Endothelium-derived nitric oxide (NO) is a mediator of angiogenesis. Vascular endothelial growth factor (VEGF) stimulates the release of NO from cultured human umbilical venous endothelial cells and upregulates the expression of nitric oxide synthase (NOS).

Segments of rabbit thoracic aorta release NO in response to VEGF; preincubation with L-arginine increases basal and VEGF-stimulated NO release 2-fold. Similar results are observed when the angiogenic stimulus is transforming growth factor β or basic fibroblast growth factor.

The release of NO by these factors plays a critical role in their angiogenic actions. In a 3D fibrin gel, human umbilical venous endothelial cells elaborate NO and form capillary-like structures when stimulated by basic fibroblast growth factor or VEGF, effects that are blocked by the NOS antagonist Nω-nitro-L-arginine methyl ester (L-NAME). Similar effects have been observed in vitro using substance P or transforming growth factor β. In the rabbit cornea model of angiogenesis, VEGF-induced angiogenesis is antagonized by L-NAME.

Angiogenesis is attenuated when NO bioactivity is reduced. Capillary-like outgrowths sprout from segments of rabbit thoracic aorta or human coronary artery explanted into a collagen matrix. This ex vivo angiogenesis is inhibited by oxidized LDL cholesterol, an agent also known to reduce NO bioactivity. In hypercholesterolemic rabbits, endothelium-dependent NO-mediated vasodilation is blunted, as is the angiogenic response to hindlimb ischemia. More definitively, the angiogenic response to hindlimb ischemia is impaired in the eNOS-deficient mice, an effect that cannot be reversed by administration of recombinant VEGF protein or adenovirus-mediated VEGF gene transfer.

How Does NO Exert Its Angiogenic Effects?
The mechanisms by which NO promotes angiogenesis are not fully elucidated. NO is an endothelial survival factor, inhibiting apoptosis and enhancing endothelial cell proliferation, perhaps in part by increasing the expression of VEGF or fibroblast growth factor. NO also enhances endothelial migration by stimulating endothelial cell podokinesis, enhancing the expression of α,β, and increasing dissolution of the extracellular matrix via the basic fibroblast growth factor–induced upregulation of urokinase-type plasminogen activator. Finally, the hemodynamic effects of this potent vasodilator may play a role in its angiogenic effects. It is known that increased flow (induced by prazosin) in the skeletal microcirculation is associated with increased endothelial cell proliferation, as indicated by uptake of bromodeoxyuridine by capillary endothelial cells.

A new mechanism by which NO may influence angiogenesis is reported in the article by Matsunaga et al in the present issue of Circulation. These investigators suggest that NO may suppress the production of angiotatin, an endogenous antagonist of angiogenesis.

NO and Angiostatin
Matsunaga and colleagues examined myocardial tissue and interstitial fluid from dogs undergoing repetitive coronary artery occlusions in the presence of vehicle or L-NAME, an antagonist of NO synthase. At 7 days, there was an increase in capillary density in the ischemic zone of those animals receiving vehicle, which was suppressed in those animals receiving L-NAME. Interstitial fluid from the ischemic myocardium of the control animals (but not sham-operated animals) induced endothelial cell proliferation and capillary tube formation in vitro. This effect of the interstitial fluid was suppressed in those animals receiving L-NAME. Western analysis of the interstitial fluid from the L-NAME–treated animals revealed a marked increase in angiotatin levels.

Angiotatin seemed to be mediating the antiangiogenic effects of L-NAME; antiangiotatin antibody restored the angiogenic effect of the interstitial fluid from the ischemic myocardium of the L-NAME–treated animals.

Angiotatin is a known inhibitor of angiogenesis that is generated from the degradation of plasminogen by matrix metalloproteinases (MMPs). The investigators noted that MMP-2 and MMP-9 activity was increased in the myocardium of L-NAME–treated animals and in the conditioned medium of endothelial cells treated with L-NAME. Presumably, the increased activity of MMP-2 and MMP-9 increased the generation of angiotatin. The same mechanism for release of angiogenic factors from the interstitium may contribute to the neovascularization that occurs in certain types of arthritis. These findings are also consistent with prior reports indicating that angiotatin is liberated by metalloelastase activity of macrophages in certain tumors. The role of MMPs in an angiogenic effect seems somewhat paradoxical, in that dissolution of extracellular matrix is needed for endothelial cell migration, a critical process in angiogenesis. Most biological processes, however, trigger countervailing effects that modulate re-
sponse. Undoubtedly, such countervailing forces, not yet defined, explain the observation by Matsunaga et al\textsuperscript{21} that, at later time points, myocardial capillary density declines in the control animals despite repetitive ischemic stimuli.

The complexities of angiogenesis and the NO synthase pathway become ever more convoluted in the context of atherosclerosis. A pathological role has been assigned to neovascularization on the basis of experimental evidence that antiangiogenic drugs inhibit plaque growth, whereas angiogenic factors accelerate it.\textsuperscript{25–27} We are presented with a paradox: The ability of the endothelium to produce NO is reflective of arterial health, yet NO mediates angiogenesis. Could NO, under certain circumstances, contribute to plaque growth—for example, by favoring neovascularization? We think not. NO is a pleiotropic factor, with many actions that oppose atherogenesis (e.g., its inhibition of platelet aggregation, monocyte adherence, and oxidant-sensitive genes involved in atherogenesis).\textsuperscript{28} When all of the actions of NO are considered in the balance, NO seems to be an antiatherosclerotic factor. Our view is supported by studies showing that genetic or pharmacological abrogation of the NO synthase pathway invariably accelerates atherosclerosis.\textsuperscript{28,29}

Of relevance to this discussion is the existence of an endogenous antagonist to NO synthase. Asymmetric dimethylarginine (ADMA) is an arginine analogue that competes with l-arginine for NOS.\textsuperscript{30} Plasma ADMA levels are elevated in animals and humans with hypercholesterolemia, diabetes mellitus, hypertension, homocystinemia, tobacco use, relatively advanced age, or congestive heart failure.\textsuperscript{30} In experimental animal models, endogenous or exogenously elevated plasma ADMA levels are associated with impaired angiogenic response to ischemia, an effect that is reversed by supplemental l-arginine.\textsuperscript{31}

**Conclusion**

To conclude, the study by Matsunaga et al\textsuperscript{21} provides more evidence that NO plays a critical role in angiogenesis. As we pursue therapeutic angiogenesis for the treatment of vascular occlusive disease, it will be important to consider the role that restoration of endothelial health may play. Certainly, VEGF can enhance endothelial regeneration, restore endothelial function, and stimulate angiogenesis.\textsuperscript{32} Aggressive treatment of comorbid conditions (e.g., hypercholesterolemia, hypertension, diabetes, and tobacco exposure) to restore endothelial function, however, may be just as important as the administration of gene therapies or recombinant angiogenic proteins in the success of therapeutic angiogenesis.

**References**


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Nitric Oxide and Angiogenesis
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