The Beneficial Effect of Intracarotid Urokinase on Acute Stroke in a Baboon Model

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SUMMARY  The capacity of intracarotid infusion of urokinase to salvage neurologic function in a baboon model of acute thrombotic stroke has been studied. The model consists of reversible eccentric balloon compression (3 hours) of the right middle cerebral artery (MCA) proximal to the take-off of the lenticulostriate arteries (LSA), resulting in in situ thrombosis of perforating branches supplying the right corpus striatum. Neurologic endpoints included quantitative assessment of neurologic function (NE), estimation of cerebral infarction volume by computerized tomographic (CT) scan, and carotid angiography. In untreated acute stroke control animals (n = 6), a persistent decrease in functional score (from 100 to 36 ± 11) at 14 days and a defined region of cerebral infarction (volume = 3.2 ± 1.5) were detected at 10 days. Intracarotid urokinase administered to five animals (1.2 x 10^6 IU over 60 min) following the 3 hour period of MCA occlusion improved neurologic function (NE = 50, 55, 85, 100, 100) and reduced infarction size (0.3, 0.5, 0.8, 0.7, 1.1 cm^3, respectively) without evidence of intracranial hemorrhage. Systemic fibrinogenolysis was produced in all five treated animals. We conclude that thrombolytic therapy given within 3 hours of experimental thrombotic occlusion may salvage neurologic function and reduce cerebral infarction volume without CT scan detectable intracranial bleeding.

IN GENERAL, studies of antithrombotic agents in the treatment of acute stroke have failed to demonstrate any beneficial effect on the outcome of stroke.1-19 Specifically, clinical studies of the safety and efficacy of fibrinolytic agents administered after acute stroke have been interpreted to indicate that intravenous infusions of urokinase or streptokinase are both unsafe and ineffective.15-17, 30 In view of data documenting the thrombotic nature of acute stroke31, 32 and acute myocardial infarction (MI)33 together with the documented benefits of early intraarterial fibrinolytic therapy in acute MI,24-29 a reexamination of the clinical studies regarding the safety and efficacy of thrombolytic therapy in acute stroke is warranted.

Although reference has been made to experiments with thrombolytic agents in acute stroke in animals, little data have been published.13 To address experimentally the issues of safety and efficacy, we have used a nonhuman primate model of in situ cerebrovascular thrombosis to test the effect of intracarotid urokinase infusions on the risk of intracerebral hemorrhage and the extent of neurologic deficit following acute stroke. The baboon model of acute cerebral ischemia secondary to reversible middle cerebral artery (MCA) occlusion and in situ lenticulostriate artery (LSA) thrombosis has been previously described.30, 31 In this report we present data supporting the postulate that early intracarotid infusion of urokinase following a 3 hour period of ischemia in this animal model may reduce the extent of the neurologic deficit and the size of cerebral infarction, without macroscopic evidence of intracerebral hemorrhage.

Methods

Animals Studied

Thirteen adolescent male baboons (Papio cynocephalus/anubis) approximately 12–14 kg weight were employed in the control and urokinase treated groups. All animals were dewormed and observed to be disease-free for at least six weeks prior to study.

In this cohort, baseline circulating platelet concentrations averaged 382,000 ± 129,000/μl (±1 SD), hematocrits were 35 ± 3%, white cell counts averaged 8,000 ± 4,100/μl. All animals had normal neurologic function prior to their involvement in the experimental procedure.

All procedures were approved by the institutional Animal Care and Use Committee and in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Device Implantation

The miniature inflatable silastic balloon cuff assembly and the method of transorbital implantation have been described previously.30 In brief, a miniature inflatable silastic balloon cuff assembly (Mentor Corporation, Goleta, CA), similar to that described by Spetzler, et al,30 was placed around the right MCA, and a silastic connector tube and plug. The miniature inflatable silastic balloon cuff assembly (Mentor Corporation, Goleta, CA) used for the right MCA was placed around the right MCA using a transorbital approach. The device consisted of an eccentric inflatable balloon (capacity 0.05 ml) with stainless steel artery hooks for stabilization about the right MCA, and a silastic connector tube and plug. The latter portion of the device was placed subcutaneously for remote post-operative inflation of the balloon. The device was loaded with diatrizoate meglumine (MD-76®, Mallinkrodt, St. Louis, MO) prior to implantation to facilitate subsequent radiographic documentation of balloon inflation in vivo.

