Hemorrhagic Transformation Following Tissue Plasminogen Activator in Experimental Cerebral Infarction

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The effect of an intravenous infusion of recombinant tissue plasminogen activator on hemorrhagic transformation early after middle cerebral artery territory ischemia was studied in an established awake nonhuman primate (baboon) model. Following 3 hours' occlusion of the middle cerebral artery and 30 minutes' reperfusion in each of 30 baboons, a 60-minute infusion of recombinant tissue plasminogen activator (at three doses: Group A, 0.3 mg/kg, n=6; Group B, 1.5 mg/kg, n=6; Group C, 10 mg/kg, n=6) or normal saline (n=12) was undertaken. The frequency and volume of intracerebral hemorrhage, the volume of infarction, and clinical alterations were determined by computed tomography at 24 hours and 10 days, neuropathology at 14 days, and serial daily neurologic evaluations, respectively. Peripheral (nonintracranial) hemorrhage (Group A, p=0.46; Group B, p=0.015; Group C, p=0.002) and peak plasma tissue plasminogen activator levels varied directly with the dose of recombinant tissue plasminogen activator. Petechial hemorrhagic infarction was a common finding among the 30 baboons. No significant differences in the incidences or volumes of infarction-related hemorrhage were apparent in any group compared with the respective saline-treated baboons. In pooled data, no significant relation between the volume of hemorrhage and the volume of infarction could be established. We conclude that the incidence and severity of hemorrhagic transformation are not related to infarction size and that recombinant tissue plasminogen activator does not increase the incidence or severity (volume) of hemorrhage when given early (≤3.5 hours) after the onset of focal cerebral ischemia in this model. (Stroke 1990;21:596-601)

A role for fibrinolytic agents in the treatment of acute thrombotic stroke requires consideration of their contribution to infarction-related hemorrhage. Early studies of the fibrin-nonselective agents urokinase and streptokinase in patients with nonacute stroke raised concerns regarding the possible deleterious effect of intracerebral hemorrhage that might accompany the use of these agents. Although hemorrhagic transformation following local intra-arterial infusion of both agents for acute carotid- and vertebrobasilar-territory thrombotic stroke has been reported,1-3 the true risk of symptomatic hemorrhage in this setting is unknown.

The relatively fibrin-selective nature of tissue plasminogen activator (t-PA) has made it an attractive thrombolytic agent for intravenous administration in the treatment of arterial thrombosis.4 Initial enthusiasm for t-PA derived from its limited fibrinolytic effect demonstrated in studies of acute myocardial infarction, which implied a somewhat lower hemorrhagic potential for t-PA than for fibrin-nonselective agents. However, a dose-dependent increase in clinically significant central nervous system hemorrhage following intravenous infusion of recombinant t-PA (rt-PA) in patients with acute myocardial infarction has been demonstrated.5 The risk of intracerebral hemorrhage following the use of t-PA in patients with acute thrombotic stroke is unknown.

We report the results of a study to assess the contribution of ischemia (and reperfusion) to the inci-
dence and extent of hemorrhagic transformation in a defined region of cerebral ischemia following early intravenous infusion of rt-PA in an awake primate model of acute middle cerebral artery (MCA) stroke.

Materials and Methods

We used 30 adolescent male baboons (Papio cynocephalus anubis) weighing approximately 11-13 kg. All were dewormed and observed to be disease-free for at least 6 weeks before study. All procedures were approved by the Institutional Animal Research Committee and were in accord with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Baseline circulating platelet counts, hematocrits, mean leukocyte counts, and fibrinogen levels for the baboons were within limits previously reported. All subjects had normal neurologic function (neurologic score of 100) after implantation of the MCA occlusion device and before entry into the study. Postimplantation cerebral computed tomography (CT) documented the absence of cerebral infarction and detectable hemorrhage in all subjects before entry into the study.

The procedure for implantation of the right MCA Silastic inflatable balloon device has been described. Sodium thiopental anesthesia (10 mg/kg induction bolus and 10 mg/kg/hr infusion) was routinely employed and was supplemented with 0.2 mg pancuronium during implantation. Recovery characteristics and functional outcome were as described elsewhere.

