Role of Endothelial Nitric Oxide Synthase in Endothelial Cell Migration

Toyoaki Murohara, Bernhard Witzenbichler, Ioakim Spyridopoulos, Takayuki Asahara, Bo Ding, Alison Sullivan, Douglas W. Losordo, Jeffrey M. Isner

Abstract—Endothelium-derived nitric oxide (NO) and its precursor L-arginine have been implied to promote angiogenesis, but little is known about the precise mechanism. The inhibition of endogenous NO formation by \(N^\text{G}\)-nitro-L-arginine methyl ester (L-NAME) (1 mmol/L) but not its inactive enantiomer D-NAME (1 mmol/L) inhibited endothelial cell sprouting from the scratched edge of the cultured bovine aortic endothelial cell monolayer. Inhibition of endogenous NO release by L-NAME was confirmed by amperometric measurement using an NO-specific electrode. In the modified Boyden chamber, L-NAME (1 mmol/L) significantly inhibited endothelial cell migration, whereas L-NAME did not affect endothelial DNA synthesis as assessed by analysis of \[^3\text{H}\]thymidine incorporation. We then examined alteration of endothelial cell adhesion molecule expression after the inhibition of NO by L-NAME in cultured human umbilical vein endothelial cells. In both normoxic and hypoxic conditions, L-NAME (1 mmol/L) inhibited surface expression of integrin \(\alpha\beta_3\), which is an important integrin facilitating endothelial cell survival and angiogenesis. However, L-NAME did not affect the expression of platelet endothelial cell adhesion molecule-1, intercellular adhesion molecule-1, vascular endothelial adhesion molecule-1, gap junction protein connexin 43, and VE-cadherin, which have been reported to potentially affect angiogenesis. In summary, inhibition of endothelial NO synthase by L-NAME attenuated endothelial cell migration but not proliferation in vitro. Furthermore, endogenous endothelium-derived NO maintains the functional expression of integrin \(\alpha\beta_3\), a mediator for endothelial migration, survival, and angiogenesis. Endothelium-derived NO, thus, may play an important role in mediating angiogenesis by supporting endothelial cell migration, at least partly, via an integrin-dependent mechanism. (Arterioscler Thromb Vasc Biol. 1999;19:1156-1161.)

Key Words: angiogenesis ■ endothelium-derived relaxing factor ■ cell adhesion molecule ■ endothelial migration

Angiogenesis is a (patho)physiological event occurring after tissue ischemia, or in tumor growth and metastasis.1 Endothelial cell proliferation and migration are critical events for angiogenesis. These processes are tightly regulated by the actions of angiogenic cytokines such as vascular endothelial growth factor/vascular permeability factor (VEGF/VPF),2 fibroblast growth factor,3 and angiopoietin-1,4 endothelial growth factor/vascular permeability factor by the actions of angiogenic cytokines such as vascular endothelial growth factor/vascular permeability factor.

Nitric oxide (NO) was first identified as an endothelium-derived relaxing factor (EDRF),5 which was originally discovered by Furchgott and Zawadzki.9 EDRF/NO not only induces vascular smooth muscle relaxation but also inhibits platelet aggregation,10 leukocyte adherence to the endothelium,11 and vascular smooth muscle cell proliferation and migration.12,13 Additionally, EDRF/NO has been recently shown to play an important role in the regulation of angiogenesis. Ziche et al14 for example, demonstrated that EDRF/NO plays an important role in angiogenesis elicited by substance P, a potent endothelium-dependent vasodilator. Guo et al15 demonstrated that the exogenous administration of an NO donor significantly stimulated endothelial proliferation in culture as assessed by incorporation of bromodeoxyuridine. Interestingly, the potent angiogenic growth factor VEGF/VPF stimulates endothelial NO production,16,17 and Morbidelli et al18 demonstrated that VEGF/VPF-induced angiogenesis indeed depends on the production of endogenous EDRF/NO. More recently, we demonstrated that spontaneous angiogenesis occurring after surgically induced hindlimb ischemia was severely impaired in mice lacking the gene for endothelial NO synthase (eNOS).19 These studies collectively support the evidence that EDRF/NO is an essential mediator for angiogenesis in vivo. However, the specific mechanisms by which endogenous NO released from endothelial cells regulates angiogenesis remains enigmatic.

