**Randomized, Placebo-Controlled, Dose-Ranging Clinical Trial of Intravenous Microplasmin in Patients With Acute Ischemic Stroke**

Vincent N.S. Thijs, MD, PhD; Andre Peeters, MD; Milan Vosko, MD, PhD; Franz Aichner, MD; Peter D. Schellinger, MD, PhD; Dietmar Schneider, MD; Tobias Neumann-Haefelin, MD, PhD; Joachim Röther, MD, PhD; Antoni Davalos, MD, PhD; Nils Wahlgren, MD, PhD; Peter Verhamme, MD, PhD

**Background and Purpose**—Microplasmin is a recombinant truncated form of human plasmin. It has demonstrated efficacy in experimental animal models of stroke and tolerability in healthy volunteers. We tested the tolerability of microplasmin in patients with acute ischemic stroke.

**Methods**—In a multicenter, double-blind, randomized, placebo-controlled Phase II trial, 40 patients with ischemic stroke were treated with either placebo or active drug between 3 and 12 hours after symptom onset in a dose-finding design. Ten patients received placebo, 6 patients received a total dose of 2 mg/kg, 12 patients received a total dose of 3 mg/kg, and 12 patients received a total dose of 4 mg/kg. We studied the pharmacodynamics of microplasmin and its effect on the clinical and hemodynamic parameters of the patients. MRI was used as a surrogate marker and matrix metalloproteinases serum concentrations were used as markers of neurovascular integrity. The study was underpowered to detect clinical efficacy.

**Results**—Microplasmin induced reversible effects on markers of systemic thrombolysis and neutralized \( \alpha_2 \)-antiplasmin by up to 80%. It was well tolerated with one of 30 treated patients developing a fatal symptomatic intracerebral hemorrhage. No significant effect on reperfusion rate or on clinical outcome was observed. Matrix metalloproteinase-2 levels were reduced in microplasmin-treated patients.

**Conclusions**—Microplasmin was well tolerated and achieved neutralization of \( \alpha_2 \)-antiplasmin. Further studies are warranted to determine whether microplasmin is an effective therapeutic agent for ischemic stroke. *(Stroke. 2009;40:3789-3795.)*

**Key Words:** acute care ■ acute stroke ■ antithrombotics ■ brain imaging ■ brain infarction ■ diffusion-weighted imaging ■ thrombolysis ■ thrombolytic Rx

---

**Tissue plasminogen activator (tPA) is the only pharmacological treatment currently approved for acute ischemic stroke.**\(^1\),\(^2\) Only a small percentage of patients with stroke are currently treated with tPA. Given the burden of stroke on society and the lack of treatments for the vast majority of patients, there is an unmet need for pharmacological therapy for acute stroke.

Microplasmin is a truncated form of human plasmin produced by recombinant technology.\(^3\) The observations that \( \alpha_2 \)-antiplasmin (\( \alpha_2 \)-AP) deficiency in mice and \( \alpha_2 \)-AP depletion by neutralizing antibodies or after administration of plasmin reduced infarct size led to the development of microplasmin for patients with ischemic stroke. Acute stroke models in mice, rats, and rabbits have generally demonstrated that intravenous microplasmin is associated with a reduction in infarct size compared with placebo and that microplasmin may have a lower propensity to cause bleeding than recombinant tPA.\(^4\),\(^5\),\(^6\),\(^7\) One study in rats did not find a reduction in infarct volumes or behavioral outcomes.\(^8\) Microplasmin has been evaluated in a Phase I clinical trial in healthy young and elderly volunteers. The cumulative doses ranged from 0.1 to 5 mg/kg. A dose response for \( \alpha_2 \)-AP neutralization was observed.\(^9\)

---

Received June 11, 2009; final revision received August 31, 2009; accepted September 11, 2009.

