ANTIOXIDANTS & REDOX SIGNALING
Volume 4, Number 5, 2002

Forum Review

Nitric Oxide and Angiogenesis in Cardiovascular Disease

TOYOAKI MUROHARA[1] and TAKAYUKI ASAHARA[2]

ABSTRACT

Ischemic heart disease and peripheral artery disease mainly develop as a consequence of atherosclerotic lesion formation. Angiogenesis, the formation of new blood vessels from the preexisting vascular bed, is of paramount importance in the maintenance of vascular integrity both in the repair process of damaged tissue (wound healing) and in the formation of collateral vessels in response to tissue ischemia. Angiogenesis is a complex process that is orchestrated by a multitude of cytokines/chemokines and growth factors. In its broadest sense, angiogenesis cannot be viewed as a single process. It is likely that different mediators are involved in different phases of angiogenesis. Vascular endothelial cells produce nitric oxide (NO), an endothelium-derived labile molecule, which maintains vascular homeostasis and thereby prevents vascular atherosclerotic changes. In patients with ischemic heart disease and peripheral artery disease, the release of endothelium-derived NO is decreased, which plays an important role in the atherosclerotic disease progression. In recent years, endothelium-derived NO has been shown to modulate angiogenesis *in vitro* and *in vivo*. In this review, we summarize recent progress in the field of the NO-mediated regulation of postnatal angiogenesis. *Antioxid. Redox Signal.* 4: 825–831.

ROLES OF ENDOTHELIUM-DERIVED NITRIC OXIDE IN THE MAINTENANCE OF VASCULAR HOMEOSTASIS

VASCULAR ENDOTHELIAL CELLS produce numerous vasoactive substances that play roles in the regulation of vascular tone, inflammatory responses, and growth and migration of vascular smooth muscle cells (VSMCs). Nitric oxide (NO) is one of the most important nonpeptide endothelium-derived vasoactive factors (23, 31, 47). Endothelium-derived NO (EDNO) was initially identified as a main molecule representing the endothelium-derived relaxing factors (EDRFs) (38), originally identified by Furchgott and Zawadzki (15). NO activates soluble guanylate cyclase, resulting in VSMC relaxation and vasodilatation (43). Subsequently, in addition to its vasodilatory capacity, NO has been shown to inhibit platelet aggregation, leukocyte–endothelium interaction, and VSMC proliferation and migration (16, 47). NO, thus, has multiple important regulatory roles in the maintenance of vascular homeostasis (Fig. 1).

Synthesis of NO is regulated by a family of isoenzymes, called NO synthase (NOS) (10), that share in common the property of converting L-arginine to L-citrulline, yielding free NO gas (31). Three isoforms of NOS have been identified so far: neuronal NOS (nNOS), inducible NOS (iNOS), and endothelial NOS (eNOS). Both nNOS and eNOS are constitutively expressed, and their activities are regulated by calcium and calmodulin, whereas iNOS is expressed in response to stimulation by inflammatory cytokines (10). Genetic analysis demonstrated that deletion of the gene encoding for eNOS has been shown to result in systemic hypertension and pulmonary vasoconstriction (22, 49), indicating that the physiological amount of NO released by eNOS plays fundamental roles in the maintenance of vascular tone.

[1]Department of Internal Medicine, Division of Cardiology, Nagoya University Graduate School of Medicine, Nagoya, Japan.
[2]Department of Medicine (Cardiology) and Cardiovascular Research, St. Elizabeth's Medical Center, Tufts University School of Medicine, Boston, MA.

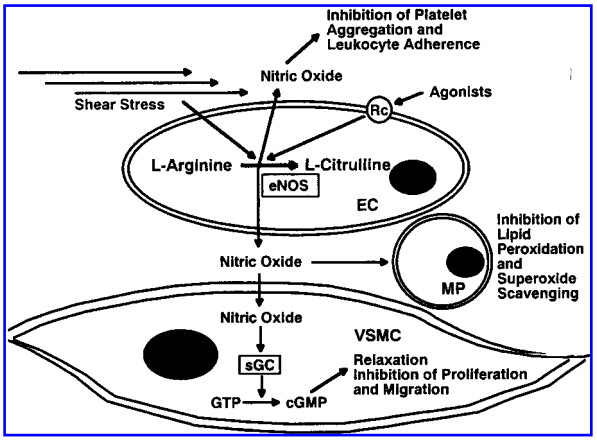

FIG. 1. NO and maintenance of vascular homeostasis. EDNO inhibits platelet aggregation, leukocyte adherence to endothelium, and VSMC proliferation and migration. NO also inhibits lipid peroxidation and foaming of macrophage (MP). Finally, NO can scavenge superoxide free radical. Disruption of the illustrated NO functions may lead to atherosclerosis. cGMP, cyclic GMP; EC, endothelial cell; Rc, receptor; sGC, soluble guanylate cyclase.

REGULATION OF POSTNATAL ANGIOGENESIS BY NO, A NEW PARADIGM IN VASCULAR BIOLOGY

Biological processes modulated by EDNO might extend to include angiogenesis, a new blood vessel formation from the preexisting vascular bed. Ziche *et al.* (61) demonstrated for the first time that NO may play a role in angiogenesis. In their study, angiogenesis mediated by substance P, a well known endothelium-dependent vasodilator, induced angiogenesis via an EDNO-dependent pathway.

