

# Reduction of Tissue Plasminogen Activator–Induced Matrix Metalloproteinase-9 by Simvastatin in Astrocytes

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**Background and Purpose**—Hemorrhagic conversion after tissue plasminogen activator (tPA) stroke therapy has been linked with elevations in matrix metalloproteinase-9 (MMP-9) at the neurovascular interface. Here, we test the idea that statins may directly ameliorate tPA-induced MMP-9 dysregulation.

**Methods**—Recombinant human tPA (5  $\mu\text{g}/\text{mL}$ ) was added to primary rat cortical astrocytes. Zymography was used to quantify MMP-9 levels in conditioned media. Effects of simvastatin or the Rho kinase inhibitor Y-27632 were assessed by pretreating cells before tPA exposure.

**Results**—Simvastatin (1 to 10  $\mu\text{mol}/\text{L}$ ) significantly reduced tPA-induced MMP-9 in cortical astrocytes. This effect may be mediated via the Rho kinase pathway because tPA-induced activation of Rho signaling was suppressed by simvastatin, and tPA-induced MMP-9 levels were similarly reduced by the Rho kinase inhibitor Y-27632 (1 to 10  $\mu\text{mol}/\text{L}$ ).

**Conclusions**—Statins reduce tPA-induced MMP-9 dysregulation by inhibiting the Rho signaling pathway. Statins may ameliorate tPA-associated MMP imbalances in stroke. (*Stroke*. 2006;37:1910-1912.)

**Key Words:** hemorrhage ■ stroke

Thrombolysis with tissue plasminogen activator (tPA) can effectively restore blood flow in the ischemic brain but increases the risk of life-threatening intracerebral hemorrhage. Because the neurovascular protease matrix metalloproteinase-9 (MMP-9) mediates blood-brain barrier leakage and edema in stroke,<sup>1,2</sup> an emerging hypothesis suggests that tPA upregulates MMP-9 after stroke, subsequently damaging the neurovascular matrix to cause hemorrhagic transformation.<sup>3-6</sup> Therefore, strategies to reduce tPA-induced MMP-9 dysregulation may improve the safety and efficacy of tPA. Here, we tested the hypothesis that statins, 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors, can directly reduce tPA-induced MMP-9 levels in astrocytes.

## Materials and Methods

### Cell Culture

Primary rat astrocyte cultures were prepared as described previously.<sup>7</sup> Cells were treated with simvastatin (gift from James Liao and Michael Moskowitz) or Y-27632 (Calbiochem) for 4 hours and then incubated for 24 hours with recombinant human tPA (Activase; Genentech). Rho kinase signaling was assessed using Western blots to look for localization of Rho to the membrane fraction of cell lysates following standard techniques.

### Gelatin Zymography

Gelatin zymography was performed as described previously.<sup>4</sup> Human MMP-2 and MMP-9 standards (Chemicon) were used as positive controls. Gelatin zymography was quantified ( $n \geq 4$  per group) as the optical density (mean  $\pm$  SEM) and expressed as a ratio to the loaded positive controls. Data were analyzed using ANOVA and Tukey honestly significant difference tests.  $P < 0.05$  was considered significant.

### Results

Our rat cortical astrocytes primarily secreted the 88-kDa form of MMP-9. Exposure of rat cortical astrocytes to tPA (5  $\mu\text{g}/\text{mL}$ ) for 24 hours upregulated 88-kDa MMP-9 levels in conditioned media. Pretreatment with simvastatin (1 to 10  $\mu\text{mol}/\text{L}$ ) significantly ameliorated this response (Figure 1A and 1B). MMP-2 levels (both 72 and 66 kDa) were upregulated by tPA, but the response tended to be variable, and simvastatin treatment had no statistically significant effects (Figure 1C and 1D).

Although statins can modulate multiple intracellular pathways, recent data suggest that inhibition of Rho kinase signaling may be an important downstream effect. Astrocytes that were exposed to tPA showed an activation in the Rho kinase pathway (Figure 2). Simvastatin prevented tPA-induced Rho activation

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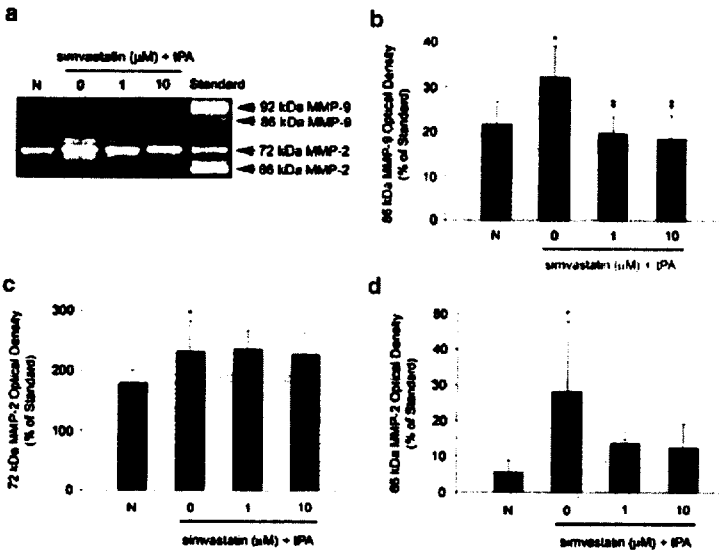
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**Figure 1.** Effects of simvastatin on tPA-induced MMP-9 levels. a, Zymogram of tPA-induced MMP-2 and MMP-9 release into culture media from primary rat astrocytes. N indicates untreated cultures. b, Quantitation of 88-kDa MMP-9. c, Quantitation of 72-kDa MMP-2. d, Quantitation of 66-kDa MMP-2. \**P*<0.05 vs untreated cells; †*P*<0.05 vs cells treated with tPA alone.

