Thorombolysis With Plasmin
Implications for Stroke Treatment

Victor J. Marder, MD; Reza Jahan, MD; Theresa Gruber, MS; Abha Goyal, MD; Vik Arora, PhD

Abstract—Plasmin is a direct-acting thrombolytic agent with a striking hemostatic safety advantage over plasminogen activators in animal models of thrombolysis and bleeding. In contradistinction to plasminogen activators, which risk bleeding at any effective thrombolytic dose, plasmin is tolerated without bleeding at several-fold higher amounts than those needed for thrombolysis. Plasmin has been safe in a current trial in patients with peripheral arterial or graft occlusion, and efforts are now directed toward therapy of stroke caused by cerebral artery occlusion. A rabbit (4 kg body weight) model of 2-hour, thrombin-induced middle cerebral artery occlusion using angiographic documentation of vascular patency and recanalization was used to perform a dose-ranging study of plasmin, delivered by catheter over a median duration of 10 minutes. Plasmin induced early recanalization in all animals (3 per group) within 10 minutes after discontinuation of 3, 2, or 1 mg of agent infusion. Control saline infusion failed to induce recanalization in 3 of 3 subjects. Plasmin rapidly induces middle cerebral artery recanalization, as determined in an angiogram-based animal model of arterial occlusion. Based on these data and other information, a phase I/IIa clinical trial of plasmin in human middle cerebral artery ischemic stroke has been initiated. (Stroke. 2010;41[suppl 1]:S45-S49.)

Key Words: thrombolysis  plasmin

There is currently only 1 Food and Drug Administration–approved treatment for ischemic stroke, namely, recombinant tissue plasminogen activator (PA; rt-PA) administered by IV infusion within 3 hours of symptom onset. Because IV administration of rt-PA rapidly neutralizes plasmin, a striking hemostatic safety advantage over plasminogen activators in animal models of thrombolysis and bleeding. In contradistinction to plasminogen activators, which risk bleeding at any effective thrombolytic dose, plasmin is tolerated without bleeding at several-fold higher amounts than those needed for thrombolysis. Plasmin has been safe in a current trial in patients with peripheral arterial or graft occlusion, and efforts are now directed toward therapy of stroke caused by cerebral artery occlusion. A rabbit (4 kg body weight) model of 2-hour, thrombin-induced middle cerebral artery occlusion using angiographic documentation of vascular patency and recanalization was used to perform a dose-ranging study of plasmin, delivered by catheter over a median duration of 10 minutes. Plasmin induced early recanalization in all animals (3 per group) within 10 minutes after discontinuation of 3, 2, or 1 mg of agent infusion. Control saline infusion failed to induce recanalization in 3 of 3 subjects. Plasmin rapidly induces middle cerebral artery recanalization, as determined in an angiogram-based animal model of arterial occlusion. Based on these data and other information, a phase I/IIa clinical trial of plasmin in human middle cerebral artery ischemic stroke has been initiated. (Stroke. 2010;41[suppl 1]:S45-S49.)

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Although rt-PA has potential for hemorrhage at any dose that has a thrombolytic effect, plasmin possesses a significant safety margin, at an approximate 4-fold greater dose than is needed to dissolve experimental thrombi (Figure 2). There is considerable confidence in this safety margin for plasmin use in peripheral arterial or graft occlusion, where plasmin is quickly and efficiently neutralized by α2-antiplasmin after perfusing the lower extremity. However, judgment must necessarily be reserved for intra-arterial treatment of ischemic stroke, because plasmin could enter a vulnerable distal cerebral artery after dissolution of a thrombo-embolus, and real-time blunting of its enzymatic effect needs to be accomplished by ambient α2-antiplasmin.

Three direct-acting thrombolytic agents have been assessed for treatment of ischemic stroke in experimental models, as described below, only 1 of which has been used in human disease trials.

Microplasmin consists of the serine protease domain of plasmin alone, and after systemic (IV) administration, showed less intracranial hemorrhage and decreased cerebral injury equal to or better than that with rt-PA in models of cerebral ischemia. Microplasmin used intravenously (not by the intra-arterial route) in middle cerebral artery (MCA) ischemic stroke showed a trend toward better recanalization compared with placebo, but further trial awaits a partner.

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S45
and phase IIa results of intra-arterial microplasmin in basilar artery stroke are not published.\textsuperscript{13}

Alfimeprase is a derivative of the Southern copperhead snake venom fibrolase, made by recombinant technology\textsuperscript{14} to slightly modify the N terminus. Preclinical studies showed superior thrombolysis compared with urokinase in the rat, pig, and dog models of the thrombosed carotid artery,\textsuperscript{4} but planned studies of alfimeprase ischemic stroke were never initiated.\textsuperscript{15}

Miniplasmin consists of the serine protease domain plus kringle 1 of plasmin,\textsuperscript{16} and reduced ischemic injury after IV administration in MCA ligation models,\textsuperscript{17} but the agent has not been tested clinically.