From the Department of Neurology (V.T.), University Hospitals Leuven, Leuven, Belgium, and the Vesalius Research Center, Katholieke Universiteit Leuven, Leuven, Belgium; UCL St Luc (A.P.), Unité neuro-vasculaire, Service de Neurologie, Bruxelles, Belgium; Abt. für Neurologie und Psychiatrie (M.V.), Allgemeines Krankenhaus der Stadt Linz, Linz, Austria; the Department of Neurology (F.A.), Wagner Jauregg Hospital, Linz, Austria; Neurologische Universitätsklinik (P.S.), Erlangen, Germany; the Department of Neurology (D.S.), University of Leipzig, Leipzig, Germany; Klinik für Neurologie (T.N.-H.), Johann Wolfgang Goethe-Universität, Frankfurt, Germany; Neurologische und Geriatrische Klinik (J.R.), Johannes Wesling Klinikum Minden, Akademisches Lehrkrankenhaus der, Medizinischen Hochschule Hannover, Minden, Germany; the Stroke Unit (A.D.), Department of Neurosciences, Hospital Universitari Germans Trias i Pujol, Universitat Autònoma de Barcelona, Badalona, Spain; the Department of Neurology (N.-G.W.), Karolinska University Hospital, Stockholm, Sweden; and the Department of Vascular Medicine and Haemostasis (P.V.), University Hospitals Leuven, Leuven, Belgium.

Correspondence to Vincent N.S. Thijs, Herestraat 49, Leuven, Belgium 3000. E-mail vincent.thijs@uz.kuleuven.ac.be © 2009 American Heart Association, Inc.

**Stroke** is available at [http://stroke.ahajournals.org](http://stroke.ahajournals.org) DOI: 10.1161/STROKEAHA.109.560201

3789
The goal of this placebo-controlled, randomized trial was to assess the safety and pharmacodynamic properties of intravenous microplasmin in patients with acute ischemic stroke. We also evaluated potential surrogate markers and performed serial MRI to assess reperfusion and recanalization.

Methods

The study was a randomized, double-blind, placebo-controlled, ascending-dose trial. The study protocol was registered at www.clinicaltrials.gov with the following identifier: NCT00123305. The trial was funded by ThromboGenics NV. The final version of the study protocol and all protocol amendments were approved by the Competent Authority and leading Independent Ethics Committee in each country and were additionally reviewed by the respective ethics review committee of each center.

Written informed consent was obtained from each patient or his or her legal representative before entry into the study. In the event that the patient was unable to provide written informed consent, verbal consent from the patient or written assent from a legally acceptable representative was accepted.

An independent Study Safety Committee (see Appendix for members) permitted proceeding to the next dose level after review of safety data.

Study Drug

Human microplasminogen, a derivative of human plasminogen that lacks the 5 kringle domains, was expressed in Pichia Pastoris. The protein is then cleaved and quantitatively converted to microplasmin with recombinant staphylokinase, which is subsequently removed during purification.3 The drug was maintained at −20°C in sealed drug packs.

The study drug was microplasmin or placebo administered intravenously as a bolus of 1 mg/kg over 15 minutes followed by a 1-hour continuous infusion of 1 mg/kg (low-dose treatment group), 2 mg/kg (medium-dose treatment group), or 3 mg/kg (high-dose treatment group) or an identically looking placebo solution administered at the same rates as the active treatment group. It was planned to treat a total of 40 eligible patients with study treatment starting with one group of 8 patients treated with the lowest dose regimen and 2 additional groups (each with 16 patients) successively treated with the 2 higher dose regimens. Patients were randomized 3:1 over the entire study population to study treatment with microplasmin or placebo. Randomization was not stratified by any variable. Randomization was done by a computerized scheme accessed through a telephone-operated interactive voice response system.

Study Population

Patients between 18 and 85 years of age with an acute ischemic stroke and a baseline National Institutes of Health Stroke Scale score ranging from 4 to 22 were recruited at 8 sites in Europe (see Appendix). Eligible patients had to be ambulatory before the stroke and have a perfusion defect of at least 2 cm in diameter as assessed by MRI perfusion imaging (PI). The baseline PI had to be assessed by relative mean transit time images on-site. Diffusion lesions did not have to be smaller than the perfusion defect. A proven arterial occlusion on MR angiography (MRA) was not an inclusion requirement. The study drug had to be administered within 1 hour after the completion of MRI and within 12 hours after stroke onset. Patients who were eligible for tPA were excluded. We also excluded patients who received vitamin K antagonists or heparin (or heparin-related products) or direct thrombin inhibitors until 24 hours after the administration of the study drug. The treatment with other concomitant medications was at the discretion of the treating physician.