Because endothelial cell proliferation and migration are early essential events for mediating angiogenesis, we inves-
tigated the role of endogenous EDHF/NO in endothelial cell proliferation and migration in vitro. Here, we demonstrate that inhibition of eNOS by the L-arginine analog N^o-nitro-L-arginine methyl ester (L-NAME) but not L-NAME significantly suppresses endothelial cell migration, and that endothelium-derived NO functions as a maintenance factor for integrin αβ3 expression, which has been shown to serve as an essential adhesive integrin for endothelial cell survival, migration, and angiogenesis.5,6,20

**Methods**

**Cell Culture**

Bovine aortic endothelial cells (BAECs) were isolated after collagenase digestion of the intimal layer of the thoracic aorta. Cells were grown in minimal essential medium (MEM) with 10% fetal bovine serum (FBS) (Life Technologies) and antibiotics. Endothelial cells were identified by their typical cobblestone appearance and factor VIII immunostaining. Human umbilical vein endothelial cells (HUVECs) were isolated from fresh umbilical cords by collagenase dissociation and grown onto 1.5% gelatin-coated plates in medium 199 (M199; Life Technologies) with 20% FBS, 100 μg/mL endo-

**Measurements of NO in Cell Culture Medium**

We examined whether L-NAME (1 mmol/L) reduces basal release of NO in cultured BAECs and HUVECs in 6-well culture plates using an NO-specific electrode (World Precision Instruments). After the regular medium was removed, cells were gently washed with Dulbecco’s phosphate-buffered saline (DPBS) (Life Technology) and antibiotics. Endothelial cells were identified by their typical cobblestone appearance and factor VIII immunostaining. Human umbilical vein endothelial cells (HUVECs) were isolated from fresh umbilical cords by collagenase dissociation and grown onto 1.5% gelatin-coated plates in medium 199 (M199; Life Technologies) with 20% FBS, 100 mg/mL endo-

**Scratch Injury Model in BAEC Monolayer**

BAECs (passages 4 to 8) were seeded onto 6-well culture plates. Once at confluence, cells were serum-starved in medium containing 0.5% FBS over night, and then scratch injury was applied using a disposable surgical scalpel. After injury, the monolayer was gently washed with DPBS, and the medium was replaced with medium containing 10% FBS. Endothelial cell sprouting from the edge of the injured monolayer was examined and photographed before and at 24 and 48 hours after scratching. Migrated endothelial cells were counted in 10 randomly selected high-power fields adjacent to the scratch injury and are expressed as cells/mm².

**Endothelial Cell Migration Assay**

In vitro endothelial cell migration assay was performed using the modified Boyden chamber system (Neuroprobe). PVP-free polycarbona
te filters with a pore size of 8 μm were coated with 0.1% gelatin for at least 6 hours at room temperature and air dried. Scattering factor, a potent stimulator of endothelial cell migration, was diluted to appropriate concentrations in M199 supplemented with 1% FBS, and 25 mL of the dilution was placed in the lower chamber of the modified Boyden apparatus. Subconfluent, early passage (2 to 6) HUVEC cultures were washed and trypsinized for the minimum time required to induce cell detachment. After the filter was placed between the lower and upper chambers, 2.5 × 10⁵ cells suspended in 50 mL of M199 containing 1% FBS were seeded in the upper component. The apparatus was then incubated for 5 hours at 37°C in a humidified incubator with 5% CO₂. After the incubation period, the filter was removed, and the upper side of the filter, with the cells that did not migrate, was scraped off with a rubber cell lifter. The filters were then fixed with methanol and stained with a Giemsa solution.

Cell migration was quantified by counting cells of 3 random microscopic fields (×100) in each well, and all experiments were performed in triplicate and expressed as the percentages of number of total cells counted per well.

**Exposure of Cultured Endothelial Cells to Hypoxia**

To achieve hypoxia, a gas mixture (95% N₂/5% CO₂) was infused into an air chamber (Billups-Rothenburg) according to previously described methods. After HUVECs were treated with 4 different conditions (normoxia and hypoxia with or without L-NAME), the medium was removed, and cells were washed with DPBS and incubated with DPBS with 1 mmol/L EDTA (pH 7.4) for 20 minutes at 37°C to induce cell detachment. Cells were collected by gently pipetting into plastic tubes (Falcon). After centrifugation, cell pellets were suspended in DPBS with 10% FBS. Aliquots of cells were then stained with primary monoclonal antibodies (MAbs) directed against integrin αβ3 (kindly provided by D. Cheresh at the Scripps Institute), platelet endothelial cell adhesion molecule-1 (PECAM-1), vascular endothelial adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), connexin 43, or VE-cadherin (Table 1). These molecules were selected on the basis of potential interest for endothelial cell-to-cell interactions that may possibly regulate angiogenesis or endothelial cell migration. After staining, these cells