Anesthesia was achieved and maintained with thiopental sodium (10 mg/kg induction bolus and 10
mg/kg/hr). The procedure involved a right transorbital approach, with enucleation, removal of the medial sphenoid and wings and the adjacent middle fossa, exposure of the internal carotid artery and MCA, and placement of the occluding device about the MCA proximal to the take-off of the lenticulostriate arteries. Fixation of the device and catheter portion of the device was completed with papaverine-impregnated Gelfoam and radiolucent methyldimethacrylate in the enucleated orbit. The connector tube and plug were placed subcutaneously near the apex of the head in a tunnel under the scalp.

Postoperative recovery typically occurred within 2–6 hours without neurologic deficit; the animals were fully active within 12–24 hours. Neurologic function was monitored for an additional 6 days prior to any experimental procedure.

Experimental Induction of Acute Stroke

Induction of acute stroke by right MCA occlusion was accomplished as previously described.30 For the MCA occlusion procedure the animals were maintained in an acute restraining device of the Davis type (Primate Products, Woodside, CA). While awake and alert, and under local anesthesia, the distal terminus of the occluder device was exposed by sterile surgical cut-down on the scalp. The right MCA balloon was inflated by infusion of 0.05 ml diatrizoate meglumine (MD-76®) via a calibrated syringe attached to the exposed terminus of the device. The balloon device was maintained inflated for three hours. Deflation of the occluding balloon was achieved by removal of the radiocontrast material and the end of the device was replaced below the scalp and the skin incision surgically closed.

All animals employed in these experiments developed a stroke syndrome within 5–10 minutes following external occlusion of the right MCA. The 3 hour period of MCA occlusion in six untreated control stroke animals produced a permanent neurological deficit that did not change during the subsequent 7–14 days.

Intracarotid Urokinase Infusion

The experimental design for the saline control of intracarotid urokinase infusions is summarized in the following table:

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Time (hours)</th>
<th>NE</th>
<th>Angio SF</th>
<th>Lat SF</th>
<th>CT Scan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placement of occluder device</td>
<td>120</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Right MCA occlusion (inflation of device)</td>
<td>0</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deflation of device; document occlusion</td>
<td>3</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placement of catheter in right carotid artery</td>
<td>3</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initiate urokinase (saline) infusion</td>
<td>0</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Discontinue urokinase (saline) infusion</td>
<td>4.5</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Documentation of cerebral infarction</td>
<td>5</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>48</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>72</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>96</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>240</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Sterile water was infused over 60 minutes via the intracarotid catheter using a Harvard pump (Harvard Apparatus, Dover, MA). Upon completion of the infusion the catheter was removed and the arteriotomy surgically closed. Animals were randomly assigned to receive saline or urokinase. Tissue culture-derived urokinase (lyophilized) for animal use was a gift from Abbott Pharmaceutical (North Chicago, IL).

Device function was confirmed in each experiment by standard lateral skull film (LAT SF).

Evaluation of hemorrhage and estimation of infarction volume in the region of ischemia were obtained from serial computerized tomographic (CT) scans of the cerebral hemispheres obtained under ketamine anesthesia at baseline (post-operative), immediately following intracarotid urokinase infusion, at 24 hours, and at 10 days following the infusion. All scans were performed on a modified EMI 1005 CT Scanner with 60 second scan time capable of resolving a hemorrhage of less than 0.5 ml (unpublished data). The volume of cerebral infarction was estimated by summation of the individual areas of low attenuation multiplied by the slice thickness from the CT scan obtained at 10 days.