Exclusion criteria for the trial were similar to other acute stroke treatment trials of thrombolytic agents. Patients with space-occupying edema or large hypodensities on CT or diffusion-weighted imaging lesions involving more than one third of the middle cerebral artery territory, patients with hemorrhagic transformation on CT or on gradient recalled echo MRI, and patients with an ischemic lesion consistent with lacunar stroke were excluded.

Study Procedures

Baseline evaluation included a clinical assessment, electrocardiogram, MRI of the brain, and laboratory tests. Repeat MRI scans were obtained 4 to 12 hours after the end of study drug administration and at Day 7. If neurological deterioration occurred during the hospital stay, an additional CT and/or MRI scan was obtained to document brain hemorrhage. Neurological deficits were evaluated with the National Institutes of Health Stroke Scale (NIHSS) score at baseline, at 6 ± 2 hours, at 24 hours, at 7 days, and at 30 days after study drug administration. The Barthel Index and the modified Rankin Scale score were obtained at baseline (prestroke estimate), 7 days, and 30 days. The follow-up examinations were performed by trained investigators who remained blinded to treatment assignment. Blood samples were drawn at baseline, at the end of treatment, and at 1, 6, 12, 24, 72, and 96 hours after the end of study drug administration.

Magnetic Resonance Imaging

MRI scans were obtained on 1.5-T scanners equipped with high-performance gradient systems and capable of echoplanar imaging. MRI sequence parameters were standardized across the centers. The baseline MRI and first follow-up MRI scans included the following sequences: diffusion-weighted imaging (DWI), dynamic susceptibility PI using a 2-dimensional gradient echo sequence after a bolus of intravenous gadolinium, fluid-attenuated inversion recovery imaging, 3-dimensional time-of-flight MRA, conventional T1, and gradient-recalled echo imaging. MRI scans at Day 7 included a fluid-attenuated inversion recovery sequence. Brain imaging studies were sent to a central MRI core laboratory, and the studies were interpreted and volumetrics performed by an independent neuroradiologist who was blinded to clinical status and treatment assignment. The neuroradiologist rated all MRI scans on a 3-point scale in which a score of 0 means complete occlusion, 1 partial occlusion, and 2 no vessel occlusion. A PI/DWI mismatch was defined as a PI volume >120% of the DWI volume and a DWI volume of at least 10 mL smaller than the PI volume and a baseline DWI not exceeding 100 mL.10 Mean transit time maps were created based on the first moment method. An arterial input function was not required. The volume of mean transit time delays was visually compared by the

Figure 1. Study flowchart.
investigators with the unaffected hemisphere to determine eligibility for study participation.

Outcome Measurements

Safety and efficacy variables were obtained at baseline, at the end of administration of study treatment, during the following in-hospital phase up to a maximum of 7 days, and at the follow-up visit approximately 30 days after the administration of the study treatment.

Safety End Points

The reporting of serious adverse events followed regulatory requirements. Deaths, including presumed cause, were recorded. Intracranial hemorrhages were considered symptomatic when a patient who deteriorated at least 2 points on the NIHSS within 36 hours had evidence of brain hemorrhage on imaging. Asymptomatic hemorrhages or hemorrhagic transformations of infarcts excluding microbleeds were recorded on gradient echo MRI scans on Day 7. Major systemic hemorrhages were defined as any bleeding resulting in death, any retroperitoneal hemorrhage, overt bleeding associated with a need for transfusion of ≥2 units of blood or which required surgical intervention, and overt bleeding associated with a decrease from baseline in hemoglobin of at least 2.0 g/dL. Progressing stroke, defined as a ≥4-point increase in NIHSS score, was a predefined serious adverse event. Vital sign alterations during treatment were recorded as were allergic reactions. Markers of systemic fibrinolysis (fibrinogen [Instrumentation Laboratories], plasminogen [Instrumentation Laboratories], D-dimer [STAGO], prothrombin time [Instrumentation Laboratories], and activated partial thromboplastin time [Instrumentation Laboratories]) were measured at baseline, the end of study treatment, and at 6, 12, 24, and 96 hours after study treatment.

