Matrix Metalloproteinase-9 in an Exploratory Trial of Intravenous Minocycline for Acute Ischemic Stroke

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Background and Purpose—Plasma matrix metalloproteinase-9 levels predict posttissue plasminogen activator (tPA) hemorrhage.

Methods—The authors investigated the effect of minocycline on plasma matrix metalloproteinase-9 in acute ischemic stroke in the Minocycline to Improve Neurological Outcome in Stroke (MINOS) trial and a comparison group.

Results—Matrix metalloproteinase-9 level decreased at 72 hours compared with baseline in MINOS (tPA, \( P = 0.0022 \); non-tPA, \( P = 0.0066 \)) and was lower than in the non-MINOS comparison group at 24 hours (tPA, \( P < 0.0001 \); non-tPA, \( P = 0.0019 \)).

Conclusions—Lower plasma matrix metalloproteinase-9 was seen among tPA-treated subjects in the MINOS trial. Combining minocycline with tPA may prevent the adverse consequences of thrombolytic therapy through suppression of matrix metalloproteinase-9 activity. (Stroke. 2011;42:2633-2635.)

Key Words: acute stroke • inflammation • neuroprotection

Plasma levels of matrix metalloproteinase (MMP)-9 are amplified by tissue plasminogen activator (tPA), correlate with neurological severity, and predict the risk of tPA-related hemorrhage. Minocycline (MC) is a potent MMP inhibitor. We recently completed a trial of intravenous MC in acute ischemic stroke (Minocycline to Improve Neurological Outcome in Stroke [MINOS]) to determine safety and tolerability. The purpose of this analysis was to determine the impact of intravenous MC on MMP-9 in acute ischemic stroke.

Methods

Subjects

MINOS was a nonrandomized, dose-escalation trial of intravenous MC for acute ischemic stroke administered within 6 hours of symptom onset. Sixty subjects were enrolled into 4 dose tiers (3.0, 4.5, 6.0, 10.0 mg/kg). MC was infused over 1 hour every 12 hours for 3 days. Blood samples for MMP-9 analysis were collected before MC treatment (baseline), 1 hour after the first infusion of MC was started, 24 and 72 hours after symptom onset, and between 4 and 7 days. If subjects received intravenous tPA, the baseline sample was drawn after tPA administration. For a comparator group, we used samples from 44 patients in a previously conducted study of blood biomarkers in acute ischemic stroke. For a comparator group, we used samples from 44 patients in a previously conducted study of blood biomarkers in acute ischemic stroke. Blood samples were collected 24 hours after symptom onset.

Laboratory Methods

MMP-9 activity was measured using a standardized zymography assay by the biomarker laboratory blinded to the clinical data. Results were expressed as percent of the recombinant standards run on each gel.

Statistical Analysis

Statistical analysis was performed using SAS 9.2 and statistical significance was assessed using an \( \alpha \) level of 0.05. MMP-9 levels were not normally distributed; data transformation was by natural log for mixed-model analysis. Post hoc pairwise comparisons within tPA groups were performed between baseline and 1, 24, and 72 hours. A Bonferroni adjustment of 0.05/6 = 0.0083 was used to determine post hoc differences between groups. To examine differences between the MINOS and non-MINOS groups and tPA status at 24 hours, 2-factor analysis of variance was used. Bonferroni adjustments of 0.05/4 = 0.0125 were used to determine post hoc differences between groups.

Results

Demographic and clinical data in MINOS and non-MINOS comparison subjects is displayed in the Table. No symptomatic intracerebral hemorrhage occurred in either cohort using Safe Implementation of Thrombolysis in Stroke-Monitoring Study (SITS-MOST) criteria and National Institutes of Health Stroke Scale score at discharge or 7 days was lower in MINOS.
### Table. Demographic and Clinical Characteristics for MINOS and Comparison Subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>MINOS (n=60)</th>
<th>Non-MINOS (n=44)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean y (SD)</td>
<td>65.0 (13.7)</td>
<td>61.8 (13.9)</td>
<td>0.2490</td>
</tr>
<tr>
<td>Male sex, %</td>
<td>53%</td>
<td>57%</td>
<td>0.8423</td>
</tr>
<tr>
<td>Black, %</td>
<td>15%</td>
<td>50%</td>
<td>0.0002</td>
</tr>
<tr>
<td>tPA, %</td>
<td>60%</td>
<td>66%</td>
<td>0.6821</td>
</tr>
<tr>
<td>Diabetes, %</td>
<td>27%</td>
<td>52%</td>
<td>0.0134</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>80%</td>
<td>89%</td>
<td>0.4850</td>
</tr>
<tr>
<td>Lacune stroke, %</td>
<td>13%</td>
<td>20%</td>
<td>0.4229</td>
</tr>
<tr>
<td>Baseline NIHSS, mean (SD)</td>
<td>8.7 (5.8)</td>
<td>9.5 (6.1)</td>
<td>0.4720</td>
</tr>
<tr>
<td>Discharge NIHSS, mean (SD)</td>
<td>4.0 (4.6)</td>
<td>7.0 (8.5)</td>
<td>0.0436</td>
</tr>
<tr>
<td>Symptomatic intracerebral hemorrhage, %</td>
<td>0%</td>
<td>0%</td>
<td>1.0000</td>
</tr>
</tbody>
</table>