Neurologic function (NE) was objectively assessed at 10 minutes, 1 hr, 2 hr, and 3 hr following MCA occlusion and daily thereafter by means of a quantitative neurological scale (table 2) developed by Specht and associates.30,31 Because the ischemic lesions produce unilateral loss of motor function, the 100-point scale was weighted toward motor loss, with less important contributions for other changes. Assessment was performed independently by two blinded observers with experience in evaluating primate neurologic functions. All animals employed in these experiments developed a stereotypic stroke syndrome (see Results).
TABLE 2  Quantitative Neurological Scoring for Stroke in Baboons

I. Motor function (70-10)

<table>
<thead>
<tr>
<th></th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal strength</td>
<td>70</td>
</tr>
<tr>
<td>favors opposite extremity</td>
<td>55</td>
</tr>
<tr>
<td>Hemiparesis</td>
<td></td>
</tr>
<tr>
<td>mild</td>
<td>25</td>
</tr>
<tr>
<td>severe</td>
<td>10</td>
</tr>
</tbody>
</table>

SCORE

II. Behavior (20-0)

<table>
<thead>
<tr>
<th></th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal aggression (swings on cage bars)</td>
<td>20</td>
</tr>
<tr>
<td>Aware of surroundings moves in response to examiner</td>
<td>15</td>
</tr>
<tr>
<td>not active</td>
<td>5</td>
</tr>
<tr>
<td>Coma</td>
<td>1</td>
</tr>
<tr>
<td>Death</td>
<td>0</td>
</tr>
</tbody>
</table>

SCORE

III. Ocular and cranial nerve function (10-2)

<table>
<thead>
<tr>
<th></th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Facial movement</td>
<td>5</td>
</tr>
<tr>
<td>paretic</td>
<td>1</td>
</tr>
<tr>
<td>Visual field</td>
<td></td>
</tr>
<tr>
<td>normal</td>
<td>5</td>
</tr>
<tr>
<td>paretic</td>
<td>1</td>
</tr>
</tbody>
</table>

SCORE

Coagulation tests (prothrombin time, partial thromboplastin time, and thrombin time) were performed by standard methods. Fibrinogen levels were determined as clottable protein using the assay of Jacobson. Plasminogen was measured by radioimmunoassay and fibrinolytic products were measured as neoantigen fragment D (fg D). Statistical comparisons were made using Student's t test (two tailed). The data in the Results section are given as the mean ± 1 SD.

Results

Acute Stroke in Untreated Control Baboons

Six animals underwent MCA occlusion without urokinase infusion and served as concurrent untreated controls. The volume of low attenuation in the corpus striatum by CT scan averaged 3.2 ± 1.5 cm³ at 10 days and the functional score was 36 ± 11 at 14 days following stroke induction (table 3).

Intracarotid Urokinase Infusion in Stroked Baboons

The effect of intracarotid infusion of urokinase following a 3 hour reversible occlusion of the proximal right MCA in awake baboons is presented in table 3. Urokinase was chosen for these experiments because it produced plasminogen activation in baboon plasma in vitro at 4,000 IU/ml that was nearly equivalent to that observed in human plasma, whereas streptokinase produced minimal activation in baboons.

The intracarotid infusion experiments employed two different infusion doses: urokinase at 4,000 IU/min for 60 minutes (2.4 × 10⁶ IU/hr) and urokinase at 20,000 IU/min for 60 minutes (1.2 × 10⁶ IU/hr). The effect of a 1 hour intracarotid infusion of urokinase on fibrinogen and plasminogen at the higher dose level (1.2 × 10⁶ IU/hr) is displayed in figure 1. Plasma fibrinogen (clottable protein) levels fell from 452 ± 130 mg/dl to 259 ± 58 mg/dl (±1 SE; p < 0.01), and plasma plasminogen (antigen) decreased from 333 ± 30 µg/ml to 266 ± 14 µg/ml (p < 0.01) one hour after the urokinase infusion had been discontinued. The infusion of 2.4 × 10⁶ IU/hr urokinase failed to produce significant systemic fibrinogenolysis (p > 0.3).