(Figure 2). Consistent with this biochemical result, pretreatment with the potent and specific Rho kinase inhibitor Y-27632 (1 to 10 μmol/L) significantly ameliorated the tPA-induced MMP-9 response (Figure 3A and 3B). tPA-induced 72-kDa MMP-2 levels were also significantly decreased (Figure 3C), but 66-kDa MMP-2 levels were statistically unaffected by Rho kinase inhibition (Figure 3D).

**Discussion**

Statins may be beneficial in ischemic stroke through their anti-inflammatory actions<sup>8</sup> and can upregulate endothelial NO synthase and tPA in the cerebral endothelium, which improves cerebral perfusion<sup>9</sup> and contributes to clot lysis,<sup>10</sup> respectively. Our data point to another possible beneficial action of statins (ie, amelioration of tPA-induced MMP-9) that is known to damage the neurovascular unit and contribute to brain injury after stroke.<sup>1-6</sup> Kilic et al recently showed that statins may reduce tPA-associated reperfusion injury in a

mouse model of focal cerebral ischemia.<sup>11</sup> More recently, Zhang et al showed that combination therapy with atorvastatin plus tPA decreased MMP-9 and extended the time window for thrombolysis in a rat embolic clot model.<sup>12</sup> A caveat with in vivo models is that it may be difficult to distinguish direct statin effects on MMPs versus multifactorial statin effects on overall tissue injury. Our data show that simvastatin directly suppresses MMP-9 in cells. Although there are important differences between different types of statins, taken together, these data indicate that statins may be protective against tPA-induced MMP-9 deregulation and the associated hemorrhagic complications in stroke.

Cardiovascular benefits of statins were historically ascribed to their cholesterol-lowering actions. However, it is now recognized that statins modulate many other cell signaling pathways, including the Rho/ROCK pathway.<sup>13</sup> In our model system, tPA activated Rho signaling, simvastatin prevented this effect, and the Rho kinase inhibitor Y-27632 downregulated MMP-9. Thus, the amelioration of tPA-induced MMP-9 by simvastatin in our study likely occurs via the Rho pathway.

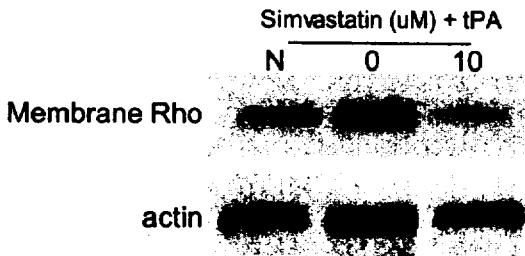
Further studies are warranted to investigate the effects of statins on all cells of the neurovascular unit<sup>14</sup> and determine whether specific statins or newly developed Rho kinase inhibitors<sup>15</sup> can indeed suppress tPA-induced MMP imbalances and improve combination therapies for stroke.

**Sources of Funding**

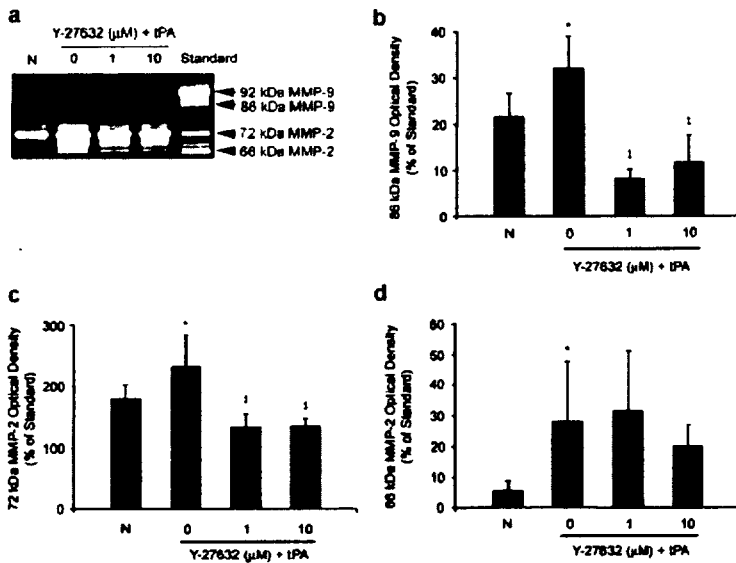
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**Disclosures**

None.



**Figure 2.** Western blots of Rho localization in membrane fractions of cell lysates. A shift toward membrane localization indicates activation of Rho signaling. tPA induced a clear increase in membrane Rho localization, which is reversed by simvastatin. N indicates untreated cultures.



**Figure 3.** Effects of Y-27632 on tPA-induced MMP-9 levels. **a**, Zymogram of tPA-induced MMP-2 and MMP-9 release in culture media from primary rat astrocytes. N indicates untreated cultures. **b**, Quantitation of 88-kDa MMP-9. **c**, Quantitation of 72-kDa MMP-2. **d**, Quantitation of 66-kDa MMP-2. \* $P < 0.05$  vs untreated cells; ‡ $P < 0.05$  vs cells treated with tPA alone.

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