MINOS indicates Minocycline to Improve Neurological Outcome in Stroke; tPA, tissue plasminogen activator; NIHSS, National Institutes of Health Stroke Scale; SD, standard deviation.

*N*=57 for the MINO group, *n*=43 for the comparison group.

In MINOS, the MMP-9 level was significantly reduced at 72 hours compared with baseline for both tPA (*P*=0.0022) and non-tPA (*P*=0.0067) -treated subjects (Figure 1) controlling for race and diabetes. In addition, in the tPA group, there was a trend toward reduced MMP-9 level as early as 1 (*P*=0.0271) and 24 hours (*P*=0.0376) compared with baseline. At 4 to 7 days, MMP-9 levels appeared to increase but had not yet returned to baseline. Compared with our non-MINOS cohort, MMP-9 level was lower at 24 hours in both tPA (*P*<0.0001) and non-tPA-treated (*P*=0.0016) subjects (Figure 2) controlling for race and diabetes.

![Figure 1. Levels of MMP-9 over time in MINOS. A, MMP-9 levels in tPA-treated and tPA-untreated subjects at each time point in MINOS. Box=25% to 75% interquartile range; horizontal line=median; vertical lines=range. *P*<0.0083. B, A representative zymogram from a MINOS patient showing MMP-9 over time. MMP-9 indicates matrix metalloproteinase-9; MINOS, Minocycline to Improve Neurological Outcome in Stroke; tPA, tissue plasminogen activator.](image1)

![Figure 2. Levels of MMP-9 in MINOS and comparison subjects at 24 hours. MMP-9 in tPA-treated and tPA-untreated MINOS and comparison subjects at 24 hours. Box=25% to 75% interquartile range; horizontal line=median; vertical lines=range. *P*<0.0125. MMP-9 indicates matrix metalloproteinase-9; MINOS, Minocycline to Improve Neurological Outcome in Stroke; tPA, tissue plasminogen activator.](image2)

### Discussion

MMPs are important mediators of blood–brain barrier disruption, edema, and hemorrhage in acute ischemic stroke. After cerebral ischemia, there is increased expression of MMP-9 within the ischemic zone, and MMP-9 levels are further amplified by tPA. In addition, elevations in plasma MMP-9 correlate with stroke severity and are predictive of tPA-related intracerebral hemorrhage. In this study, we observed lower levels of MMP-9 in MC-treated subjects at 24 hours, and levels of MMP-9 decreased in MINOS from baseline to 72 hours. Interestingly, in intravenous tPA-treated subjects, we saw a trend toward reduced MMP-9 as soon as 1 hour after initiating MC infusion.

There are several limitations of our study. First, this was not a randomized controlled trial. Although subjects in the 2 groups were similar in terms of age, sex, stroke severity, and subtype, there were imbalances in diabetes and race and bias from an unrecognized confounder cannot be excluded. Second, the small sample size of our study limited our power to make numerous comparisons between time points and precludes more definitive conclusions about the impact of MC on biomarker levels. Last, the significance of lower levels of MMP-9 on risk of hemorrhagic conversion and clinical outcome is unclear.

In summary, plasma MMP-9 decreased in the hours to days after intravenous MC treatment and levels of MMP-9 were lower at 24 hours in subjects in our MINOS study when compared with a separate cohort who had not received MC. These results require confirmation in an ancillary study of a randomized clinical trial and support further investigation of the strategy of combining MC with thrombolytic therapy to reduce post-tPA hemorrhage and ameliorate outcome.

### Sources of Funding

This study was supported by National Institutes of Health/National Institute of Neurological Disorders and Stroke grants to D.C.H. (R01 NS053728) and S.C.F. (NS063965), Veteran Affairs Merit Review to S.C.F. and A.E., American Heart Association Established Investigator Award to A.E., and the Medical College of Georgia Intramural Grants Program Scientist Training Program to J.A.S.
Disclosures

None.

References


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*Stroke.* 2011;42:2633-2635; originally published online July 7, 2011;
doi: 10.1161/STROKEAHA.111.618215

*Stroke* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0039-2499. Online ISSN: 1524-4628

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