Editorial

Therapeutic Angiogenesis
New Indication for Endothelial NO Synthase Gene Transfer

Zvonimir S. Katusic

Evidence continues to accumulate on the importance of NO in angiogenesis.1–7 A number of angiogenic substances, including vascular endothelial growth factor (VEGF), stimulate production of NO in endothelial cells.8,9 In vivo biosynthesis of NO is essential for angiogenesis induced by tissue ischemia.3 Angiogenesis is severely impaired in ischemic hindlimb of endothelial NO synthase (eNOS)-deficient mice.3,10 This impairment is not corrected by administration of VEGF, strongly suggesting that NO is downstream signal for angiogenic effect of VEGF. Indeed, in vascular endothelial cells, VEGF increases eNOS enzymatic activity via activation of protein kinase Akt and subsequent phosphorylation of eNOS.11,12 More recent study demonstrated that 3-hydroxyl-3-methyl coenzyme A (HMG-CoA) reductase inhibitor simvastatin also promotes angiogenesis by activation of protein kinase Akt.13 This effect seems to be mediated by stimulation of eNOS enzymatic activity. The importance of protein kinase Akt in regulation of NO production in vivo was demonstrated by adenovirus-mediated delivery of active Akt into the vascular wall. Overexpression of Akt increased resting blood flow, whereas expression of dominant negative Akt inhibited endothelium-dependent relaxation mediated by NO.14 Thus, phosphorylation of eNOS by protein kinase Akt seems to be a major molecular mechanism underlying the angiogenic effect of VEGF and statins.

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Structural adaptation of the vascular tree in response to increased shear stress is of fundamental importance for normal function of cardiovascular system. For instance, exercise training increases cross-sectional area of coronary arteries and stimulates angiogenesis in the heart.15 This adaptive response appears to be designed to maintain normal shear stress in coronary circulation. Increasing capillary shear stress by vasodilatation of resistance arteries due to chronic administration of the α1-adrenergic antagonist prazosin stimulates expression of VGEF and angiogenesis in skeletal muscle capillaries.16 It is unclear whether this angiogenic effect is specific for α1-adrenergic blockade; however, this observation suggests that pharmacologically induced vasodilatation and subsequent increase in blood flow may stimulate angiogenesis. It is of major interest that shear stress activates protein kinase Akt leading to phosphorylation of eNOS and production of NO in endothelial cells.11,12 Protein kinase Akt and NO are apparently key molecules responsible for adaptation to high shear stress and structural remodeling of vascular tree.

Loss of NO biological activity and/or biosynthesis is a central mechanism responsible for pathogenesis of vascular endothelial dysfunction.17 Restoration of normal NO levels in diseased arteries is a major therapeutic goal and could be achieved by supplementation with exogenous NO or by strategies designed to increase concentration of endogenous NO, including exercise, L-arginine, tetrahydrobiopterin, antioxidants, statins, angiotensin-converting enzyme inhibitors, and estrogen replacement. During the past decade, gene transfer technology emerged as a new approach in restoration of NO production. All three isoforms of NOS, endothelial (eNOS), neuronal (nNOS), and inducible (iNOS), have been patented and tested for potential therapeutic value in animal models of vascular diseases.18–20 In 2001, the first Phase I NOS clinical trial was initiated. Plasmid based formulation of iNOS is being tested for treatment of coronary artery restenosis after balloon angioplasty.

Numerous preclinical studies of NOS isoforms suggest that wide variety of vascular diseases (eg, atherosclerosis, hypertension, vasospasm, diabetic vascular disease, impotence) could be prevented or treated with NOS gene therapy.19 The study by Smith and colleagues21 published in this issue of Arteriosclerosis Thrombosis and Vascular Biology provided the first experimental evidence that recombinant eNOS may stimulate therapeutic angiogenesis. The investigators used rat model of hindlimb ischemia and delivered eNOS or luciferase adenovirus into adductor skeletal muscle. Four weeks later, blood flow in ischemic limb was almost normalized in rats treated with recombinant eNOS but remained significantly lower in rats treated with vehicle or adenovirus encoding luciferase. eNOS gene delivery increased the number of capillaries, strongly suggesting that high local concentration of NO may stimulate angiogenesis in skeletal muscle.

The mechanisms by which NO stimulates angiogenesis are not completely understood. Overexpression of eNOS in the arterial wall causes vasodilatation and increase in local blood flow.19 These hemodynamic changes may lead to adaptive angiogenic response and explain findings reported in the study by Smith and colleagues.21 It would be of interest to determine whether adenovirus-mediated gene delivery of a vasodilator gene other than eNOS (eg, prostacyclin, natriuretic peptides, calcitonin gene-related protein) can also stimulate angiogenesis. These experiments will certainly help deter-

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© 2002 American Heart Association, Inc.
Arterioscler Thromb Vase Biol is available at http://www.atvbaha.org
DOI: 10.1161/01.ATV.0000026860.08501.0F
mine if the effect of recombinant eNOS is specific or could it be mimicked by other vasodilators. Besides vasodilatation, increased local concentration of NO may stimulate proliferation and migration of endothelial cells. Both proliferation and migration of endothelial cells are essential for formation of new microvessels or larger arteries. Obviously, further investigation is needed to completely characterize molecular mechanisms underlying angiogenesis in response to overexpression of eNOS.

If indeed increased local concentration of NO is sufficient for angiogenesis, recombinant eNOS may have therapeutic advantage over VGEF. NO is essential for angiogenic effect of VGEF; however, biological availability or therapeutic advantage over VGEF. NO is essential for angiogenesis and migration of endothelial cells are essential for formation of new microvessels or larger arteries. Obviously, further investigation is needed to completely characterize molecular mechanisms underlying angiogenesis in response to overexpression of eNOS.

Acknowledgments

This work was supported in part by National Heart, Lung, and Blood Institute grants HL-53524, HL-58080, and HL-066958, by National Institute for Neurological Disorders and Stroke grant NS-37491, by the American Heart Association Bugher Foundation Award for the Investigation of Stroke, and by the Mayo Foundation. The secretarial assistance of Janet Beckman is gratefully acknowledged.

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doi: 10.1161/01.ATV.0000026860.08501.0F
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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