In five animals, intracarotid urokinase infusion of 20,000 IU/min (1.2 × 10⁶ IU/hr) for 1 hour, begun 1 hour following 3 hours of MCA occlusion (acute stroke), produced partial or complete correction of the acute neurological deficits during the subsequent 24

Table 3. Effects of 1 Hour Intracarotid Infusion of Urokinase Following 3 Hour Reversible Occlusion of the Right Middle Cerebral Artery in Baboons

<table>
<thead>
<tr>
<th>Animal no.</th>
<th>Baseline</th>
<th>Post MCA Occlusion</th>
<th>Infarction volume (cm³)</th>
<th>Hemorrhage CT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 hr*</td>
<td>24 hr</td>
<td>14 d</td>
</tr>
<tr>
<td>Control untreated</td>
<td>100</td>
<td>33 ± 7</td>
<td>36 ± 11</td>
<td>36 ± 11</td>
</tr>
<tr>
<td>Urokinase treated</td>
<td>7</td>
<td>100</td>
<td>25</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>100</td>
<td>27</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>100</td>
<td>55</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>100</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>100</td>
<td>27</td>
<td>85</td>
</tr>
<tr>
<td>mean</td>
<td>100</td>
<td>37 ± 14 $</td>
<td>72 ± 20</td>
<td>78 ± 23 $</td>
</tr>
</tbody>
</table>

*Following the onset of MCA occlusion.
†Baseline volumes reflect the presence of the uninflated balloon occlusion device.
‡Difference significant at p < 0.01.
§Difference significant at p < 0.05.
UROKINASE THERAPY IN A STROKE MODEL/DEL ZOPPO ET AL

by Del Zoppo et al

The demonstration that cerebral arterial occlusion by thrombotic material underlies stroke in the majority of patients makes the use of early intraarterial infusion of a thrombolytic agent an approach to be assessed. A prospective angiographic study of 68 patients with documented retinal or cerebral hemispheric infarction demonstrated occlusive lesions of the appropriate cerebral vessel during the acute phase (<24 hours) in 93% of patients.21 This observation is similar to the findings of thrombotic coronary artery occlusion during the initial 4 to 6 hours following acute myocardial infarction.23 Intracoronary artery streptokinase or urokinase infusion in patients with acute MI have achieved coronary artery reperfusion in 69–100% of occluded coronary arteries.24-27 In one randomized prospective trial involving 250 patients with acute MI, streptokinase

hours (p < 0.05; table 3). Furthermore, there was marked reduction in the detectable region of low attenuation by CT cerebral scan (p < 0.01), and no evidence of intracerebral hemorrhage.

Urokinase infusion of 4,000 U/min (2.4 x 10^5 IU/hr) 1 hour following the 3 hours of MCA occlusion (acute stroke) failed to produce systemic fibrinogenolysis or to modify either the neurological deficit or the development of the expected CT scan-demonstrable region of infarction at 10–14 days in two animals. No evidence of hemorrhage was detected.

No animals died during these studies.

Discussion

In the present study improvement in neurologic function and reduction in infarction volume were observed in a group of 5 stroke baboons receiving intracarotid arterial urokinase at a dose-rate sufficient to produce detectable systemic fibrinogenolysis (1.2 x 10^6 U/hr for 1 hour) when compared with six control animals.

In these experiments, a model of acute cerebral infarction in the baboon was employed that involved in situ microcirculatory thrombotic occlusion in the corpus striatum following proximal ipsilateral MCA compression. The advantages of this model have been described.30, 31, 37, 38 Because MCA patency is preserved following deflation of the occluding silastic balloon, it is possible to achieve perfusion of the dependent arteri-al bed and ischemic corpus striatal tissue using a thrombolytic agent delivered from a fixed intracarotid catheter. A dose of the thrombolytic agent was chosen for these experiments that produced evidence of plasmin effect. In our studies there is a dose dependence of plasminogen activation and fibrinogenolysis by intracarotid urokinase infusion in the baboon.