More recently, vascular endothelial growth factor (VEGF), a potent endothelial cell-specific mitogen and vascular permeability factor (9), was found to induce angiogenesis via an EDNO-dependent pathway (32, 39, 61). VEGF has been shown to induce EDRF/NO-dependent vasorelaxation in isolated canine coronary arteries *in vitro* (27). Direct measurement of NO released from cultured human endothelial cells as well as isolated vascular strips confirmed that VEGF stimulates endothelial NO release *in vitro* (55). VEGF treatment resulted in both an acute (1 h) and chronic (>24 h) stimulation of NO production (21). Furthermore, VEGF elicited a dose-dependent increase in the cellular content of eNOS messenger RNA and protein that may account for the chronic up-regulation of NO production by VEGF. VEGF thus up-regulates eNOS enzyme and elicits a biphasic stimulation of endothelial NO production. Consistently, VEGF reduces systemic blood pressure via an EDNO-dependent mechanism *in vivo* (19). Interestingly, the VEGF-mediated increase in vascular permeability also depends at least in part on EDNO (33).

Currently, the precise mechanism by which VEGF stimulates NO release is unknown. Brock *et al.* (5) found that VEGF increases cytosolic Ca^{2+} in human umbilical vein endothelial cells (HUVECs), thereby activating calcium/calmodulin-dependent enzymes, including eNOS. It is not clear which of the VEGF receptors (VEGFR-1 or VEGFR-2) mediates the signal for induction of eNOS protein expression and NO release in endothelial cells. Placenta growth factor, a ligand that exclusively binds to fms-like tyrosine kinase 1 (Flt-1) but not to fetal liver kinase 1 (Flk-1), failed to stimulate NO release in human coronary microvascular endothelial cells (33). Thus, Flk-1/kinase insert domain receptor (KDR) likely plays a main role in VEGF-induced NO release in endothelial cells. Consistently, vascular endothelial growth factor-C (VEGF-C or VEGF-2), a specific ligand for the endothelial receptor tyrosine kinases VEGFR-2 and VEGFR-3, stimulated release of NO from endothelial cells (57). VEGF-C also increased vascular permeability in the Miles assay, which was attenuated by pretreatment with the NOS inhibitor L-nitro-L-arginine methyl ester (L-NAME). Recent studies also confirmed that VEGF-induced NO release depends on the VEGFR-2 on endothelial cells (20, 26).

We recently demonstrated that endogenous EDNO is a key molecule for physiologic angiogenesis that constitutes a naturally occurring, compensatory response to tissue ischemia (34). The first experiment using a rabbit ischemic hindlimb model showed that oral administration of L-arginine alone, a substrate for NOS, is sufficient to augment spontaneous angiogenesis in the setting of hindlimb ischemia. Angiography performed *in vivo* and histologic examination performed at necropsy revealed anatomic evidence of enhanced neovascularity by oral L-arginine supplementation. In the second part of our study, angiogenesis after surgically induced hindlimb ischemia was markedly impaired in mice lacking the gene for eNOS (34). Consistently, wound healing and related angiogenesis were impaired in eNOS-deficient mice (29). Although these studies clearly showed that eNOS is an important regulatory molecule for ischemia-induced angiogenesis in the postnatal period, eNOS-deficient mice are normally born, and they grow and are fertile (22, 49). Therefore, eNOS deficiency alone is not critical for vasculogenesis, the *de novo* synthesis of blood vessels from hemangioblasts, which is typically observed in the early stages of the embryonic process. Taken together, eNOS and EDNO seem to be important effector molecules for the regulation of postnatal angiogenesis.

CORONARY RISK FACTORS AND ANGIOGENESIS

Endothelial dysfunction is associated with atherosclerotic (coronary) risk factors, including hypercholesterolemia, hypertension, diabetes mellitus, and aging. In the presence of various risk factors, endothelial dysfunction occurs and release and/or production of EDNO is impaired (47). It is then conceivable that EDNO-mediated angiogenesis is impaired in the presence of risk factors. In fact, ischemia-induced angiogenesis was attenuated in various animal models of different coronary risk factors. Van Belle *et al.* (54), for example, showed that ischemia-induced angiogenesis was impaired in Watanabe heritable hyperlipidemic rabbits. In these animals, however, treatment with VEGF restored the impaired angiogenic responses, indicating that VEGF receptors

were still intact and functional. As hypercholesterolemia impairs endothelium-dependent vasorelaxation (12), the hypercholesterolemia-related impairment of angiogenesis may be explained at least in part by reduced EDNO formation. Consistently, the impaired ischemia-induced angiogenesis was rescued by oral administration of L-arginine, the substrate of NOS, in hypercholesterolemic rats (8). It was also shown that ischemia-induced angiogenesis was impaired in a mouse model of type I diabetes mellitus (45). Similarly, coronary collateral vessel formation was shown to be impaired in patients with diabetes mellitus (1). Rivard *et al.* (45) also showed that angiogenesis became suppressed with aging, which may be associated with an age-related impairment of EDNO release (52). Taken together, reduced EDNO formation may account at least in part for the impaired angiogenesis in the presence of various atherosclerotic (coronary) risk factors.