**Flow Cytometric Analysis of Endothelial Cell Adhesion Molecule Expression**

After HUVECs were treated with 4 different conditions (normoxia and hypoxia with or without L-NAME), the medium was removed, and cells were washed with DPBS and incubated with DPBS with 1 mmol/L EDTA (pH 7.4) for 20 minutes at 37°C to induce cell detachment. Cells were collected by gently pipetting into plastic tubes (Falcon). After centrifugation, cell pellets were suspended in DPBS with 10% FBS. Aliquots of cells were then stained with primary monoclonal antibodies (MAbs) directed against integrin αβ3 (kindly provided by D. Cheresh at the Scripps Institute), platelet endothelial cell adhesion molecule-1 (PECAM-1), vascular endothelial adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), connexin 43, or VE-cadherin (Table 1). These molecules were selected on the basis of potential interest for endothelial cell-to-cell interactions that may possibly regulate angiogenesis or endothelial cell migration. After staining, these cells

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**TABLE 1. Monoclonal Antibodies Used in the Study of FACS Analysis**

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Clone or Catalog</th>
<th>Source</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Integrin αβ3</td>
<td>LM609</td>
<td>Scripps Research Institute</td>
<td>Mouse IgG</td>
</tr>
<tr>
<td>PECAM-1</td>
<td>5.6E</td>
<td>Biodesign</td>
<td>Mouse IgG-FITC</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>BBIG-I1</td>
<td>R&amp;D System</td>
<td>Mouse IgG-FITC</td>
</tr>
<tr>
<td>VCAM-1</td>
<td>BBIG-V3</td>
<td>R&amp;D System</td>
<td>Mouse IgG-FITC</td>
</tr>
<tr>
<td>Connexin 43</td>
<td>MAB3068</td>
<td>Chemicon International</td>
<td>Mouse IgG</td>
</tr>
<tr>
<td>VE-cadherin</td>
<td>MAB1989</td>
<td>Chemicon International</td>
<td>Mouse IgG</td>
</tr>
</tbody>
</table>

The monoclonal anti-αβ3 antibody LM609 was kindly provided by Dr D.A. Cheresh at The Scripps Institute (San Diego, CA). The primary anti-ICAM-1, anti-VCAM-1, and anti-PECAM-1 MAbs were conjugated with FITC, thus FITC-conjugated mouse IgG (Sigma) was used as a control. The anti-integrin αβ3, anti-connexin 43, and anti-VE-cadherin MAbs were mouse IgG with phycocyanin fluorescence; thus these molecules were further stained with the secondary polyclonal anti-mouse IgG conjugated with phycoerythrin (Biosource). In the latter case, mouse IgG was used as a control for primary MAbs followed by staining with anti-mouse IgG-phycoerythrin conjugate (see also Methods).
were fixed with 4% paraformaldehyde in PBS (pH 7.5) and analyzed by flow cytometry (FACScan; Beckton-Dickinson).

**Western Blot Analysis**

Endothelial integrin β3 protein levels were examined by Western blot analysis. After treatment with various agents and conditions, cells were washed and lysed with lysis buffer (10 mmol/L Tris-HCl, pH 7.5, with 1% NP-40, 0.1% sodium deoxycholate) containing proteinase inhibitors (Complete, Boehringer Mannheim), followed by centrifugation at 15,000g for 15 minutes at 4°C. Supernatants were separated and used as whole cell extracts. Total protein concentrations were determined using bovine serum albumin as a standard (Protein Assay ESL, Boehringer Mannheim). Samples (100 mg protein) were separated on 12% denaturing SDS-PAGE, and transferred to a PVDF membrane (Bio-Rad). The membranes were incubated with a 1:200 dilution of a mouse anti-arginine. Rates of DNA synthesis were determined by incorporation of [3H]thymidine (Dupont-NEN) during the last 16 hours of pulse exposure, and amount of [3H]thymidine incorporated into DNA was determined using a liquid scintillation counter (Beckman). Data are expressed as ΔNO (picomoles/mg protein) increased or decreased (−) from the baseline values (before addition of l-NAME or d-NAME). **P<0.01 vs baseline values of each study.