The improvement in functional score in animals receiving intracarotid urokinase immediately following the 3 hour period of MCA occlusion and reperfusion implies a causal role for induced fibrinolysis. Because clinical endpoints were monitored for 14 days after stroke induction, it was not possible to obtain the acute pathologic changes associated with the functional improvement. However, these experiments suggest that the infused urokinase produced dissolution of thrombi in the occluded lenticulostriate arteries thereby allowing sufficient marginally perfused ischemic corpus striatal tissue to be salvaged such that detectable neuronal function could be restored. Electrophysiologic (evoked potential), flow monitoring, and neuropsychologic correlations would be useful for further characterization of the mechanism underlying the functional recovery.

No evidence of intracerebral hemorrhage by CT scan or of clinical deterioration was observed in any animal receiving urokinase at either dose-rate during the 14 day period of observation. Because of the potential clinical relevance of the effects of a thrombolytic agent in acute cerebral ischemia, clinical endpoints only were chosen. Although the possibility of a corpus striatal hemorrhage of less than 0.5 ml volume could not be excluded by the CT scanning equipment available, such an event, if it occurred, had no detectable clinical functional consequence in this series. Since intracarotid infusion of urokinase was performed immediately following the 3 hour period of MCA occlusion, the risk of hemorrhage into infarcted cerebral tissue may have been less than when thrombolytic agents are delivered late following completed stroke.12, 14 The lack of intracranial bleeding in a limited number of patients with acute stroke who received urokinase or streptokinase within 4–6 hours following the onset of symptoms is consistent with this possibility (unpublished data, and references 39–44).

FIGURE 1. Urokinase-induced fibrinogenolysis. The intra-carotid infusion of 1.2 x 10^6 U of urokinase into baboons over 60 minutes produced significant destruction of fibrinogen (solid circles) and plasminogen (solid triangles) (p < 0.01) with reciprocal appearance of fibrinogenolytic products in plasma (open circles). The urokinase-mediated changes normalized by 48 hours after completion of urokinase infusion. Fibrinogen was measured as thrombin clottable protein (mg/dl). Plasminogen was measured as plasminogen antigen (µg/ml), and fibrinogenolytic products were measured as neoantigen fragment D (fg Dneo).
reduced mortality from 11% (control) to 3% (treated).18 Because clinical trials with anticoagulant and antiplatelet agents in the treatment of acute stroke or the secondary prevention of stroke have been inconclusive, measures directed at thrombus dissolution may be of more than theoretical interest.1-19, 45-54

Treatment with intravenous infusion of fibrinolytic agents (urokinase or streptokinase) in patients with stroke have been reported.12-19 Meyer, et al., in an angiographic study, demonstrated that thrombi were lysed with greater frequency in the group receiving intravenous streptokinase, but no neurologic improvement was evident.13, 14 Unfortunately, the theoretical benefit of reperfusion was associated with a "small but significant risk of cerebral hemorrhage." Other studies have implied that intravenous urokinase or streptokinase infusions are unsafe, providing little clinical improvement.20 A critical reexamination of these data demonstrates serious shortcomings in study design: 1) Because of the unavailability of CT scanning equipment, patients with intracerebral hemorrhage could not be excluded from any study. 2) With rare exception patients were not treated within 6 hours of presentation. 3) The dose-rate of urokinase/streptokinase administration was variable from patient to patient and was not controlled in each case. In contrast, recent experience with early infusion of fibrinolytic agents in a limited number of patients with complete carotid thrombosis55-56 or vertebrobasilar occlusion demonstrates the potential safety and efficacy of this approach (unpublished data, and references 39-44).

Those reported benefits are in accord with the safety and efficacy of early intraarterial infusion of fibrinolytic agents in this baboon model of acute cerebral ischemia, and suggest that this approach may be useful in acute thrombotic stroke patients. It should be noted, however, that because adolescent baboons such as those employed in this study do not have evident extra- or intracranial carotid arteriosclerosis or intracerebral vascular disease, they are not entirely comparable to the patient population presenting with acute stroke.

In summary, we have shown in baboons that neurologic salvage without CT scan detectable intracerebral hemorrhage can be achieved by early intracarotid infusion of urokinase following a 3 hour proximal M1 segment middle cerebral artery occlusion. It has previously been shown that thrombi form in situ in the lenticulostriate arterial supply of the ipsilateral corpus striatum following the 3 hour MCA occlusion.

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