EFFECTS OF NO ON ENDOTHELIAL PROLIFERATION AND/OR MIGRATION

The precise mechanisms by which EDNO regulates angiogenesis are still unclear. However, studies documented that EDNO is important for endothelial cell proliferation and/or migration, the essential early steps requisite for neovascularization. Guo *et al.* (18) showed that CAS 1609, a froxan class of NO donor, stimulated proliferation of rat aortic endothelial cells cultured under low-serum conditions (0.5% calf serum) *in vitro*. More recently, Parenti *et al.* (40) showed that exogenous administration of sodium nitroprusside, an NO donor, significantly increased mitogen-activated protein kinase activity in capillary endothelial cells. This feature of NO may be linked to direct mitogenic actions of NO and VEGF on endothelial cells (61). However, we recently found that addition of neither L-NAME nor NOS substrate L-arginine (to upregulate endogenous EDNO) influenced bovine aortic endothelial cell proliferation *in vitro* as assessed by [^{3}H]thymidine incorporation (35). Thus, the role of EDNO in endothelial proliferation is still unclear. In contrast, L-NAME significantly inhibited endothelial migration in a modified Boyden chamber system (35). Indeed, several studies have demonstrated that EDNO is an important promoter of endothelial cell migration (36). EDNO contributed to endothelial migration by facilitating endothelial podokinesis (scalar motion), which is required for directed migration of endothelial cells. Therefore, EDNO may be necessary for a switch from stationary to migrating and/or locomoting phenotype in endothelial cells.

NO DONOR AND ANGIOGENESIS

Although the endogenous L-arginine/NO pathway is likely to play a key role as an effector system for postnatal angiogenesis, there are some conflicting reports regarding NO donors and angiogenesis. Lau and Ma (28) reported that exogenously administered NO donors inhibited migration of cultured endothelial cells. In contrast, inhibition of EDNO by L-NAME inhibited endothelial migration in culture (35). Pipili-Synetos *et al.* (41) demonstrated that nitrovasodilators, such as sodium nitroprusside, isosorbide mononitrate, and isosorbide dinitrate, inhibited angiogenesis both *in vitro* (matrigel tube formation assay) and *in vivo* (chick chorioallantoic membrane and tumor implantation models). From the results of a study in which eNOS-deficient mice were used, it became evident that endogenous eNOS activity was critical for angiogenesis in the setting of tissue ischemia *in vivo*. However, in the same experimental model, exogenously administered NO donor failed to restore the decreased angiogenesis in eNOS-knockout mice *in vivo* (34). A similar phenomenon was recently observed in a different experimental model. eNOS-deficient mice developed increased total pulmonary vascular resistance, and the resulting pulmonary hypertension was not reversed by inhaled NO (51). These studies suggest that exogenously administered NO donors may not simply substitute the function of endogenous and physiological amounts of EDNO regarding the promotion of angiogenesis.

The precise reason for the discrepancy of the efficacy between exogenous NO donors and endogenous EDNO in terms of angiogenesis is currently unknown. There are, however, several possible explanations. First, it is possible that NO donors may have different effects on the microvascular versus macrovascular endothelial cells. Therefore, experimental results may depend on what type of endothelial cells is used. Second, concentrations of NO in and around endothelial cells may be critical for the regulation of angiogenesis. Exogenously administered NO donors may often deliver uncontrollable high concentrations of NO to target tissues, eliciting direct damage and/or antiproliferative action in endothelial cells (48). Moreover, high enough concentrations of NO attenuate VEGF production in underlying VSMCs (53), whereas physiological concentrations of NO produced from eNOS are considered protective for endothelial cells (*i.e.,* antiapoptosis) (30). A recent study indeed showed that controlled delivery of small doses of NO donors inhibited endothelial apoptosis *in vivo* (46). Third, vascular structure formation requires not only endothelial proliferation and migration, but also participation of other cell types, such as pericytes. Pericytes have been reported to derive from VSMCs. There are studies showing that exogenous administration of NO donors can inhibit VSMC proliferation and migration (16) and induce smooth muscle cell apoptosis (42). Thus, one possible hypothesis is that exogenous NO donors may deliver high concentrations of NO to vascular tissue, which might inhibit VSMC-derived pericyte formation and migration, and thereby they would inhibit angiogenesis.