Clinical End Points

The following clinical outcome parameters were studied. The percentages of patients who had achieved an NIHSS of ≤1 or who had shown an improvement from baseline of at least 8 points at 30 days were compared between treatment groups. The percentages of patients who had achieved a total NIHSS score of ≤2 were compared. We analyzed the change in NIHSS score between baseline and 30 days and compared the modified Rankin Scale and the Barthel Index score after 30 days. Deaths were imputed as worst possible score.

Imaging End Points

The rate of reperfusion was assessed by MRI. Reperfusion was defined as either the reduction of PI abnormality by >30% (or absence of perfusion deficits for patients with baseline PI <10 mL),

<table>
<thead>
<tr>
<th>Feature</th>
<th>Microplasmin 1 mg/kg (n=6)</th>
<th>Microplasmin 2 mg/kg (n=12)</th>
<th>Microplasmin 3 mg/kg (n=12)</th>
<th>Microplasmin Total (n=30)</th>
<th>Placebo (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deaths, n (%)</td>
<td>0 (0)</td>
<td>1 (8)</td>
<td>0 (0)</td>
<td>1 (3)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Symptomatic ICH, n (%)</td>
<td>0 (0)</td>
<td>1 (8)</td>
<td>0 (0)</td>
<td>1 (3)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Asymptomatic ICH, n (%)</td>
<td>3 (50)</td>
<td>4 (33)</td>
<td>6 (50)</td>
<td>13 (43)</td>
<td>4 (40)</td>
</tr>
<tr>
<td>Major systemic hemorrhage, n (%)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Progressive stroke, n (%)</td>
<td>2 (33)</td>
<td>3 (25)</td>
<td>3 (25)</td>
<td>8 (27)</td>
<td>4 (40)</td>
</tr>
</tbody>
</table>

ICH indicates intracerebral hemorrhage.
improvement on the MRA scale by 2 points, or both of the aforementioned on a follow-up scan performed between 4 and 12 hours after the start of study treatment. The reperfusion rate was additionally calculated for those patients who had an improvement on the MRA scale score by at least 1 point.

Neutralization $\alpha_2$-AP and Biomarker End Points
The degree of $\alpha_2$-AP neutralization (Instrumentation Laboratories) was determined at the end of study drug administration and 6, 12, 24, and 4 days.

Surrogate biomarkers were assessed serially. Matrix metalloproteinase (MMP)-2, MMP-9, and tissue inhibitor of metalloproteinase 1 (enzyme-linked immunosorbent assay kit; R & D Systems) were measured at baseline, 24 hours, and 4 days after study drug administration. The surrogate markers neuron-specific enolase (enzyme-linked immunosorbent assay kit; IDS), and S100 (enzyme-linked immunosorbent assay kit; Cambridge Life Sciences) were measured at baseline, 12 hours, 24 hours, 3 days, and 4 days.

Statistical Analysis
Because this was an exploratory trial, no formal sample size was calculated. Intention-to-treat analysis was used. An independent statistician performed statistical analysis according to a statistical analysis plan that was finalized before breaking of the randomization code. For the analysis of fibrinogen and the degree of inhibition of $\alpha_2$-AP, analysis of variance was performed at every time point and post hoc tests were performed with correction for multiple comparisons using Dunnett’s procedure, which compares every dose level with placebo. For analysis of MMP, tissue inhibitor of metalloproteinase 1, S100, and neuron-specific enolase data, analysis of covariance was performed with baseline value as a covariate and post hoc tests among different dose levels performed with corrections for multiple comparisons using Dunnett’s procedure. A $\chi^2$ test for trend across dose tiers was used for the imaging end points as a post hoc analysis. A probability value of <0.05 was considered significant.

Role of the Funding Source
The sponsor was involved in the design of the study, the collection, analysis, and interpretation of the data and in writing the study report. The corresponding author had access to all data in the study and had final responsibility for the decision to submit for publication.