Plasmin is currently under preclinical study as a foundation for clinical trial. To assess its potential, we have established a model for angiographically documented recanalization of the rabbit MCA,\textsuperscript{18} and now use this selective MCA occlusion model to evaluate the recanalization efficacy of 3 dosages of plasmin.

### Methods

Animal studies were approved by the David Geffen School of Medicine at the University of California at Los Angeles Animal Research Committee and conducted on 3.5- to 4.3-kg New Zealand white rabbits.

A 4F sheath (Terumo Pinnacle, Boston Scientific) was placed into the right femoral artery and a 4F glide catheter (Terumo Glidecath, Boston Scientific) was inserted to reach the right common carotid artery. A 1.2F Magic Balt flow-directed microcatheter and Sorcerer guide wire (BALT extrusion) were introduced into the right common carotid artery coaxially through the Terumo glide catheter using the roadmap technique. Coagulant mixture of bovine thrombin (10 National Institutes of Health U/100 \textmu L; Enzyme Research) with 1:10 saline-diluted rabbit brain thromboplastin (Neoplastine CI PLUS, Diagnostica Stago) was administered over 10 minutes into the MCA, and occlusion was documented by angiography immediately and at 2 hours to rule out spontaneous MCA recanalization. Plasmin (Human, TAL-05-00018, Talecris Biotherapeutics) was administered over 10 to 30 minutes by infusion pump just proximal to the MCA occlusion, after which angiography was performed at 15, 30, 45, and 60 minutes to determine whether arterial recanalization had occurred. Angiographic perfusion was classified according to Thrombolysis in Myocardial Infarction grades.\textsuperscript{19}

### Figure 1

Schematic representation of mode of action of systemic (IV) and local (intra-arterial) administration of plasminogen activators (eg, tissue PA [TPA]) and plasmin. Modified from Marder and Novokhatny.\textsuperscript{4} The top panel shows systemic (IV) administration, and the bottom panel shows local (catheter) administration of tissue-type plasminogen activator (t-PA; left) and plasmin (right), as typical representatives of direct and indirect fibrinolytic agents. PAI-1 indicates plasminogen activator inhibitor type 1.

<table>
<thead>
<tr>
<th>TPA</th>
<th>PLASMIN</th>
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<tbody>
<tr>
<td><strong>BLEEDING</strong></td>
<td>Bleeding at all doses, in proportion to thrombolytic potential.</td>
</tr>
<tr>
<td><strong>SAFETY MARGIN</strong></td>
<td>None</td>
</tr>
</tbody>
</table>

### Figure 2

Margin of safety against bleeding for rt-PA and plasmin. Conclusions based on data provided by experimental models of intra-arterial thrombolysis and systemic circulation of agent.\textsuperscript{5,8}
Results
MCA occlusion (Thrombolysis in Myocardial Infarction 0) was successfully accomplished in all 12 of the animals receiving IA thrombin, and the 2-hour follow-up angiograms showed no instance of spontaneous recanalization (Table 1).

Table 1. Plasmin Infusions Into Occluded MCA of the Rabbit

| No. | Duration of MCA Occlusion, min | Agent         | Infusion Details | Time Until Recanalization, min From Start of Infusion From End of Infusion |
|-----|-------------------------------|---------------|-----------------|-----------------------------|-----------------------------|
|     |                               |               | mg/mL mL/min min mL | Total | Mean | Total | Mean |
| 1   | 128                           | Plasmin (1 mg)| 0.2 0.17 30 5 | 42    | 23.3 | 12    | 7.7  |
| 2   | 132                           | Plasmin (1 mg)| 0.2 0.5 10 5 | 15    | 5    |       |      |
| 3   | 132                           | Plasmin (1 mg)| 0.2 0.5 10 5 | 13    | 3    |       |      |
| 4   | 130                           | Plasmin (2 mg)| 1 0.2 10 2 | 10    | 22.3 | 0     | 5.7  |
| 5   | 136                           | Plasmin (2 mg)| 0.2 0.5 20 10 | 34    | 14   |       |      |
| 6   | 120                           | Plasmin (2 mg)| 0.2 0.5 20 10 | 23    | 3    |       |      |
| 7   | 101                           | Plasmin (3 mg)| 0.6 0.5 10 5 | 20    | 21.0 | 10    | 11.0 |
| 8   | 113                           | Plasmin (3 mg)| 0.6 0.5 10 5 | 26    | 16   |       |      |
| 9   | 113                           | Plasmin (3 mg)| 0.6 0.5 10 5 | 17    | 7    |       |      |
| 10  | 97                            | Saline        | NA 0.5 10 5 | >94   | >69.0| >84   | >59.0|
| 11  | 130                           | Saline        | NA 0.5 10 5 | >58   | >48  |       |      |
| 12  | 132                           | Saline        | NA 0.5 10 5 | >55   | >45  |       |      |