SIGNAL TRANSDUCTION PATHWAYS IN VEGF-INDUCED NO RELEASE AND ANGIOGENESIS

Binding of VEGF to its receptors, Flk-1 and Flt-1, causes receptor dimerization and autophosphorylation on tyrosine residues (9). Although the precise postreceptor signaling pathways underlying VEGF actions on endothelial cells are unclear, several signaling molecules that associate with VEGF receptors have been identified, such as phosphatidyl-

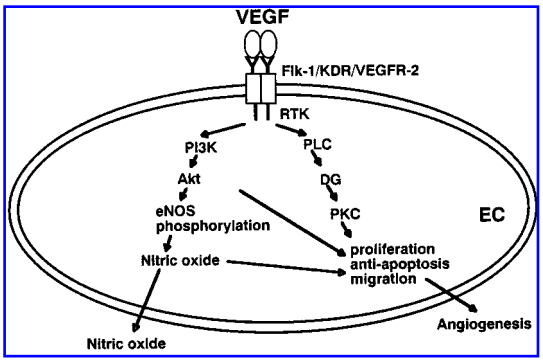

FIG. 2. VEGF-induced signal transduction, NO formation, and angiogenesis. Dimeric VEGF acts through the dimeric Flk-1/KDR/VEGFR-2 on the endothelial cell (EC) surface. Subsequently, receptor tyrosine kinase (RTK) is activated, which results in the activation of both PI3K and PLC/PKC pathways. DG, diacylglycerol.

inositol 3-kinase (PI3K) and phospholipase C (PLC) (56, 58) (Fig. 2). PLC activation leads to increased formation of diacylglycerol and inositol 1,4,5-triphosphate, increased intracellular calcium concentration, followed by activation of protein kinase C (PKC) and calcium/calmodulin-dependent kinases. Activation of the PI3K leads to generation of phosphatidylinositol species, which can activate the PKC and the serine-threonine kinase Akt (also known as protein kinase B) (11). Recently, VEGF has been shown to stimulate PI3K and Akt and thereby promotes endothelial cell survival via the Flk-1/KDR/VEGFR-2 (13, 17). This is consistent with the evidence that Akt plays a role in promoting the survival of a wide spectrum of cell types (25). Because apoptosis of endothelial cells counteracts angiogenesis, the suppression of endothelial apoptosis by the PI3K/Akt pathway may result in angiogenesis. In fact, Wortmannin, a PI3K inhibitor, has been shown to inhibit angiogenesis in the chick chorioallantoic membrane, and lipid products of PI3K increase cell migration through PKC (37).

Interestingly, PI3K inhibitors, LY294002 or Wortmannin, attenuate the release of NO in response to VEGF in human endothelial cells, suggesting that PI3K activation mediates, at least in part, the VEGF-induced release of NO (39). This is consistent with the evidence that PI3K inhibition blocks insulin- and insulin-like growth factor-1-induced NO production in HUVECs (59). Although the mechanism of PI3K-mediated NO release is unclear, recent studies have demonstrated that Akt phosphorylates eNOS leading to persistent enzymatic activation to release NO (7, 14). Taken together, VEGF-induced angiogenesis seems to be supported at least in part by PI3K/Akt-dependent eNOS activation and NO release, because EDNO is angiogenic and has an antiapoptotic effect on endothelial cells.

ENDOTHELIAL FUNCTION IN COLLATERAL VESSELS

Endothelium-dependent vasodilatation (EDNO production) is generally attenuated in the collateral circulation in ischemic lesions (4) despite increased expression of eNOS mRNA (3). The collateral vessels have regenerated proliferating endothelial cells. Although Arnal *et al.* (2) showed that eNOS mRNA is expressed more in proliferating endothelial cells than confluent cells, eNOS protein expression and NO-producing capacity did not necessarily parallel (24). In fact, the response of regenerating endothelial cells, such as in a reendothelialized vessel after balloon injury, to endothelium-dependent vasodilators is impaired (50). Taken together, these data indicate that it would be ideal to augment the physiological amount of EDNO to induce therapeutic angiogenesis in ischemic tissues.

AUGMENTATION OF ENDOGENOUS NO AS A POTENTIAL THERAPEUTIC ANGIOGENESIS FOR ISCHEMIC DISEASE

NO biosynthesis is attenuated in patients with ischemic heart disease and peripheral artery occlusive disease. Because physiological concentrations of NO produced by eNOS are important for postnatal angiogenesis, enhancement of the activity of this enzyme may become an effective therapeutic means for improving angiogenesis in patients with ischemic diseases. Previous *in vivo* studies have demonstrated that vascular function improves in response to L-arginine administration, independent of the presence or absence of L-arginine deficiency (6). Then it is conceivable that L-arginine administration induces therapeutic angiogenesis via augmenting EDNO. In fact, L-arginine has improved ischemia-induced angiogenesis in experimental animal models (8, 34). Therefore, the therapeutic potential of agents augmenting endogenous NO bioactivity (*i.e.,* NOS substrate, L-arginine, eNOS cofactor such as tetrahydrobiopterin, or eNOS gene transfer) may be considered in patients with ischemic cardiovascular diseases (Fig. 3). Parenthetically, recent studies demonstrated that statin, HMG-CoA reductase inhibitors, also augmented ischemia-induced angiogenesis in an experimental model, which was in part mediated through statin-induced activation of endothelial eNOS.

