

# Spatial Heterogeneity in VEGF-induced Vasodilation: VEGF Dilates Microvessels but Not Epicardial and Systemic Arteries and Veins

Roger J. Laham, MD, Jian Li, MD, PhD, Motohisa Tofukuji, MD, PhD, Mark Post, MD, PhD, Michael Simons, MD, and Frank W. Sellke, MD, Boston, Massachusetts

This study was designed to investigate the site of vascular endothelial growth factor (VEGF)-induced vasodilation in the systemic and coronary vasculature. Intracoronary infusion of VEGF in Yorkshire pigs resulted in a significant drop in the mean arterial blood pressure, with a decline in the left ventricular left end-diastolic pressure, and no change in the heart rate. Coronary blood flow increase after intracoronary infusion of 10  $\mu\text{g}$  VEGF ( $2.63 \pm 0.49\times$ ) was comparable to that seen after 40  $\mu\text{g}$  of intracoronary adenosine ( $2.5 \pm 0.53\times$ ,  $p = 0.67$ ) and was significantly higher than after 200  $\mu\text{g}$  of intracoronary nitroglycerine ( $1.9 \pm 0.12\times$ ,  $p = 0.0005$ ). At the same time, intracoronary VEGF did not result in a significant increase in coronary cross-sectional area determined using intravascular ultrasound. In vitro, VEGF produced dose-dependent relaxation of myocardial and systemic arterioles and venules (arterioles: 60-100  $\mu\text{m}$  and venules: 120-200  $\mu\text{m}$  in internal diameter) that was partially inhibited by L-NNA, but had no effect on epicardial coronary arteries, systemic arteries, or veins. Both VEGF receptors (*flt-1* and *flk-1*) were identified on endothelial cells of epicardial arteries and veins. We conclude that this spatial heterogeneity of VEGF vasomotor effects cannot be explained by the absence VEGF receptors and suggests differential patterns of signal transduction in the vascular tree.

## INTRODUCTION

Vascular endothelial growth factor/vascular permeability factor (VEGF/VPF) is one of the most commonly studied heparin-binding growth factors, particularly with regard to its role in physiologic

and pathologic angiogenesis, as well as its therapeutic potential for promoting functionally significant angiogenesis.<sup>1-5</sup> As with other angiogenic factors, several animal studies have shown that VEGF administration induces functionally significant angiogenesis in myocardial ischemia<sup>2,3,6,7</sup> and limb ischemia.<sup>8-10</sup>

The promising results of these preclinical studies rapidly lead to the investigation of VEGF clinically for therapeutic angiogenesis.<sup>11,12</sup> The therapeutic use of VEGF, however, has been limited by its hypotensive effect observed in animals<sup>13,14</sup> and in clinical studies.<sup>12</sup> These effects have been attributed to profound vasodilation due to effects of VEGF on nitric oxide (NO) release<sup>13,15-17</sup> and vascular permeability.<sup>17</sup>

Angiogenesis Research Center, Department of Medicine and Surgery, Harvard Medical School and Beth Israel Deaconess Medical Center, Boston, MA.

Correspondence to: R.J. Laham, MD, Angiogenesis Research Center, Interventional Cardiology Section, BIDMC/Harvard Medical School, 330 Brookline Avenue, Boston, MA 02215, USA, E-mail: rlaham@bidmc.harvard.edu

*Ann Vasc Surg* 2003; 17: 245-252  
DOI: 10.1007/s10016-001-0299-x  
© Annals of Vascular Surgery Inc.  
Published online: 22 April 2003

We have previously reported that, in Yorkshire pigs, intracoronary VEGF produced a dose-dependent increase in coronary blood flow and systemic hypotension, effects partially inhibited by pretreatment with  $N^G$ -nitro-L-arginine (L-NNA).<sup>13</sup> The underlying mechanisms of VEGF-induced vasodilation and its site of action in the vascular tree are not well defined. To this end, we set out to examine in vivo and in vitro the components of the coronary and systemic vasculature that are responsible for VEGF hemodynamic effects.

