Nitric Oxide in Vascular Endothelial Growth Factor-Induced Focal Angiogenesis and Matrix Metalloproteinase-9 Activity in the Mouse Brain

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Background and Purpose—Vascular endothelial growth factor (VEGF) can induce matrix metalloproteinase (MMP)-9 activities and focal angiogenesis. We hypothesized that VEGF activation of cerebral MMP-9 would require nitric oxide participation.

Methods—We compared the in vivo effects of: (1) N\textsubscript{G}-monomethyl-L-arginine, a nonspecific nitric oxide synthase inhibitor; (2) L-N\textsuperscript{6}-(1-iminoethyl)lysine, an inducible nitric oxide synthase selective inhibitor; and (3) doxycycline, a known nonspecific inhibitor of MMP in the mouse brain, using in situ zymography and endothelial marker CD31. 3-nitrotyrosine was used as a surrogate for nitric oxide activity. Inflammatory cell markers CD68 and MPO were used to confirm leukocyte infiltration.

Results—VEGF-stimulated MMP-9 activity expressed primarily around cerebral microvessels. N\textsubscript{G}-monomethyl-L-arginine suppressed cerebral angiogenesis (P<0.05), especially those microvessels associated with MMP-9 activation (P<0.02) induced by VEGF, comparable to the effect of doxycycline. L-N\textsuperscript{6}-(1-iminoethyl)lysine showed similar inhibitory effects. 3-nitrotyrosine confirmed nitric oxide levels in the brain. Compared with the lacZ control, VEGF increased inflammatory cell infiltration, especially macrophages, in the induced brain angiogenic focuses.

Conclusions—Inhibition of nitric oxide production decreased MMP-9 activity and focal angiogenesis in the VEGF-stimulated brain. Both specific and nonspecific inhibition of nitric oxide synthase resulted in similar reductions, suggesting that VEGF-stimulated cerebral MMP activity and angiogenesis are predominantly mediated through inducible nitric oxide synthase, a specific nitric oxide synthase isoform mediating inflammatory responses. (Stroke. 2009;40:2879-2881.)

Key Words: matrix metalloproteinase | nitric oxide | vascular endothelial growth factor
Results

Under VEGF hyperstimulation, gelatinolytic (MMP) activities colocalized with microvessels in the angiogenic focus in brain caudate putamen. Merged images (yellow): MMP activity (in situ zymography, green) and microvessels (CD31, red). B, Inhibitors decreased mouse cerebral MMP gelatinolytic activity. Bar=20 μm. C, Quantitation of capillary density (mean±SD, n=5 to 6; *P<0.05).

Discussion

We demonstrated that inhibition of NOS suppressed VEGF-stimulated MMP activity and angiogenesis in the mouse brain. Although in vitro studies have shown that NO can either inhibit or promote MMP activation depending on cell types and stimulants,3,4 our data from this in vivo mouse model provide direct evidence linking VEGF-induced NO production to increased MMP activity and cerebral angiogenesis.

Although in situ zymography may detect both MMP-2 and -9 activity, increased MMP-9 activation (but not MMP-2 in either the pro- or activated form) has been detected by gelatin zymography under VEGF stimulation in vivo5 or in vitro7 despite MMP-2 involvement in other neurovascular degradation pathways.8 The failure of VEGF to stimulate angiogenesis in MMP-9 knockout mice9 further supports its importance in the VEGF pathway.

Figure 1. A, VEGF-stimulated MMP gelatinolytic activities colocalized with microvessels in the angiogenic focus in brain caudate putamen. Merged images (yellow): MMP activity (in situ zymography, green) and microvessels (CD31, red). B, Inhibitors decreased mouse cerebral MMP gelatinolytic activity. Bar=20 μm. C, Quantitation of capillary density (mean±SD, n=5 to 6; *P<0.05).

Figure 2. A, 3-NT expression in the brain angiogenic focus (n=3). Arrows point to positive stainings. Inset shows larger magnification. Bar=50 μm. B, 3-NT in cells close vicinity to and colocalizing with cerebral microvessels (lectin). Bar=100 μm.

Figure 3. A, Increased inflammatory cells in the VEGF-stimulated brain angiogenic focus (n=3). Arrows point to positive stainings. Insets show larger magnification. Bar=100 μm. B, Macrophage marker CD68 colocalized with 3-NT-expressing cells. Bar=100 μm.
Inducible NOS inhibition showed similar effects as non-specific NOS inhibition, suggesting a predominant role of inducible NOS in VEGF-induced cerebral angiogenesis, although endothelial NOS has been found in vascular endothelial cells in response to VEGF. Although NO and MMP can increase VEGF release from tissues, recent evidence indicates that NOS inhibition mainly alters VEGF downstream effector capacity, in our case, cerebral MMP activation and angiogenic response. Given that inducible NOS is a potent source of NO in leukocytes and vascular cells, increased leukocyte infiltration is consistent with VEGF-induced inflammation in brain angiogenesis.

As an important mechanism underlying VEGF-stimulated MMP activity, further studies are needed to explore anti-inflammatory manipulation by local NO inhibition to decrease pathological angiogenesis and stabilize abnormal vasculature to decrease spontaneous hemorrhage risk. On the other hand, accumulating evidence suggests that VEGF–MMP cascade augmentation may be beneficial during stroke recovery. The demonstrated participation of NO in the VEGF-stimulation pathway may also open a new window for therapeutic interventions to promote functional revitalization after central nervous system insults.

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Disclosures
None.

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