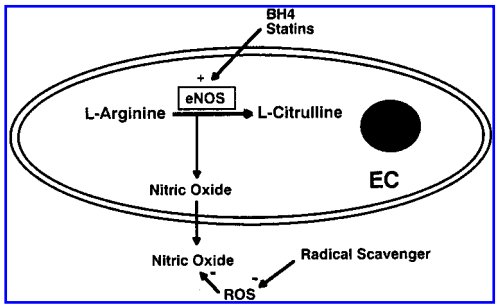

FIG. 3. Possible therapeutic strategy for neovascularization by augmenting endogenous EDNO. Supplementation of L-arginine, the substrate for eNOS, stimulates NO formation. Similarly, eNOS cofactor, tetrahydrobiopterin (BH4), will augment NO formation. Because reactive oxygen species (ROS) inactivate NO, free oxygen radical scavengers will augment endogenous NO. EC, endothelial cell.

ACKNOWLEDGMENTS

We dedicate this review article to Dr. Jeffrey M. Isner, who died on October 31, 2001. A part of the work documented in this review was supported by a grant from the Ministry of Education, Science and Culture (MESC) of Japan.

ABBREVIATIONS

EDNO, endothelium-derived nitric oxide; EDRF, endothelium-derived relaxing factor; eNOS, endothelial nitric oxide synthase; Flk-1, fetal liver kinase 1; Flt-1, fms-like tyrosine kinase 1; HUVEC, human umbilical vein endothelial cell; iNOS, inducible nitric oxide synthase; KDR, kinase insert domain receptor; L-NAME, N^G-nitro-L-arginine methyl ester; nNOS, neuronal nitric oxide synthase; NO, nitric oxide; NOS, nitric oxide synthase; PI3K, phosphatidylinositol 3-kinase; PKC, protein kinase C; PLC, phospholipase C; VEGF, vascular endothelial growth factor; VSMC, vascular smooth muscle cell.

REFERENCES

1. Abaci A, Oguzhan A, Kahraman S, Eryol NK, Unal S, Arinc H, and Ergin A. Effect of diabetes mellitus on formation of coronary collateral vessels. *Circulation* 99: 2239–2242, 1999.
2. Arnal JF, Yamin J, Dockery S, and Harrison DG. Regulation of endothelial nitric oxide synthase mRNA, protein, and activity during cell growth. *Am J Physiol* 267: C1381–C1388, 1994.
3. Arnet UA, McMillan A, Dinerman JL, Ballermann B, and Lowenstein CJ. Regulation of endothelial nitric-oxide synthase during hypoxia. *J Biol Chem* 271, 15069–15073, 1996.
4. Bauters C, Asahara T, Zheng LP, Takeshita S, Bunting S, Ferrara N, Symes JF, and Isner JM. Recovery of disturbed endothelium-dependent flow in the collateral-perfused rabbit ischemic hindlimb after administration of vascular endothelial growth factor. *Circulation* 91, 2802–2809, 1995.
5. Brock TA, Dvorak HF, and Senger DR. Tumor-secreted vascular permeability factor increases cytosolic Ca^{2+} and von Willebrand factor release in human endothelial cells. *Am J Pathol* 138, 213–221, 1991.
6. Creager MA, Gallagher SJ, Girerd XJ, Coleman SM, Dzau VJ, and Cooke JP. L-arginine improves endothelium-dependent vasodilation in hypercholesterolemic humans. *J Clin Invest* 90, 1248–1253, 1992.
7. Dimmeler S, Fleming I, Fisslthaler B, Hermann C, Busse R, and Zeiher AM. Activation of nitric oxide synthase in endothelial cells by Akt-dependent phosphorylation. *Nature* 399: 601–605, 1999.
8. Duan J, Murohara T, Ikeda H, Katoh A, Shintani S, Sasaki K, Kawata H, Yamamoto N, and Imaizumi T. Hypercholesterolemia inhibits angiogenesis in response to hindlimb ischemia: nitric oxide-dependent mechanism. *Circulation* 102: III370–III376, 2000.
9. Ferrara N, Houck K, Jakeman L, and Leung DW. Molecular and biological properties of the vascular endothelial growth factor family of proteins. *Endocr Rev* 13: 18–32, 1992.
10. Forstermann U, Closs E, Pollock JS, Nakane M, Schwarz P, Gath I, and Kleinert H. Nitric oxide synthase isoenzymes: characterization, purification, molecular cloning, and functions. *Hypertension* 23: 1121–1131, 1994.
11. Franke TF, Kaplan DR, and Cantley LC. PI3K: downstream AKTion blocks apoptosis. *Cell* 88: 435–437, 1997.
12. Freiman PC, Mitchell GG, Heistad DD, Armstrong ML, and Harrison DG. Atherosclerosis impairs endothelium-dependent vascular relaxation to acetylcholine and thrombin in primates. *Circ Res* 58: 783–789, 1986.
13. Fujio Y and Walsh K. Akt mediates cytoprotection of endothelial cells by vascular endothelial growth factor in an anchorage-dependent manner. *J Biol Chem* 274: 16349–16354, 1999.
14. Fulton D, Gratton JP, McCabe TJ, Fontana J, Fujio Y, Walsh K, Franke TF, Papapetropoulos A, and Sessa WC. Regulation of endothelium-derived nitric oxide production by the protein kinase Akt. *Nature* 399: 597–601, 1999.
15. Furchgott RF and Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* 288, 373–376, 1980.
16. Garg UC and Hassid A. Nitric oxide-generating vasodilators and 8-bromo-cyclic guanosine monophosphate inhibit mitogenesis and proliferation of cultured rat vascular smooth muscle cells. *J Clin Invest* 83, 1774–1777, 1989.
17. Gerber HP, McMurtrey A, Kowalski J, Yan M, Keyt BA, Dixit V, and Ferrara N. Vascular endothelial growth factor regulates endothelial cell survival through the phosphatidylinositol 3′-kinase/Akt signal transduction pathway. Requirement for Flk-1/KDR activation. *J Biol Chem* 273: 30336–30343, 1998.
18. Guo JP, Murohara T, Panday MM, and Lefer AM. Nitric oxide promotes endothelial cell proliferation: role in inhibiting restenosis. *Circulation* 92: I-750, 1996.
19. Hariawala M, Horowitz JR, Esakof D, Sheriff DD, Walter DH, Chaudhry GM, Desai V, Keyt B, Isner JM, and Symes JF. VEGF improves myocardial blood flow but produces EDRF-mediated hypotension in porcine hearts. *J Surg Res* 63, 77–82, 1996.
20. He H, Venema VJ, Gu X, Venema RC, Marrero MB, and Caldwell RB. Vascular endothelial growth factor signals endothelial cell production of nitric oxide and prostacyclin through Flk-1/KDR activation of c-Src. *J Biol Chem* 274: 25130–25135, 1999.
21. Hood JD, Meininger CJ, Ziche M, and Granger HJ. VEGF upregulates ecNOS message, protein, and NO production in human endothelial cells. *Am J Physiol* 274, H1054–H1058, 1998.
22. Huang PL, Huang Z, Mashimo H, Bloch KD, Moskowitz MA, Bevan JA, and Fishman MC. Hypertension in mice lacking the gene for endothelial nitric oxide synthase. *Nature* 377: 239–242, 1995.
23. Ignarro LJ. Biosynthesis and metabolism of endothelium-derived nitric oxide. *Annu Rev Pharmacol Toxicol* 30: 535–560, 1990.
24. Kanazawa K, Kawashima S, Mikami S, Miwa Y, Hirata K, Suematsu M, Hayashi Y, Itoh H, and Yokoyama M.