**Statistical Analysis**

All results are expressed as mean±standard error. Statistical significance was evaluated using unpaired Student’s t test for comparisons between 2 groups. The multiple comparison among >3 groups was performed with the use of ANOVA. When a significant difference was detected, multiple-comparison analysis was performed using Fisher’s analysis. P<0.05 was considered to denote statistical significance.

**Results**

**Diminished Basal Levels of Nitric Oxide After Treatment With l-NAME in Cultured BAEC and HUVEC**

We examined whether l-NAME indeed inhibits basal release of NO in cultured BAECs and HUVECs. Amperometric measurement of NO revealed that basal levels of NO rapidly decreased after incubation with l-NAME (1 mmol/L) but not with d-NAME in both cell types (Table 2).

**1-NAME Inhibits Endothelial Cell Sprouting in a Scratch Injury Model of Endothelial Cell Monolayer**

After serum starvation, confluent BAEC monolayer was given a scratch injury by a cell lifter, and endothelial cell sprouts were then induced in either regular medium (MEM with 10% FBS), medium containing l-NAME (1 mmol/L) or medium containing d-NAME (1 mmol/L). l-NAME significantly inhibited endothelial cell sprouting examined at 24 or 48 hours after applying the scratch injury. In contrast, d-NAME did not affect endothelial cell sprouting (Figure 1A and 1B).

**Assay of Endothelial Cell Migration in Experiments Using a Modified Boyden Chamber**

In experiments using a modified Boyden chamber, HUVEC migration in response to scatter factor (0.1 to 1000 ng/mL), a potent endothelial stimulant, was significantly impaired in the presence of l-NAME (1 mmol/L) as compared with untreated control cells (Figure 1C).

**Endothelial DNA Synthesis in Response to Serum Stimulation: Effects of l-NAME**

Using [3H]thymidine incorporation assay, neither l-NAME (1 mmol/L) nor d-NAME (1 mmol/L) significantly altered [3H]thymidine incorporation into BAEC stimulated with medium containing 10% FBS. In addition, l-arginine

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**Table 2. Inhibition of Nitric Oxide Release From Cultured Endothelial Cells by l-NAME**

<table>
<thead>
<tr>
<th></th>
<th>l-NAME</th>
<th>d-NAME</th>
</tr>
</thead>
<tbody>
<tr>
<td>HUVEC</td>
<td>32±5*</td>
<td>2±2</td>
</tr>
<tr>
<td>BAEC</td>
<td>48±6**</td>
<td>3±4</td>
</tr>
</tbody>
</table>

*Data are expressed as ΔNO (picomoles/mg protein) increased or decreased (−) from the baseline values (before addition of l-NAME or d-NAME). **P<0.01 vs baseline values of each study.*

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**Figure 1. A. Study in scratch injury model using cultured BAEC monolayer demonstrates impaired endothelial cell sprouting from the injured edge in l-NAME (1 mmol/L)-treated cells but not in d-NAME-treated cells. B. Quantitative analysis revealed that number of sprouting endothelial cells was significantly lower in l-NAME-treated group than those of control untreated or d-NAME-treated groups. C. In modified Boyden chamber experiments, l-NAME significantly attenuated endothelial cell migration in response to scatter factor compared with untreated control endothelial cells. D. [3H]thymidine incorporation analysis revealed no significant changes in DNA synthesis among treatments with l-NAME, d-NAME, or l-NAME+l-arginine compared with 10% FBS-treated cells.**
Figure 2. A, In 4 different conditions in HUVEC (normoxia for 24 hours, hypoxia for 24 hours, normoxia+L-NAME, and hypoxia+L-NAME), the surface expressions of integrin αβ3, PECAM-1, ICAM-1, VCAM-1, VE-cadherin, and gap junction protein connexin 43 were analyzed using FACS analysis. All monoclonal antibodies used are listed in Table 1. Among the 6 molecules examined, only integrin αβ3 expression was inhibited by eNOS inhibition with L-NAME (1 mmol/L). B, Magnified view of the integrin αβ3 expression analyzed by flow cytometry. C, Western blot analysis in HUVECs shows no change in the protein levels of integrin β3 after treatment with L-NAME with (right) or without (left) serum stimulation.