## MATERIALS AND METHODS

### In Vivo Studies

Six Yorkshire pigs of either sex weighing 40-45 kg were anesthetized with intramuscular ketamine (10 mg/kg) and isoflurane inhalation anesthesia. A right femoral cut down was performed and an 8 French sheath was inserted, through which an 8 French JR4 guiding catheter (Cordis, Miami, FL) was introduced and was used to engage the left main coronary artery. Under angiographic guidance, a 0.014-inch Doppler Flow Wire (Cardiometrics, Mountain View, CA) was advanced to the left anterior descending artery (LAD). A 3.2 French 30 MHz intravascular ultrasound (IVUS) catheter (CVIS, SCIMED Life Systems, Maple Grove, MN) was delivered over the Doppler flow wire and positioned in the mid-LAD allowing simultaneous measurement of the LAD cross-sectional area (CSA) and average spectral peak velocity<sup>18</sup> (APV) after the bolus intracoronary administration of 10  $\mu$ g of recombinant human VEGF (rhVEGF; Genentech, San Francisco, CA), adenosine (40  $\mu$ g), and nitroglycerine (200  $\mu$ g). Animals were treated according to National Institute of Health guidelines and the protocol was approved by the Institutional Animal Care and Utilization Committee of the Beth Israel Deaconess Medical Center.

### In Vitro Studies

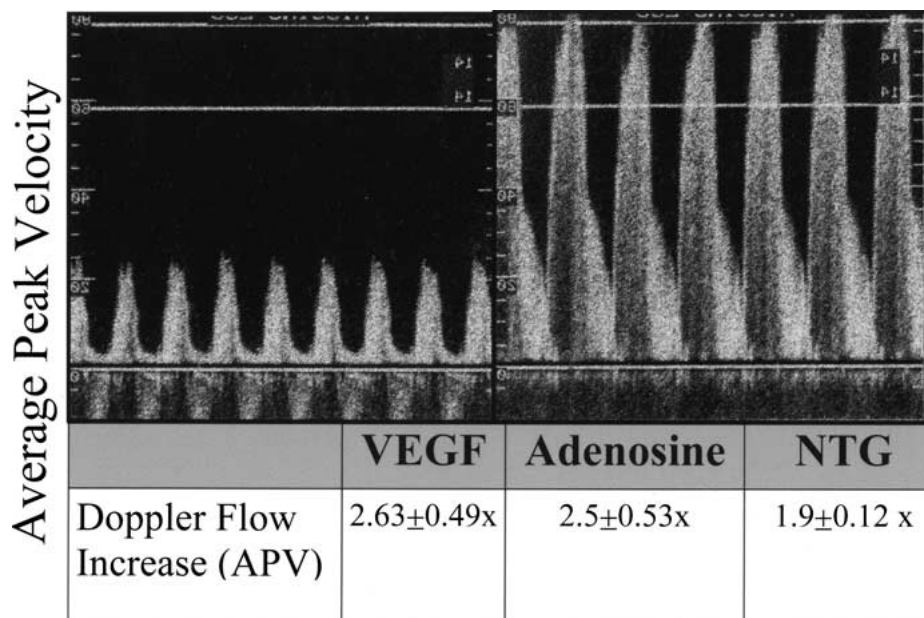
**Microvessel analysis.** Myocardial microvessels (arterioles: 60-100  $\mu$ m and venules: 120-200  $\mu$ m in internal diameter) and systemic arterioles were dissected from the myocardium and quadriceps muscle, respectively. Microvessels were placed in an isolated plexiglass chamber, cannulated with dual glass micropipettes measuring 30-80  $\mu$ m in diameter, and secured with 10-0 nylon monofilament suture (Ethicon, Somerville, NJ). Oxygenated (95% O<sub>2</sub>, 5% CO<sub>2</sub>) Krebs buffer solution warmed to 37°C was continuously circulated through the organ chamber using a reservoir containing 100 mL.

The vessels were pressurized to 40 mmHg in a no-flow state using a burette manometer filled with Krebs buffer solution.<sup>15,16</sup> Using an inverted microscope (40-200 $\times$ , Olympus, Japan) connected to a video camera, the vessel image was projected onto a video monitor. An electronic dimension analyzer (Living System Instrumentation, Burlington, VT) was used to measure internal lumen diameter.<sup>15,16</sup> Measurements were recorded with a stripchart recorder. Vessels were allowed to equilibrate for 30 min in Krebs buffer solution before an intervention and for 15 min between applications of each drug. Microvessels were precontracted by 30-60% of baseline with the thromboxane analogue U46619 (10<sup>-9</sup>-10<sup>-6</sup> M). Microvascular responses to rhVEGF and adenosine diphosphate (ADP, an endothelium-dependent vasodilator) were measured, both before and after pretreatment with 3  $\mu$ M L-NNA.