Results
The study flow is detailed in Figure 1. Forty patients were enrolled and randomized in 8 European centers between October 18, 2005, and May 5, 2008. All patients received the study drug according to the defined protocol. The baseline characteristics of the enrolled patients are described in Table 1. Microplasmin-treated patients had slightly more severe neurological impairment and were treated slightly earlier than the placebo-treated group. The lesion sizes of the low-dose, high-dose, and placebo groups were similar. In one patient in the microplasmin group, the PI imaging at baseline was not interpretable. There were 27 patients with a PI/DWI mismatch at baseline (62% of included patients). There were slightly more patients with a PI/DWI mismatch and with an MRA occlusion in the placebo group (Table 1).

Safety End Points
Safety results up to day 30 are shown in Table 2. One patient developed a symptomatic intracranial hemorrhage shortly after study drug administration and subsequently died. The family withdrew consent and no additional invasive assessments (laboratory, MRI) were performed after symptomatic
intracranial hemorrhage diagnosis. One patient died 2 months after study drug administration due to pneumonia.

The frequency of serious adverse events was similar for patients treated with microplasmin combined across all groups \( (n=7 \text{ [23%]}) \) and in the placebo group \( (n=3 \text{ [30%]}) \).

There was no evidence of any impairment of hematology and clinical chemistry variables by microplasmin. Vital signs, respiratory rate, body temperature, and electrocardiographic findings were similar in microplasmin and placebo patients. There were no reported allergic reactions.

Figure 3 shows the plasma fibrinogen concentrations over the first 24 hours. Differences between treatment groups were significant at all time points except at baseline and at 96 hours \( (P<0.003) \). Post hoc analysis revealed that significant decreases occurred in the medium-dose group at Hours 6 and 12 after the end of infusion and in the highest dose group from the end of infusion until Hour 24. There were transient concomitant increases in D-dimer concentrations, prothrombin time, and activated partial thromboplastin time in the treatment groups compared with the placebo group (data not shown).

### Efficacy End Points

#### Clinical End Points

Table 3 shows the clinical outcomes at Day 30. There were no significant changes in any outcome parameter based on neurological impairment, disability, and handicap scales.

#### Imaging Outcomes

Table 4 shows the imaging end points. There was no significant difference in recanalization/reperfusion rates. There was also no effect on lesion growth.

#### Biomarker Outcomes

There were no clear differences in neuron-specific enolase expression levels between the treatment and placebo groups over time. The protein S100 levels evolved differently over time compared with placebo \( (P=0.001) \). Protein S100 levels were significantly increased in the medium-dose group, but not in the lower or the higher dose groups compared with placebo.

Microplasmin had a significant effect on MMP-2, but not on MMP-9 or tissue inhibitor of metalloproteinase 1, as shown in Table 5. Analysis of covariance showed that

### Table 4. Imaging End Points

<table>
<thead>
<tr>
<th>Feature</th>
<th>Microplasmin 1+1 mg/kg (n=6)</th>
<th>Microplasmin 1+2 mg/kg (n=12)</th>
<th>Microplasmin 1+3 mg/kg (n=12)</th>
<th>Microplasmin Total (n=30)</th>
<th>Placebo (n=10)</th>
<th>P Value for Linear Trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reduction of PI abnormality with 30%, n (%)</td>
<td>1 (17)</td>
<td>2 (17)</td>
<td>1 (8)</td>
<td>4 (13)</td>
<td>0 (0)</td>
<td>0.53</td>
</tr>
<tr>
<td>TIMI improved by 2 points, n (%)</td>
<td>1 (17)</td>
<td>2 (17)</td>
<td>1 (8)</td>
<td>4 (13)</td>
<td>1 (10)</td>
<td>0.92</td>
</tr>
<tr>
<td>TIMI improved by ≥ 1 point, n (%)</td>
<td>1 (17)</td>
<td>3 (25)</td>
<td>1 (8)</td>
<td>5 (17)</td>
<td>1 (10)</td>
<td>0.97</td>
</tr>
<tr>
<td>PI reduction and/or TIMI 2 points, n (%)</td>
<td>1 (17)</td>
<td>4 (33)</td>
<td>2 (17)</td>
<td>7 (23)</td>
<td>1 (10)</td>
<td>0.54</td>
</tr>
<tr>
<td>PI reduction and/or TIMI ≥ 1-point improvement, n (%)</td>
<td>1 (17)</td>
<td>5 (42)</td>
<td>2 (17)</td>
<td>8 (27)</td>
<td>1 (10)</td>
<td>0.48</td>
</tr>
</tbody>
</table>