The long-term (14-day) experimental format employed was similar to that in previous studies. Following 3 hours of MCA occlusion and 30 minutes of reperfusion (deflation of the MCA occlusion device), all baboons received a 60-minute intravenous infusion of rt-PA (at one of three doses) or placebo (equal volume of 0.9% saline, USP). In the first experimental set, an open study, each of 18 baboons was treated with 0.3 mg/kg rt-PA (Group A, n=6), 10.0 mg/kg rt-PA (Group C, n=6), or saline (Group A/C placebo, n=6). In a second experimental set, 12 baboons were randomized to treatment with 1.5 mg/kg rt-PA (Group B, n=6) or saline (Group B placebo, n=6); the investigators were blinded to the treatment. The doses of rt-PA were chosen to encompass a range from subtherapeutic (A) to excessive (C), the latter presumably capable of producing significant hemorrhage. All baboons received 0.9% saline at 50 ml/hr continuous infusion through a separate intravenous port during MCA occlusion and for 6 hours thereafter. No anticoagulants or antiplatelet agents were employed. All subjects underwent pressure perfusion-fixation at 14 days with 10% phosphate-buffered formalin at 90–100 torr intraarterial perfusion pressure.

Neurologic function in each baboon was measured serially according to the weighted quantitative neurologic scale previously described. Serial cerebral CT scans were performed on a GE 9800 scanner (Milwaukee, Wisconsin) after implantation of the MCA occlusion device, and at 24 hours and 10 days after MCA occlusion.

The volume of infarction was determined from the regions of encephalomalacia and cystic formation in the corpora striata (and adjacent temporal cortex) ipsilateral to the MCA occlusion on serial 2-mm coronal slices of whole perfusion-fixed brains. The rostral surface area of the appropriate defect from each slice was computed from the weight of tracings cut from the projected 35-mm photographic images of infarction and hemorrhage (normalized and adjusted for magnification). The volume of infarction was determined as the summation of the rostral surface area of infarction x 0.2 cm for all coronal slices. This method caused mean control volumes of infarction to be consistently lower than those previously reported.

Intracerebral hemorrhage on the rostral surface of each coronal slice was defined as petechial (0.2–2 mm), confluent petechial (<1.0 cm), hematoma (hemorrhage with mass effect), or other (e.g., subarachnoid). The volume of hemorrhage associated with each infarction was determined as the summation of the rostral surface area of hemorrhage x 0.2 cm for all coronal slices. Venepuncture-site hemorrhage, surgical-site hemorrhage, and/or peripheral hematoma formation constituted extracerebral peripheral hemorrhage.

Plasma was sampled at selected times. Serial rt-PA antigen levels were measured by IRMA, and serial circulating fibrinogen levels were determined using a modification of the method of Jacobson (samples were collected in e-aminocaproic acid).

The rt-PA preparations were predominantly two-chain and were obtained as generous gifts from two sources. That used in Group A was a predominantly two-chain preparation (G11021) from Genentech, Inc. (South San Francisco, California), while those used in Groups B and C were provided by the Wellcome Research Laboratories (Research Triangle Park, North Carolina). No attempt was made to compare or adjust relative specific activities; the doses employed were based on the manufacturers' labeling.

The data are expressed as mean ± standard deviation (SD). Neurologic scores and plasma concentrations of the groups were compared using Student's t test (unpaired data) and serial χ² analyses. Infarction and hemorrhage-related volume changes were compared using logistic regression analysis and analysis of covariance.

Results

Neurologic scores for the Group A/C and Group B placebo cohorts did not differ significantly from each other and from those previously published (data not shown). Despite an apparent significant improvement over placebo in neurologic score for Group A, Groups B and C fared no better than their respective
placebo cohorts (data not shown). No neurologic deterioration was evident in any subject during or after infusion of rt-PA (data not shown).

Peak levels of t-PA antigen in plasma reflected rt-PA doses (Figure 1, top); t-PA antigen levels fell to near baseline soon after termination of the infusion, consistent with the known pharmacokinetic characteristics of rt-PA. At each rt-PA dose, the measured decrease in fibrinogen level followed the measured increase in t-PA antigen level (Figure 1, bottom). The decrease in fibrinogen level after infusion of placebo was due to hemodilution. In the two placebo cohorts, no significant changes in t-PA or fibrinogen levels during MCA occlusion or reperfusion were evident.

A significant dose-dependent increase in the number of baboons with venipuncture-site hemorrhages and 7-day-old surgical-site hemorrhages was noted among Groups B and C compared with Group A and either placebo cohort (Table 1).