**Epicardial and systemic arteries and veins.** The epicardial coronary and femoral arteries and veins were carefully dissected and immediately placed in 37°C modified Krebs solution (oxygenated 95% O<sub>2</sub>, 5% CO<sub>2</sub>). Circular strips approximately 2 mm in width were fastened at one end. The other end was attached to an isometric force transducer (Kent TRN 002), using a 7-0 nylon monofilament suture (Ethicon, Somerville, NJ). Temperature was maintained at 37  $\pm$  0.2°C. Experiments were conducted at  $I_{max}$ , which was determined to be 1.5 times the slack length. Strips were precontracted with 10<sup>-8</sup> M U46619. Upon the development of a maximal contraction, strips were treated with rhVEGF (10<sup>-14</sup>-10<sup>-3</sup> M). Relaxation to 10<sup>-8</sup>-10<sup>-4</sup> M ADP was performed before and after the addition of rhVEGF to confirm viability and intact endothelial function.

### Immunocytochemical Analysis

Porcine epicardial coronary arteries and veins were carefully dissected and fixed in 2% paraformaldehyde at room temperature for 30 min and then in 30% sucrose overnight at 4°C. Specimens were then frozen and stored at -80°C. Sections cut 5  $\mu$ m thick were mounted on glass slides. The slides were washed with phosphate-buffered saline (PBS) and incubated in 5% normal goat serum for 20 min to reduce background staining. Affinity-purified rabbit polyclonal anti-*flt-1* and anti-*flk-1* (Biotechnology, Inc., Santa Cruz, CA) antibodies were applied overnight at 4°C in a humidity chamber at a 1:50 dilution. The slides were washed in PBS  $\times$  3 and a biotinylated anti-rabbit antibody (Vector



**Fig. 1.** Representative Doppler flow mapping at baseline (*left*) and after drug administration (*right*) showed similar increase in average peak flow velocity (APV) with VEGF and adenosine, with much lower increase in APV with nitroglycerine. Data presented as mean  $\pm$  standard deviation.

Laboratories, New Castle, UK) was applied for 30 min at 37°C. The slides were rinsed in PBS  $\times$ 3, followed by 30-min incubation with Avidin-DH-biotinylated alkaline phosphatase H (Vector Laboratories). Following washing in PBS  $\times$ 3, the alkaline phosphatase substrate Vector Red) was applied (Vector Laboratories) for 30 min. The slides were then rinsed in water, lightly counterstained with methyl green, and mounted for microscopic analysis.

### Statistical Analysis

Data are expressed as mean  $\pm$  standard deviation. Continuous variables were compared by unpaired Student's *t*-test and ANOVA, while categorical variables were compared by chi-square analysis. Vascular responses were examined with two-factor ANOVA. All reported *p*-values were two-tailed, and a *p*-value  $\leq 0.05$  was considered statistically significant.

## RESULTS

### In Vivo Studies

Six Yorkshire pigs were studied in this portion of the investigation. Systemic blood pressure, heart rate, left ventricular end-diastolic pressure, coronary flow velocity (APV), and LAD CSA were recorded at baseline and after the intracoronary administration of VEGF (10  $\mu$ g), adenosine (40  $\mu$ g), and nitroglycerine (200  $\mu$ g). VEGF infusion resulted in a significant drop in the mean arterial blood pressure from  $79 \pm 15$  mmHg to  $61 \pm 14$  mmHg ( $p = 0.05$ ), with a slight decline in the left ventricular left end-diastolic

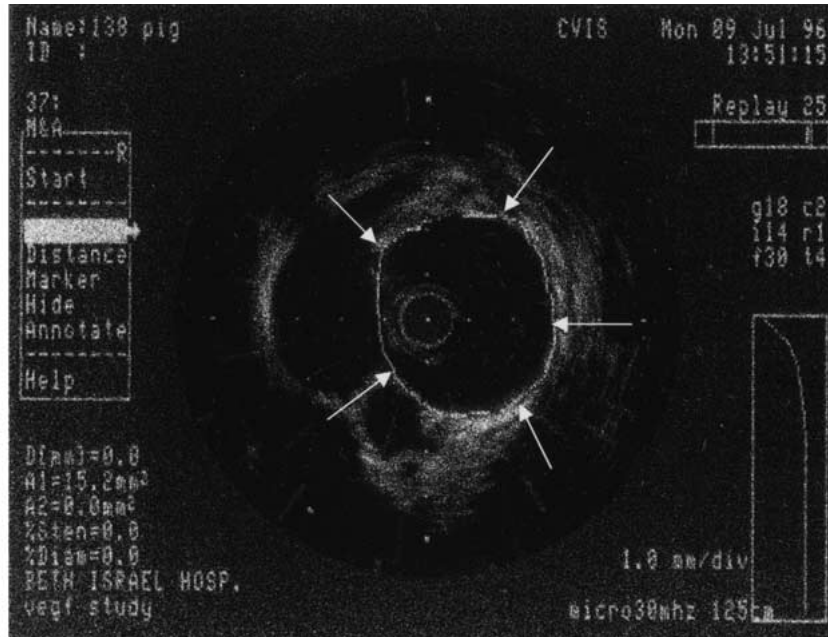
pressure ( $8.8 \pm 2.7$  mmHg to  $5.6 \pm 3.2$  mmHg,  $p = 0.09$ ) and no change in the heart rate ( $90 \pm 19$  to  $92 \pm 24$  beats/min,  $p = 0.88$ ).