Endothelial constitutive nitric oxide synthase protein and mRNA increased in rabbit atherosclerotic aorta despite impaired endothelium-dependent vascular relaxation. *Am J Pathol* 148: 1949–1956, 1996.
25. Khwaja A. Akt is more than just a Bad kinase. *Nature* 401: 33–34, 1996.
26. Kroll J and Waltenberger J. VEGF-A induces expression of eNOS and iNOS in endothelial cells via VEGF receptor-2 (KDR). *Biochem Biophys Res Commun* 252: 743–746, 1998.
27. Ku DD, Zaleski JK, Liu S, and Brock TA. Vascular endothelial growth factor induces EDRF-dependent relaxation in coronary arteries. *Am J Physiol* 265: H586–H592, 1993.
28. Lau YT and Ma WC. Nitric oxide inhibits migration of cultured endothelial cells. *Biochem Biophys Res Commun* 221: 670–674, 1996.
29. Lee PC, Salyapongse AN, Bragdon GA, Shears LL, Watkins HD, Edington SC, and Billiar TR. Impaired wound healing and angiogenesis in eNOS-deficient mice. *Am J Physiol* 277: H1600–H1608, 1999.
30. Lopez-Farre A, De Miguel LS, Caramelo C, Gomez-Macias J, Garcia R, Mosquera JR, De Frutos T, Millas I, Rivas F, Echezarreta G, and Casado S. Role of nitric oxide in autocrine control of growth and apoptosis of endothelial cells. *Am J Physiol* 272: H760–H768, 1997.
31. Moncada S and Higgs A. The L-arginine–nitric oxide pathway. *N Engl J Med* 329: 2002–2012, 1993.
32. Morbidelli L, Chang CH, Douglas JG, Granger HJ, Ledda F, and Ziche M. Nitric oxide mediates mitogenic effect of VEGF on coronary venular endothelium. *Am J Physiol* 270: H411–H415, 1995.
33. Murohara T, Asahara T, Silver M, Bauters C, Masuda H, Kalka C, Kearney M, Chen D, Symes JF, Fishman MC, Huang PL, and Isner JM. Nitric oxide synthase modulates angiogenesis in response to tissue ischemia. *J Clin Invest* 101: 2567–2578, 1998.
34. Murohara T, Horowitz JR, Silver M, Tsurumi Y, Chen D, Sullivan A, and Isner JM. Vascular endothelial growth factor/vascular permeability factor enhances vascular permeability via nitric oxide and prostacyclin. *Circulation* 97, 99–107, 1998.
35. Murohara T, Witzenbichler B, Spyridopoulos I, Asahara T, Ding B, Sullivan A, Losordo DW, and Isner JM. Role of endothelial nitric oxide synthase in endothelial cell migration. *Arterioscler Thromb Vasc Biol* 19: 1156–1161, 1999.
36. Noiri E, Hu Y, Bahou WF, Keese CR, Giaever I, and Goligorsky MS. Permissive role of nitric oxide in endothelin-induced migration of endothelial cells. *J Biol Chem* 272: 1747–1752, 1997.
37. Oikawa T and Shimamura M. Potent inhibition of angiogenesis by wortmannin, a fungal metabolite. *Eur J Pharmacol* 318: 93–96, 1996.
38. Palmer RMJ, Ashton DS, and Moncada S. Vascular endothelial cells synthesize nitric oxide from L-arginine. *Nature* 333: 664–666, 1988.
39. Papapetropoulos A, Garcia-Cardena G, Madri JA, and Sessa WC. Nitric oxide production contributes to the angiogenic properties of vascular endothelial growth factor in human endothelial cells. *J Clin Invest* 100: 3131–3139, 1997.
40. Parenti A, Morbidelli L, Cui XL, Douglas JG, Hood JD, Granger HL, Ledda F, and Ziche M. Nitric oxide is an upstream signal of vascular endothelial growth factor-induced extracellular signal-regulated kinase1/2 activation in postcapillary endothelium. *J Biol Chem* 273: 4220–4226, 1998.
41. Pipili-Synetos E, Sakkoula E, Haralabopoulos G, Andriopoulou P, Peristeris P, and Maragoudakis ME. Evidence that nitric oxide is an endogenous antiangiogenic mediator. *Br J Pharmacol* 111: 894–902, 1994.
42. Pollman MJ, Yamada T, Horiuchi M, and Gibbons GH. Vasoactive substances regulate vascular smooth muscle cell apoptosis. Countervailing influences of nitric oxide and angiotensin II. *Circ Res* 79: 748–756, 1996.
43. Rapoport RM, Draznin MB, and Murad F. Endothelium-dependent relaxation in rat aorta may be mediated through cyclic GMP-dependent protein phosphorylation. *Nature* 306: 174–176, 1983.
44. Rivard A, Silver M, Chen D, Kearney M, Magner M, Annex B, Peters K, and Isner JM. Rescue of diabetes-related impairment of angiogenesis by intramuscular gene therapy with adeno-VEGF. *Am J Pathol* 154: 355–363, 1999.
45. Rivard A, Fabre JE, Silver M, Chen D, Murohara T, Kearney M, Magner M, Asahara T, and Isner JM. Age-dependent impairment of angiogenesis. *Circulation* 99: 111–120, 1999.
46. Rossig L, Fichtlscherer B, Breitschopf K, Haendeler J, Zeiher AM, Mulsch A, and Dimmeler S. Nitric oxide inhibits caspase-3 by *S*-nitrosation in vivo. *J Biol Chem* 274: 6823–6826, 1999.
47. Sessa WC. The nitric oxide synthase family of proteins. *J Vasc Res* 31: 131–143, 1994.
48. Shen YH, Wang XL, and Wilcken DE. Nitric oxide induces and inhibits apoptosis through different pathways. *FEBS Lett* 433: 125–131, 1998.
49. Shesely EG, Maeda N, Kim HS, Desai KM, Krege JH, Laubach VE, Sherman PA, Sessa WC, and Smithies O. Elevated blood pressures in mice lacking endothelial nitric oxide synthase. *Proc Natl Acad Sci U S A* 93: 13176–13181, 1996.
50. Shimokawa H, Flavahan NA, and Vanhoutte PM. Natural course of the impairment of endothelium-dependent relaxations after balloon endothelium removal in porcine coronary arteries. Possible dysfunction of a pertussis toxin-sensitive G protein. *Circ Res* 65: 740–753, 1989.
51. Steudel W, Ichinose F, Huang PL, Hurford WE, Jones RC, Bevan JA, Fishman MC, and Zapol WM. Pulmonary vasoconstriction and hypertension in mice with targeted disruption of the endothelial nitric oxide synthase (NOS 3) gene. *Circ Res* 81: 34–41, 1997.
52. Tschudi MR, Barton M, Bersinger NA, Moreau P, Cosentino F, Noll G, Malinski T, and Lucher TF. Effect of age on kinetics of nitric oxide release in rat aorta and pulmonary artery. *J Clin Invest* 98: 899–905, 1996.
53. Tsurumi Y, Murohara T, Krasinski K, Chen D, Witzenbichler B, Kearney M, Couffinhal T, and Isner JM. Reciprocal relationship between VEGF and NO in the regulation of endothelial integrity. *Nat Med* 3: 879–886, 1997.
54. Van Belle E, Rivard A, Chen D, Silver M, Bunting S, Ferrara N, Symes JF, Bauters C, and Isner JM. Hypercholes-