No significant difference was observed in the number of rt-PA- and placebo-treated baboons displaying intracerebral hemorrhages. Most baboons in each group displayed petechial hemorrhages (Table 1); confluent petechial hemorrhages were unusual. Cerebral CT scans suggested right corpus striatal hemorrhagic transformation in the one Group B placebo-treated baboon with only confluent hemorrhage. In Group C, no intracerebral hematomas were noted. No relation between the volume of hemorrhage and the rt-PA dose ($p=0.53$, Table 1) or the peak level of plasma t-PA ($p=0.43$) was found.

No significant difference in mean volume of infarction was noted between the rt-PA-treated and their respective placebo-treated groups at any dose, and no difference among the rt-PA-treated groups was apparent (Table 1). No relation between volume of infarction and rt-PA dose or peak level of plasma t-PA was apparent. When the data were pooled, no relation between the presence or volume of infarction-related hemorrhage and the volume of infarction (independent of treatment) was noted (Figure 2).

Discussion

Intracerebral hemorrhage with clinical deterioration represents the most important major complication of and safety issue in the use of thrombolytic agents in patients with thrombotic disorders, including acute thrombotic stroke. Few studies employing animal models of acute cerebral ischemia have investigated the incidence and mechanism of intracerebral hemorrhage following the infusion of fibrinonselective (urokinase and streptokinase) or fibrin-selective (t-PA, scu-PA) thrombolytic agents and their effects on outcome. This study evaluated the contribution of territorial ischemia alone to the risk of hemorrhage after rt-PA in acute stroke, and in this way differs from most studies.

Early infusion of rt-PA did not increase the incidence or volume of hemorrhagic transformation.

FIGURE 1. Top: Graph of mean±SD concentration of tissue plasminogen activator (t-PA) antigen in plasma of baboons treated with saline (placebo, n=6 for each group) or 0.03 (rt-PA A, n=6), 1.5 (rt-PA B, n=6), or 10.0 (rt-PA, n=6), mg/kg. Bottom: Graph of mean±SD fibrinogen ratio (mean plasma fibrinogen concentration+ baseline plasma fibrinogen concentration) in the same baboons.
TABLE 1. Effect of rt-PA on Cerebral Infarction and Related Hemorrhage in Baboons after Middle Cerebral Artery Occlusion

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Group</th>
<th>Dose (mg/kg/hr)</th>
<th>Peripheral hemorrhage</th>
<th>Intraparenchymal hemorrhage</th>
<th>Infarction volume</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>n</td>
<td>No.</td>
<td>p</td>
</tr>
<tr>
<td>Saline</td>
<td>A/C</td>
<td>0</td>
<td>6</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>0.3</td>
<td>6</td>
<td>2</td>
<td>0.455</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>1.5</td>
<td>6</td>
<td>5</td>
<td>0.015</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>10.0</td>
<td>6</td>
<td>6</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Data are mean±SD. rt-PA, recombinant tissue plasminogen activator; P, petechial; CP, confluent petechial; H, hematoma. Saline-treated (placebo) groups compared with each other; rt-PA-treated groups compared with respective placebo using serial Student's t tests.

associated with focal cerebral ischemia following reperfusion, and associated hemorrhage-related neurologic deterioration was not apparent. Three recent studies of t-PA in rabbit models of carotid-territory infarction are pertinent. In a ligation/hypotension model, t-PA (melanoma cell-derived, 2x10^5 IU) given 24 hours after cerebral ischemia in four rabbits was associated with macroscopic hemorrhage in 75% (0% of untreated controls had macroscopic hemorrhages). However, hemorrhage was no more frequent in rabbits treated with rt-PA (Wellcome, 5 mg/kg) than in controls when given early (30 minutes or 4 hours) following the infusion of autologous thrombi. In a study of similar design, hemorrhagic transformation was equally frequent if rt-PA was given 10 minutes, 8 hours, or 24 hours after the thromboembolic event. Other animal studies of cerebral ischemia have suggested that intracerebral hemorrhage does not significantly complicate infusions of fibrin-nonselective agents in those species.