(1 mmol/L) supplementation did not alter thymidine incorporation in the presence of L-NAME in BAECs (Figure 1D).

Flow Cytometric Analysis of Expression of Angiogenesis-Related Endothelial Cell Adhesion Molecules

Because endothelial cell migration may require interactions between cell adhesion molecules located on endothelial surface, we investigated the effects of NO inhibition by L-NAME on expression of several cell adhesion molecules in both normoxic and hypoxic conditions in HUVECs. Fluorescence-activated cell sorter (FACS) analysis revealed that inhibition of endogenous NO synthesis by L-NAME (1 mmol/L) inhibited endothelial expression of integrin αβ3 in both normoxic and hypoxic conditions (Figure 2B). In contrast, L-NAME did not alter the degree of expression of other potentially angiogenesis-related cell adhesion molecules such as PECAM-1, VCAM-1, ICAM-1, connexin 43, or VE-cadherin (Figure 2A).

Western Blot Analysis of Integrin β3 Protein Expression in Endothelial Cells After Inhibition of NO Synthase

Because the antibody (LM609) that we used to detect αβ by FACS analysis recognizes only αβ complex, we decided to examine whether inhibition of NO synthesis reduced protein level of one of the integrin subunits. We examined the effects of L-NAME (1 mmol/L) on β3 protein levels by Western blot analysis. Although L-NAME inhibited αβ3 expression on endothelial surface (by FACS analysis) as shown above, L-NAME did not alter the total protein levels of β3 subunit. Thus, endogenous NO may mediate assembly of αα and β3 on endothelial surface or maintain αα protein level (Figure 2C).

Discussion

Recent studies demonstrated that endogenous endothelium-derived NO plays an important role in angiogenesis in vitro and in vivo. The present study demonstrates that endothelium-derived NO (endogenous NO) contributes to endothelial cell migration in vitro, which is at least in part because of NO-mediated maintenance of integrin αβ3 expression, a critical regulator for endothelial cell migration, survival, and angiogenesis.

Angiogenesis consists of endothelial proliferation and migration, remodeling of extracellular matrix, and tubular structure formation. These processes are tightly regulated by actions of angiogenic cytokines such as VEGF/VPF, fibroblast growth factor, and angiopoietin-1. Angiogenesis also requires endothelial cell-to-cell, and cell-to-matrix interactions, which are mediated by various cell adhesion molecules. VEGF/VPF is a potent endothelial cell-specific mitogen and thus elicits angiogenesis. VEGF/VPF also has been shown to stimulate endothelial NO production. Recent studies have shown that eNOS lies downstream of VEGF/VPF. Similarly, eNOS plays an important role in angiogenesis mediated by substance P, a potent endothelium-dependent vasodilator NO releaser. In contrast, Pipilisynetos et al demonstrated that an NO donor, isosorbide nitrate, inhibited angiogenesis in the chick chorioallantoic membrane. Thus, there are controversial results about the regulatory role of NO in angiogenesis. Importantly, the experimental models used by Morbidelli et al and Ziche et al or by Pipilisynetos et al are the rabbit corneal assay or the chick chorioallantoic membrane assay, which may not mimic ischemia-induced angiogenesis in vivo. We recently found that eNOS is a critical mediator of in vivo angiogenesis in response to tissue ischemia using 2 animal models. First, oral l-arginine, an NO precursor, significantly enhanced angiogenesis after severe hindlimb ischemia in rabbits. Second, spontaneous angiogenesis after surgical induction of hindlimb ischemia was severely impaired in mice lacking the gene for eNOS. These studies provide evidence that eNOS is a critical molecule for angiogenesis in vitro and in vivo. The present study demonstrates that eNOS-derived NO facilitates endothelial cell migration. In the scratch injury model of the endothelial monolayer, endothelial cell sprouting is significantly attenuated by L-NAME but not by its inactive enantiomer D-NAME. We confirmed that there was diminished NO in the cell culture media after the L-NAME but not...
d-NAME treatment using the NO-specific electrode (Table 1). Because the scratch injury assay cannot distinguish which process (proliferation or migration or both) is inhibited, we further analyzed the effects of l-NAME on endothelial DNA synthesis, a marker of endothelial cell proliferation, using [3H]thymidine incorporation in similar culture conditions as performed in the scratch model. However, neither l-NAME, d-NAME, nor l-NAME+l-arginine treatments inhibited endothelial DNA synthesis in response to serum stimulation as compared with untreated cells. Thus, our results suggest that eNOS-derived endogenous NO is likely more critical for endothelial migration than for proliferation. Guo et al. recently showed that a novel froxan class NO donor, CAS-1609, stimulated rat aortic endothelial cell proliferation as assessed by bromodeoxyuridine incorporation. However, their study used cell culture medium containing 1% FBS, which is lower than the concentrations of FBS (10%) used in our present study. Therefore, in lower serum conditions, exogenously administered NO donor may be more important for endothelial proliferation. Alternatively, NO may function as a survival factor for endothelial cells in a low serum condition. In this regard, we recently demonstrated that VEGF/VPF can prevent endothelial apoptosis induced by growth factor withdrawal.29 It is important to know whether inhibition of endothelial cell apoptosis by VEGF/VPF is mediated by VEGF-stimulated eNOS-derived NO.