terolemia attenuates angiogenesis but does not preclude augmentation by angiogenic cytokines. *Circulation* 96: 2667–2674, 1997.
55. van der Zee R, Murohara T, Luo Z, Zollmann F, Passeri J, Lekutat C, and Isner JM. Vascular endothelial growth factor/vascular permeability factor augments nitric oxide release from quiescent rabbit and human vascular endothelium. *Circulation* 95: 1030–1037, 1997.
56. Waltenberger J, Claesson-Welsh L, Siegbahn A, Shibuya M, and Heldin CH. Different signal transduction properties of KDR and Flt-1, two receptors for vascular endothelial growth factor. *J Biol Chem* 269: 26988–26995, 1994.
57. Witzenbichler B, Asahara T, Murohara T, Silver M, Spyridopoulos I, Magner M, Principe N, Kearney M, Hu JS, and Isner JM. Vascular endothelial growth factor-C (VEGF-C/VEGF-2) promotes angiogenesis in the setting of tissue ischemia. *Am J Pathol* 153: 381–394, 1998.
58. Xia P, Aiello LP, Ishii H, Jiang ZY, Park DJ, Robinson GS, Takagi H, Newsome WP, Jirousek MR, and King GL. Characterization of vascular endothelial growth factor's effect on the activation of protein kinase C, its isoforms, and endothelial cell growth. *J Clin Invest* 98: 2018–2026, 1996.
59. Zeng G and Quon MJ. Insulin-stimulated production of nitric oxide is inhibited by wortmannin. Direct measurement in vascular endothelial cells. *J Clin Invest* 98: 894–898, 1996.
60. Ziche M, Morbidelli L, Masini E, Amerini S, Granger HJ, Maggi CA, Geppetti P, and Ledda F. Nitric oxide mediates angiogenesis in vivo and endothelial cell growth and migration in vitro promoted by substance P. *J Clin Invest* 94: 2036–2044, 1994.
61. Ziche M, Morbidelli L, Choudhuri R, Zhang HT, Donnini S, Granger HJ, and Bicknell R. Nitric oxide synthase lies downstream from vascular endothelial growth factor-induced but not fibroblast growth factor-induced angiogenesis. *J Clin Invest* 99: 2625–2634, 1997.