Methodologic concerns may adversely affect outcome in animal studies of fibrinolytic agents. Regarding hemorrhage size, criteria for petechial and confluent petechial hemorrhages in fixed tissue have been adopted and extended. Our sample sizes were sufficient to detect a hemorrhage volume difference of 4x10^-3 cm³ between groups with a power of 0.5 using a one-sided test at an α level of 0.05. In retrospect, our sample sizes were sufficient to detect a difference in mean hemorrhage volume of 1.0 cm³ with a power of >0.99 (assuming that a clinically significant hemorrhage would be as large as the infarct and would occur in three of six baboons at any rt-PA dose). The risk of hemorrhage caused by rt-PA is expected to be related to its thrombolytic effect. The thrombolytic effect of rt-PA at the doses used was indirectly confirmed by the dose-dependent increase in measured plasma t-PA levels, together with the dose-dependent increase in the incidence of peripheral hemorrhages. The disparity between the incidence of peripheral hemorrhages and the absence of significant ischemia-related hemorrhagic transformation in this study may reflect differences in the responses of the respective vascular beds to different injuries (surgical interruption of cutaneous vessels with thrombosis versus ischemia in a cerebral end-arteriolar bed) in the presence of rt-PA.

Infarction-related petechial hemorrhages often accompany untreated thrombotic/thromboembolic stroke, occurring in up to 60% of patients, a finding analogous to the frequency of petechial hemorrhages in our placebo cohorts. It has been suggested that progressive endothelial disruption, capillary expansion and rupture during perivascular ischemia, and/or exposure of the ischemic end-arteriolar bed to systemic blood pressure may contribute to ischemia-related hemorrhage. Garcia et al have suggested that hemorrhagic transformation is more frequent following MCA reperfusion, depending on the duration of the occlusion (in cats), and is associated with capillary blood stasis. However, the actual
mechanism of hemorrhagic transformation remains unknown.

In patients, intracerebral hemorrhage and subsequent neurologic deterioration have accompanied the use of fibrin-nonselective and fibrin-selective fibrinolytic agents. Additionally, CT-detectable infarction-related hemorrhage has been reported in patients treated with local infusions of urokinase or streptokinase & 8 hours after carotid or vertebral arteries territory ischemia and in patients receiving rt-PA early after focal ischemia.

Curiously, hemorrhagic transformation observed in stroke patients receiving fibrinolytic agents and in this model occurs in ischemic end-arteriolar (lenticulostriate artery [LSA] or pontine perforating artery) beds, suggesting some importance of vascular anatomy and structure. The inability of high doses of rt-PA to substantially increase the volume of ischemia-related hemorrhage in this model may result from 1) significant (unproven) differences between primate and human microvessels and their responses to ischemic injury, 2) the inability of rt-PA to penetrate the LSA bed (e.g., due to the “no-reflow” phenomenon), or 3) the very early use of rt-PA, before vascular integrity has been completely disrupted.

The absence of any association between the total volume of hemorrhage and the volume of infarction in a single vascular territory are in accord with the observations of Fisher and Adams, who found no relation between the size of cerebral infarcts and the frequency of hemorrhagic transformations. These data also suggest that rt-PA does not substantially decrease the volume of MCA-dependent infarction when given early after the onset of symptoms, at any dose. Whether rt-PA alters the incidence or distribution of microvascular occlusions that may follow ischemic injury/reperfusion in this model can be determined only by careful short-term experiments.

The hemostatic systems in baboons are, with some exceptions, similar to those in humans. No significant alterations in the concentrations of endogenous t-PA antigen or fibrinogen were observed during or after MCA occlusion in the placebo groups. While elevated fibrinogen levels may represent an acute-phase reaction, the significance of plasminogen activator alterations requires simultaneous measurements of plasminogen activator inhibitor levels. Serial postevent studies in patients suffering stroke have demonstrated changes in plasminogen activator levels, plasma fibrinogen, serum fragment E, soluble fibrin monomer, and D-dimer, suggesting activation of the fibrinolytic system. Whether the alterations observed are secondary to cerebrovascular thrombosis or are epiphenomena cannot be determined from published data; however, our prospective experience in a primate model indicates that ongoing cerebral ischemia perse did not apparently produce immediate changes in the concentrations of fibrinogen or t-PA antigen.

At extreme doses, rt-PA does not increase the severity of hemorrhagic transformation associated with only focal ischemia and early reperfusion in the LSA territory in this model. Species differences in the extent of thrombus lysis by t-PA in in vitro assays suggest that dose data from nonhuman primates and lower mammals may not be directly applicable to patients. Despite these differences, we suggest that in patients receiving thrombolytic agents for thromboembolic stroke, additional as-yet undefined factors may contribute to hemorrhagic risk.

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References


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