Middle Cerebral Artery Occlusion in the Rabbit Using Selective Angiography
Application for Assessment of Thrombolysis

Reza Jahan, MD; Daphne Stewart, MD; Harry V. Vinters, MD; William Yong, MD; Fernando Vinuela, MD; Pete Vandeberg, MD; Victor J. Marder, MD

Background and Purpose—An animal model of selective middle cerebral artery (MCA) occlusion is needed for evaluation of intra-arterial (IA) delivery of thrombolytic agents. We describe a technique for MCA thrombo-occlusion in the rabbit with real-time angiographic documentation of occlusion and thrombolytic recanalization.

Methods—After femoral artery cutdown, a microcatheter was advanced from the internal carotid artery to the MCA. MCA occlusion was achieved by IA thrombin and reperfusion by IA plasmin.

Results—The terminal internal carotid artery was successfully catheterized in 12 of 13 animals. Stable (2-hour) MCA occlusion was induced and verified angiographically in all 12 animals; 2 animals also had distal internal carotid artery thrombus. Recanalization was achieved rapidly after IA plasmin in 3 of 3 animals.

Conclusions—We describe a new animal model of selective MCA occlusion documented by real-time angiography and used to demonstrate recanalization with IA plasmin. (Stroke. 2008;39:1613-1615.)

Key Words: animal model ■ ischemic stroke ■ middle cerebral artery ■ thrombolysis

A n animal model of selective middle cerebral artery (MCA) occlusion to evaluate intra-arterial (IA) thrombolytic therapy should be amenable to thrombolysis, allow regional infusions, and provide documentation of angiographic results. We have developed a rabbit stroke model of selective MCA occlusion using microcatheter technology and IA thrombin infusion. We use the model for studying IA-delivered thrombolysis with plasmin, using a direct acting fibrinolytic agent.

Methods

Studies were conducted on 13 consecutive New Zealand white rabbits (Charles River Laboratories, Wilmington, Mass). Animals were sedated with intramuscular ketamine HCl (35 mg/kg) and xylazine (5 mg/kg; Phoenix Pharmaceuticals), and anesthetized with isoflurane (Isothesia, Butler Animal Health Supply, Dublin, OH) via endotracheal intubation. A 4F sheath (Terumo Pinnacle, Boston Scientific) was placed into the right femoral artery and the right common carotid artery was catheterized with a Terumo 4F glide-catheter (Terumo Glidecath, Boston Scientific). The terminal right internal carotid artery (ICA) was catheterized with a 1.2F Magic Balt microcatheter and Sorcerer guide wire (BALT extrusion, Montmorency, France). Bovine thrombin (2063 NIH units/mg; Enzyme Research, South Bend, Ind) was reconstituted with saline to 1 NIH unit/μL, and mixed with rabbit brain thromboplastin (1:10 with saline; Neoplastine CI PLUS, Diagnostica Stago, Asnieres, France), as 0.3 mL thrombin plus 3.0 mL thromboplastin. MCA occlusion was induced by slow thrombin infusion (10 NIH units/100 μL) into the distal ICA above the posterior communicating artery (P-comm). There was minimal flow of thrombin into the right anterior cerebral artery (ACA), which filled from the contralateral ACA via the anterior communicating artery. Plasmin (Talecris Biotherapeutics) was reconstituted as 1 mg/mL sterile nonbuffered saline, and administered just proximal to the MCA occlusion. Angiography was performed to document MCA occlusion and recanalization. Animals were euthanized with 1 mL Euthasol (Virbac AH, Inc), after which the brain was perfused with paraformaldehyde, removed in toto and sectioned for hematoxylin-eosin staining.

Results

The ICA above the P-comm was successfully catheterized in 12 of 13 (93%) animals (Figure 1). After thrombin infusion, angiography documented MCA occlusion in all 12 animals; one animal also had thrombus in the distal ICA (Table). Follow-up angiography at 2 hours showed no spontaneous recanalization; one animal had extension of MCA clot into the ICA. The model was evaluated for thrombolysis in 3 animals treated with IA plasmin into the distal ICA (Figure 2). Postplasmin angiography showed complete recanalization in all 3 animals (Table). Histology of the ischemic right hemisphere showed vacuolization of the neuropil, diffuse edema and loss of cellular integrity (not shown).