### Table 5. Evolution of MMP-2, MMP-9, and TIMP-1 at Baseline, 24 Hours, and 96 Hours After the End of Treatment

<table>
<thead>
<tr>
<th>Time of Measurement, Hours</th>
<th>1+1 mg/kg</th>
<th>1+2 mg/kg</th>
<th>1+3 mg/kg</th>
<th>All Doses</th>
<th>Placebo</th>
<th>Analysis of Covariance</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP-2, ng/mL</td>
<td></td>
<td></td>
<td></td>
<td>All Doses</td>
<td>Placebo</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>292±131</td>
<td>268±121</td>
<td>279±124</td>
<td>277±120</td>
<td>207±116</td>
<td></td>
<td>0.001</td>
</tr>
<tr>
<td>24</td>
<td>232±84</td>
<td>225±124</td>
<td>196±90</td>
<td>214±101</td>
<td>288±151</td>
<td></td>
<td></td>
</tr>
<tr>
<td>96</td>
<td>254±75</td>
<td>183±99</td>
<td>201±44</td>
<td>205±77</td>
<td>254±132</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMP-9, ng/mL</td>
<td></td>
<td></td>
<td></td>
<td>All Doses</td>
<td>Placebo</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>376±540</td>
<td>1944±1685</td>
<td>2505±1685</td>
<td>1854±1444</td>
<td>1505±1072</td>
<td></td>
<td>0.17</td>
</tr>
<tr>
<td>24</td>
<td>1093±1149</td>
<td>1795±2181</td>
<td>2134±1101</td>
<td>1790±1601</td>
<td>2061±2326</td>
<td></td>
<td></td>
</tr>
<tr>
<td>96</td>
<td>417±400</td>
<td>926±727</td>
<td>2208±2117</td>
<td>1351±1597</td>
<td>2273±2205</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TIMP-1, ng/mL</td>
<td></td>
<td></td>
<td></td>
<td>All Doses</td>
<td>Placebo</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>308±105</td>
<td>230±120</td>
<td>466±295</td>
<td>346±227</td>
<td>398±229</td>
<td></td>
<td>0.24</td>
</tr>
<tr>
<td>24</td>
<td>294±95</td>
<td>349±207</td>
<td>401±185</td>
<td>359±179</td>
<td>422±95</td>
<td></td>
<td></td>
</tr>
<tr>
<td>96</td>
<td>305±59</td>
<td>304±233</td>
<td>481±343</td>
<td>377±272</td>
<td>586±249</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
MMP-2 serum concentrations were lower or remained constant in the microplasmin group, whereas there was an increase in the placebo group ($P=0.001$). Pairwise comparisons showed that the medium- and high-dose microplasmin had significantly lower values compared with placebo after correction for multiple comparisons.

**Interaction of Microplasmin with $\alpha_2$-AP**

Figure 2 shows the evolution of $\alpha_2$-AP levels over time. $\alpha_2$-AP was inhibited in the microplasmin dose groups at 0, 6, and 24 hours postdosing and at 96 hours in the highest dose group compared with placebo ($P<0.05$). The $\alpha_2$-AP inhibition was approximately 62% in the microplasmin 1+1 mg/kg treatment group and approximately 80% in the microplasmin 1+2 mg/kg and 1+3 mg/kg treatment groups at the end of study treatment.

**Discussion**

Microplasmin was well tolerated with only one treated patient who developed a fatal symptomatic intracranial hemorrhage. No dose effect was seen on the rate of asymptomatic intracerebral hemorrhage, and there were no major systemic bleedings.

We observed potentially relevant biological effects of administration of microplasmin in patients with acute ischemic stroke 3 to 12 hours after the onset of symptoms.