Figure 3. Representative results of local (intra-arterial) infusion of plasmin into the thromboc俱 occluded rabbit MCA. Prethrombin image (top left) shows the patent right MCA (arrow), which, on the immediate postthrombin image (top right), is occluded (arrow) and remains occluded on the 2-hour postthrombin image (bottom left). After intra-arterial plasmin infusion, flow through the MCA is re-established (arrow, bottom right).
fraction 0 MCA occlusion. The mean elapsed times from the start of plasmin infusion until MCA recanalization varied between 13 and 42 minutes, depending on technical delays before performing the angiogram, but were the same for the 1-, 2-, and 3-mg dosages (23.3, 22.3 and 21.0 minutes, respectively; Figure 4). Recanalization after termination of plasmin infusion was always documented with the first follow-up angiogram, at 7.7, 5.7, and 11.0 minutes for the 1-, 2-, and 3-mg dosages, respectively (Figure 4).

**Discussion**

Our focus on MCA recanalization is clinically relevant, because analysis of human ischemic stroke trial data indicates that arterial recanalization is the key to successful clinical outcome.20 Specifically, a clinical (functional) advantage at 3 months occurs in patients in whom the occluded vessel had recanalized (58.1% versus 24.8%; odds ratio: 4.43), and more importantly, the overall mortality was reduced in patients whose occluded artery had recanalized (14.4% versus 41.6%; odds ratio: 0.24). Our model is ideal for demonstrating recanalization efficacy, because it is based on routine angiographic documentation of MCA occlusion and patency.

The safety of these dosages of plasmin relative to complicating intracranial hemorrhage must still be assessed in comparative preclinical studies. Nevertheless, our results provide guidance for selecting appropriate initial dosages for clinical trials of ischemic stroke. Considering that a 1-mg dose of plasmin administered into the occluded MCA of the rabbit induced rapid (within 10 minutes) recanalization, one could calculate a starting dose for human MCA occlusion based on relative body weight, thrombus histology, and delay until recanalization, as indicated in Table 2. On the basis of body weight and relative size of the MCA thrombus, the human dose would be ≈10- to 20-fold greater than in the rabbit (20 mg instead of 1 mg). However, the induced MCA occlusion in the rabbit was caused by thrombin-clotted whole blood, whereas human ischemic stroke is caused by vascular occlusion of more mature and organized thrombo-embolic material.21 The latter are, therefore, likely to be more resistant to and require relatively larger amounts of any thrombolytic agent than is effective in our model. As to the duration of plasmin infusion into or near the human thrombo-embolus, it is difficult to predict an appropriate regimen, but based on rt-PA infusions of ≈1 hour, it is reasonable to use 30-minute infusions, ascertain vessel patency status at 15 minutes, and continue to monitor by angiogram for 30 minutes after the end of plasmin infusion.

Based on these and other considerations, a phase I safety and dose-finding study of intra-arterial plasmin has been initiated for patients with ischemic MCA stroke at ≥8.5 hours after onset, with focal deficit and a rather broad National Institutes of Health Stroke Scale score of 4 to 25.22 MCA occlusion of the M1, M2, or M1-2 branches must be documented by arteriography, and escalating dosages of 20, 40, and 60 mg are planned, administered as 50% over 15 minutes, followed by the second infusion, with angiograms at 15, 30, and 60 minutes after the start of infusion. End points for safety are intracranial hemorrhage and functional neurological changes, and, for efficacy, partial or complete recanalization (Thrombolysis in Myocardial Infarction scores of 2a, 2b, or 3).

**Conclusions**

Plasmin is the prototypical direct-acting thrombolytic agent and has shown impressive hemostatic safety in comparison with rt-PA in an experimental model of fibrinolytic hemorrhage.5 Efficacy of plasmin is documented in the rabbit MCA recanalization model18 (Figures 3 and 4), and a phase I/IIa clinical trial of plasmin has been initiated in acute ischemic stroke.22

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![Figure 4. Mean delays until MCA recanalization after the start and from the end of plasmin infusions (1-, 2-, or 3-mg doses).](http://stroke.ahajournals.org/)

**Table 2. Dose Considerations for Plasmin in Human Ischemic Stroke**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Rabbit</th>
<th>Human</th>
<th>Implication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight</td>
<td>4 kg</td>
<td>70 to 80 kg</td>
<td>20-fold higher dose needed in humans (20 vs 1 mg)</td>
</tr>
<tr>
<td>Thrombus histology</td>
<td>Simple whole blood clot</td>
<td>Mature, complex thrombo-embolus</td>
<td>More plasmin needed in humans than on weight basis alone</td>
</tr>
<tr>
<td>Delay until recanalization</td>
<td>20 to 25 min from start of infusion</td>
<td>t-PA infusion time 60 min</td>
<td>Plasmin infusion time of 30 min (early look at 15 min)</td>
</tr>
</tbody>
</table>
Disclosures

V.J.M. and R.J. are consultants for and receive research support from, and V.A. is an employee of, Talecris Biotherapeutics, Inc.

References


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