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Discussion

We describe the technique and feasibility of selective MCA thrombo-occlusion in the rabbit, using angiography to confirm both occlusion and thrombolytic-induced reperfusion. All 12 animals that were catheterized to the terminal ICA had successful MCA thrombo-occlusion, with no spontaneous recanalization after 2 hours. Occlusion was limited to the MCA in 10, with distal ICA occlusion also present in 2. All 3 animals treated with IA plasmin showed rapid angiographic recanalization.

The major advantages of our model are reliable occlusion of the target artery, real-time angiographic confirmation of vessel status, and occlusive material that is amenable to thrombolysis. Prior attempts to access the distal ICA were hindered by catheter size, because selective occlusion of the MCA is unreliable without access to the ICA beyond the P-comm. Injection of clots into the rabbit common carotid artery fails to occlude a cerebral artery or terminates outside of the ICA circulation in 25%. Use of thrombin-induced clot that can be dissolved with thrombolytic agents provides an advantage over suture occlusions or injection of particulates. Our demonstration of rapid lysis of MCA clot with plasmin, a newly-developed direct-acting thrombolytic, warrants further study to assess safety and efficacy for eventual IA clinical use.

Further characterization is needed to assess the extent and variation of induced ischemic damage, because even reliable

Table. Results

<table>
<thead>
<tr>
<th>Procedure</th>
<th># (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animals studied</td>
<td>13</td>
</tr>
<tr>
<td>Catheter into CCA</td>
<td>13 (100%)</td>
</tr>
<tr>
<td>Catheter into ICA above P-comm</td>
<td>12 (92%)</td>
</tr>
<tr>
<td>Thrombin infusion into ICA</td>
<td>12</td>
</tr>
<tr>
<td>MCA occlusion</td>
<td>12 (100%)</td>
</tr>
<tr>
<td>Thrombus in MCA + ICA</td>
<td>1 (8%)</td>
</tr>
<tr>
<td>Follow-up angiogram (2 hours)</td>
<td>12</td>
</tr>
<tr>
<td>MCA occlusion</td>
<td>12 (100%)</td>
</tr>
<tr>
<td>Thrombus in MCA + ICA</td>
<td>2 (17%)</td>
</tr>
<tr>
<td>IA infusion of plasmin</td>
<td>3</td>
</tr>
<tr>
<td>Successful thrombolysis</td>
<td>3 (100%)</td>
</tr>
</tbody>
</table>

CCA indicates common carotid artery.

Figure 1. Baseline cerebral angiograms. Baseline angiograms. A, Lateral view. Catheter (thick arrow) is in the common carotid artery (CCA). Arrowhead indicates the ICA take-off and double arrows the P-comm. B, Lateral view. The microcatheter tip (thick arrow) is in the ICA which narrows significantly at the skull base (dashed arrow). C, Submental vertex view. The microcatheter is in the ICA above the P-comm. ICA bifurcation into the MCA and ACA noted by the thick arrow, distal ACA by double arrows. The left ACA and MCA fill via the anterior communicating artery (A-comm). ECA indicates external carotid artery; RT, right; LT, left.
MCA occlusion may not cause similar sized infarcts. Slow delivery of coagulant mixture may result in more complete occlusion of distal MCA branches than occurs in human stroke, where proximal MCA occlusion allows some collateral flow from the ACA. Stroke induction under general anesthesia may have a neuroprotective effect.

Conclusions
We describe a new technique for angiographically confirmed MCA thrombo-occlusion in the rabbit with a high degree of success and demonstrate the use of this model for IA-induced thrombolytic recanalization.

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Disclosures
Dr Marder serves as a consultant to Talecris Biotherapeutics, which provided an unrestricted grant for pursuance of this study. Dr Vandeberg is an employee of Talecris Biotherapeutics.

References

Figure 2. Thrombin-induced MCA occlusion and plasmin-induced recanalization. MCA fills normally at baseline (left panel), is absent after IA thrombin (center panel) and is recanalized after IA plasmin (right panel).
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