Intravenous administration of microplasmin does not result in a direct thrombolytic effect on clots given the rapid inactivation of microplasmin by circulating inhibitors. Transient depletion of $\alpha_2$-AP activity may, however, lead to increased endogenous fibrinolytic activity, which may protect the vascular integrity of the microcirculation in the penumbral area. Experimental depletion of $\alpha_2$-AP by plasmin or immunoneutralization reduced infarct volumes in permanent occlusion models, suggesting that an additional neuroprotective mechanism may also be present.4

The strong reduction in $\alpha_2$-AP levels observed in the higher doses tested in this study resulted in a systemic effect of microplasmin as demonstrated by the reduced fibrinogen levels, reflecting the nonfibrin-specific lytic activity of microplasmin due to the lack of kringle domains. This may have positive effects on viscosity and enhance microperfusion. Stronger defibrinogenating drugs like ancrod have, however, had mixed results in the treatment of acute ischemic stroke.11,12 Finally, we observed less expression of MMP-2 compared with placebo. Increased levels of MMPs have been linked to intracerebral hemorrhages associated with tPA use.13,14 One potential advantage of microplasmin from preclinical work is the lower risk of intracerebral hemorrhage, which is one of the reasons why tPA is given infrequently.3

The rate of reperfusion in microplasmin-treated patients was not significantly different from placebo. It is difficult to compare the recanalization rates in this study with the rates reported for tPA because recanalization rates with tPA have not been tested in this late time window.15

As expected from a trial with a small sample size, no effect on clinical outcome was observed.

In view of the preclinical data, the safety profile, and encouraging imaging findings, we think further clinical studies are warranted to determine whether microplasmin is an effective therapeutic agent for acute ischemic stroke.

**Appendix**

**Study Safety Committee**

Nils Wahlgren, MD, PhD (Committee Chair), Neurologiska Kliniken, Karolinska Universitetssjukhuset, Stockholm (Solna), Sweden; Antoni Dávalos, MD, PhD, Department of Neurosciences, Hospital Universitari Germans Trias i Pujol, Barcelona, Spain; and Raymond Verhaeghe, MD, PhD, Department of Vascular Medicine, UZ Gasthuisberg, Leuven, Belgium.


**Recruiting Investigators**

Belgium: Thijs Peeters; Germany: Röther Neuman-Haefelin and Schneider Schellinger; and Austria: Aichner Vosko.
Disclosures
V.N.S.T.’s academic institution has a financial interest in Thrombo- 
genics NV. He is also supported by FWO Flanders. P.V. serves as a 
consultant for Thrombogenics NV. His academic institution has a 
financial interest in Thrombogenics NV. There are no other conflicts 
to report.

References
1. Tissue plasminogen activator for acute ischemic stroke. The National 
Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group. 
2. Hacke W, Kaste M, Bluhmki E, Brozman M, Davalos A, Guidetti D, 
Larrue V, Lees KR, Medeghri Z, Machning T, Schneider D, von 
Kummer R, Wahlgren N, Toni D. Thrombolysis with alteplase 3 to 4.5 
Collen D. Recombinant human microplasmin: production and potential 
4. Nagai N, De Mol M, Van Hoef B, Verstreken M, Collen D. Depletion of 
circulating alpha2-antiplasmin by intravenous plasmin or immunoneu-
tralization reduces focal cerebral ischemic injury in the absence of arterial 
reduces ischemic brain damage and improves neurological function in a 
system components in focal cerebral ischemic infarction: a gene targeting and 
Randomized, Placebo-Controlled, Dose-Ranging Clinical Trial of Intravenous Microplasmin in Patients With Acute Ischemic Stroke

Vincent N.S. Thijs, Andre Peeters, Milan Vosko, Franz Aichner, Peter D. Schellinger, Dietmar Schneider, Tobias Neumann-Haefelin, Joachim Röther, Antoni Davalos, Nils Wahlgren and Peter Verhamme

Stroke. 2009;40:3789-3795; originally published online October 15, 2009;
doi: 10.1161/STROKEAHA.109.560201

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/40/12/3789

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org/subscriptions/