Address reprint requests to:
Toyoaki Murohara, M.D., Ph.D.
Department of Internal Medicine
Division of Cardiology
Nagoya University Graduate School of Medicine
65 Tsurumai, Showa-ku
Nagoya 466-8550, Japan

E-mail: murohara@med.nagoya-u.ac.jp

Received for publication February 20, 2002; accepted July 11, 2002.

This article has been cited by:

1. Bin Wang, Lin Sun, Ye Tian, Zhanying Li, Huijie Wei, Dong Wang, Zhuo Yang, Jieli Chen, Jianning Zhang, Rongcai Jiang. 2012. Effects of atorvastatin in the regulation of circulating EPCs and angiogenesis in traumatic brain injury in rats. *Journal of the Neurological Sciences* . [CrossRef]
2. C. G. Kevil, D. J. Lefer. 2011. Review focus on inorganic nitrite and nitrate in cardiovascular health and disease. *Cardiovascular Research* **89**:3, 489-491. [CrossRef]
3. C. B. Pattillo, S. Bir, V. Rajaram, C. G. Kevil. 2011. Inorganic nitrite and chronic tissue ischaemia: a novel therapeutic modality for peripheral vascular diseases. *Cardiovascular Research* **89**:3, 533-541. [CrossRef]
4. Monique C. de Waard, Rien van Haperen, Thomas Soullié, Dennie Tempel, Rini de Crom, Dirk J. Duncker. 2010. Beneficial effects of exercise training after myocardial infarction require full eNOS expression. *Journal of Molecular and Cellular Cardiology* **48**:6, 1041-1049. [CrossRef]
5. Hongtao Wu, Dunyue Lu, Hao Jiang, Ye Xiong, Changsheng Qu, Bo Li, Asim Mahmood, Dong Zhou, Michael Chopp. 2008. Increase in phosphorylation of Akt and its downstream signaling targets and suppression of apoptosis by simvastatin after traumatic brain injury. *Journal of Neurosurgery* **109**:4, 691-698. [CrossRef]
6. D. Kumar, B. G. Branch, C. B. Pattillo, J. Hood, S. Thoma, S. Simpson, S. Illum, N. Arora, J. H. Chidlow, W. Langston, X. Teng, D. J. Lefer, R. P. Patel, C. G. Kevil. 2008. Chronic sodium nitrite therapy augments ischemia-induced angiogenesis and arteriogenesis. *Proceedings of the National Academy of Sciences* **105**:21, 7540-7545. [CrossRef]
7. Shampa ChatterjeeReactive Oxygen Species and Angiogenesis **20072347**, 389-402. [CrossRef]
8. Dunyue Lu , Anton Goussev , Jieli Chen , Paul Pannu , Yi Li , Asim Mahmood , Michael Chopp . 2004. Atorvastatin Reduces Neurological Deficit and Increases Synaptogenesis, Angiogenesis, and Neuronal Survival in Rats Subjected to Traumatic Brain Injury. *Journal of Neurotrauma* **21**:1, 21-32. [Abstract] [Full Text PDF] [Full Text PDF with Links]
9. Nilanjana Maulik . 2002. Redox Regulation of Vascular Angiogenesis. *Antioxidants & Redox Signaling* **4**:5, 783-784. [Citation] [Full Text PDF] [Full Text PDF with Links]