We further examined how NO derived from eNOS facilitates endothelial cell migration. Recently, Brooks and coworkers5,6 and Stromblad and associates29 reported that integrin αβ3 on the endothelial cells is a critical mediator for angiogenesis, and functions as an endothelial survival factor. In addition, there are multiple reports suggesting that endothelial cell adhesion molecules participate in angiogenesis.7,30 Thus, using FACS analysis, we examined the effects of eNOS inhibition by l-NAME on the expression of several cell adhesion molecules such as integrin αβ3, immunoglobulin gene superfamily (PECAM-1, VCAM-1, ICAM-1), gap junction protein connexin 43, and VE-cadherin, which are all potentially related to the regulation of endothelial cell-to-cell interactions and angiogenesis. Interestingly, l-NAME downregulated integrin αβ3 expression on the endothelial cell both in normoxic and hypoxic conditions as examined by binding of the MAb LM609, which recognizes only functional αβ3. However, l-NAME did not alter the expression of other cell adhesion molecules examined. In particular, l-NAME did not enhance the expression of VCAM-1 in our present study. This result is somewhat different from those reported by DeCaterina and coworkers,31 who showed that a different NO synthase inhibitor, N-monomethyl-l-arginine (l-NMMA), increased VCAM-1 mRNA expression in human saphenous vein endothelial cells. In contrast, Khan and coworkers32 showed that l-NMMA alone did not enhance VCAM-1 mRNA expression in human dermal microvascular endothelial cells but l-NMMA greatly enhanced the cyto-kine-induced VCAM-1 mRNA expression. Thus, our result is consistent with those reported by Khan et al.32 The reason for discrepancies observed in these studies is currently unknown, and further study will be required.

Okada and coworkers33 recently demonstrated that focal cerebral ischemia enhanced integrin αβ3 expression in endothelial cells. Because ischemia is one of the major stimuli for inducing angiogenesis in vivo, the enhanced expression of αβ3 could in part account for hypoxia-stimulated angiogenesis. In the present study, hypoxia mildly upregulated integrin αβ3 expression in HUVECs. Furthermore, inhibition of NO synthase by l-NAME inhibited integrin αβ3 expression in both normoxic and hypoxic conditions. Interestingly, αβ3 expression after NO synthase inhibition was still higher in hypoxia than normoxia, suggesting that both NO and hypoxia are independent modulators for αβ3 expression in endothelial cells. We further analyzed whether the decrease in integrin αβ3 expression is because of a decrease in the protein levels of the integrin subunit in endothelial cells. We examined the effects of l-NAME on β3 integrin protein levels by Western blot analysis. However, there was no difference in β3 integrin protein abundance in HUVECs after treatment with l-NAME. Because LM609 reacts only with functional αβ3, these results suggest that endogenous NO may function as a maintenance factor for assembly of αv and β3 subunits, which facilitate endothelial cell migration.

In conclusion, the present study further supports the notion that eNOS is essential for angiogenesis; specifically, endothelial-derived NO maintains integrin αβ3 expression and promotes endothelial cell migration. In this context, we recently demonstrated that oral supplementation of l-arginine, the precursor of NO, enhances angiogenesis in response to limb ischemia in rabbits.19 Because endogenous NO production is impaired in patients with peripheral artery occlusive disease,34 it may be useful to further evaluate the therapeutic potential of NO-generating agents (eg, NO donors, l-arginine,35 or eNOS cofactors such as tetrahydrobiopterin) in patients with apparently defective angiogenesis.

Acknowledgment

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References


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