences in percent reflow in classes 4 to 7.5 μm (P=.118) and 50 to 100 μm (P=.603) in diameter did not reach significance. TF, tissue factor; MoAb, monoclonal antibody. *SD=173.4.
dothelial and parenchymal concentration of TF. Extravascular fibrin formation would also be expected to occur. Microvascular permeability may therefore provide an important regulatory mechanism for control of microvascular thrombosis.

Two other sources of TF may contribute to cerebral microvascular occlusion. TF expression on the endothelial cell surface may be induced in vitro by a number of pathophysiologically relevant mediators, including thrombin, interleukin-1, endothelin, tumor necrosis factor, immune complexes, and minimally oxidized low-density lipoproteins. Maximal TF expression occurs by 4 to 6 hours after induction. More intriguing to the problem of cerebrovascular ischemia is the finding that procoagulant activity is expressed on the cultured endothelial cell surface after hypoxia induced by decreased Po2 in the range of in vivo hypoxic states. Expression of procoagulant activity is increased further by subsequent reoxygenation.

Circulating monocytes express TF after exposure to endotoxin, immune complexes, and plasma lipoproteins. A recent study by Gregory et al suggests that TF mRNA is present within 15 minutes after stimulation of monocytes with endotoxin and peaks at 2 to 4 hours. In this setting, TF antigen appears on the monocyte surface by 2 hours, well within the limits of this study. TF transported from the cytoplasm to the plasma membrane may be shed in membrane vesicles. Once TF is exposed to the extracellular environment, activation of factor VII and formation of the TF-factor VIIa complex in the presence of coagulation factors lead to thrombin generation and fibrin formation. Fibrin strands have been observed with activated monocytes, including those traversing vessels at sites of cerebral infarction. Recent work has demonstrated that TF release is significantly increased from stimulated cultured endothelial cells in the presence of monocytes over either cell type alone, suggesting that a cell-to-cell interaction may be important in enhancing the magnitude of thrombin generation in vivo.

We cannot exclude that among cells classified as PMN leukocytes in a recent report, a small number of monocytes may have been present, which also contributed to occlusion formation after focal ischemia-reperfusion in the primate.

The anti-TF MoAb TF9-6B4 has been shown to inhibit 96% of plasma coagulation induced by purified human brain TF. Similar inhibition of coagulation by TF9-6B4 was found using baboon brain extract as a source of the TF. Complete inhibition of coagulation and factor X activation was seen with anti-TF antibody concentrations on the order of 1 μg/mL in vitro. Considering an average baboon plasma volume of 500 mL (10 kg) and a TF9-6B4 dose of 10 mg/kg, a concentration of approximately 500 μg/mL appears sufficient to achieve complete inhibition of TF activity. This is consistent with the plasma anti-TF antibody levels measured here.

The use of an anti-TF antibody to block TF-dependent coagulation carries a theoretically increased risk of parenchymal hemorrhage, especially in focal ischemia. The increased presence of TF in cerebral gray matter parenchyma may protect this territory from such expansive hemorrhages. In this study, no parenchymatous hematoma was seen in either group and hemorrhagic infarction, a natural consequence of focal cerebral ischemia and reperfusion, was not different from the untreated group. Because vascular permeability and hemorrhage may be related to ischemia duration, more prolonged MCA occlusion in the presence of TF9-6B4 may lead to significant intracerebral hemorrhage.

As with previous experiments, although microvascular patency was significantly improved after TF9-6B4 infusion, the short-term nature of this study did not afford an adequate assessment of neurological recovery. Therefore, long-term studies will be necessary to further clarify a role of this approach in stroke management.

In addition to a role of PMN leukocyte adherence, we describe here a second important potential contributor to impaired microvascular reflow after focal ischemia-reperfusion. The preischemia use of TF9-6B4 does not allow one to determine when TF-dependent fibrin formation may have occurred. The experimental paradigm used in this study maximized the vascular distribution and early effect of TF9-6B4 by infusion before MCA occlusion. At what point during ischemia or reperfusion TF may contribute to such perfusion defects remains to be elucidated.

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