The effect of VEGF administration on coronary blood flow was measured using Doppler flow mapping (Fig. 1). Following the intracoronary (left main coronary artery) administration of 10  $\mu$ g of VEGF, Doppler-measured APV increased by  $2.63 \pm 0.49\times$  compared to  $2.5 \pm 0.53\times$  after intracoronary adenosine ( $p = 0.67$ ) and  $1.9 \pm 0.12\times$  after intracoronary nitroglycerine ( $p = 0.005$ ). VEGF's effect on coronary blood flow and systemic pressure was partially inhibited by pretreatment with 100  $\mu$ g of L-NNA, indicating that these effects are, in part, NO mediated.

To investigate the *in vivo* effects of VEGF, adenosine, and nitroglycerine on the epicardial coronary arteries, IVUS was performed in conjunction with Doppler flow measurements. On-line coronary CSA (Fig. 2) was measured 15 and 30 sec after intracoronary administration and compared to baseline CSA. Baseline LAD CSA was  $11.8 \pm 0.5$  cm<sup>2</sup>. Intracoronary VEGF resulted in a nonsignificant increase in CSA to  $12.3 \pm 0.8$  ( $p = 0.2$ ) compared with a much more pronounced CSA increase after nitroglycerine ( $15.5 \pm 0.8$ ,  $p < 0.005$ ). LAD CSA failed to increase after intracoronary adenosine ( $12.4 \pm 0.7$ ,  $p = 0.1$ ).

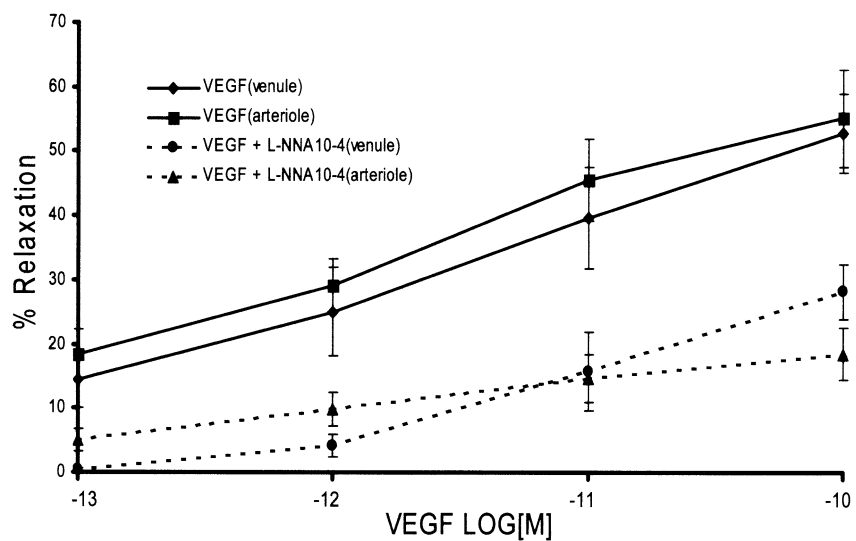
### In Vitro Studies

To localize the site of VEGF vascular effects, six additional Yorkshire pigs were utilized. Microvessels (arterioles: 60-100  $\mu$ m and venules: 120-200  $\mu$ m in



**Fig. 2.** Representative intravascular ultrasound (IVUS). The coronary lumen area (CSA) was measured on line (arrows) at baseline and after the administration of VEGF and nitroglycerine (NTG). VEGF administration did not result in any significant epicardial dilation compared to baseline. Data presented as mean  $\pm$  standard deviation.

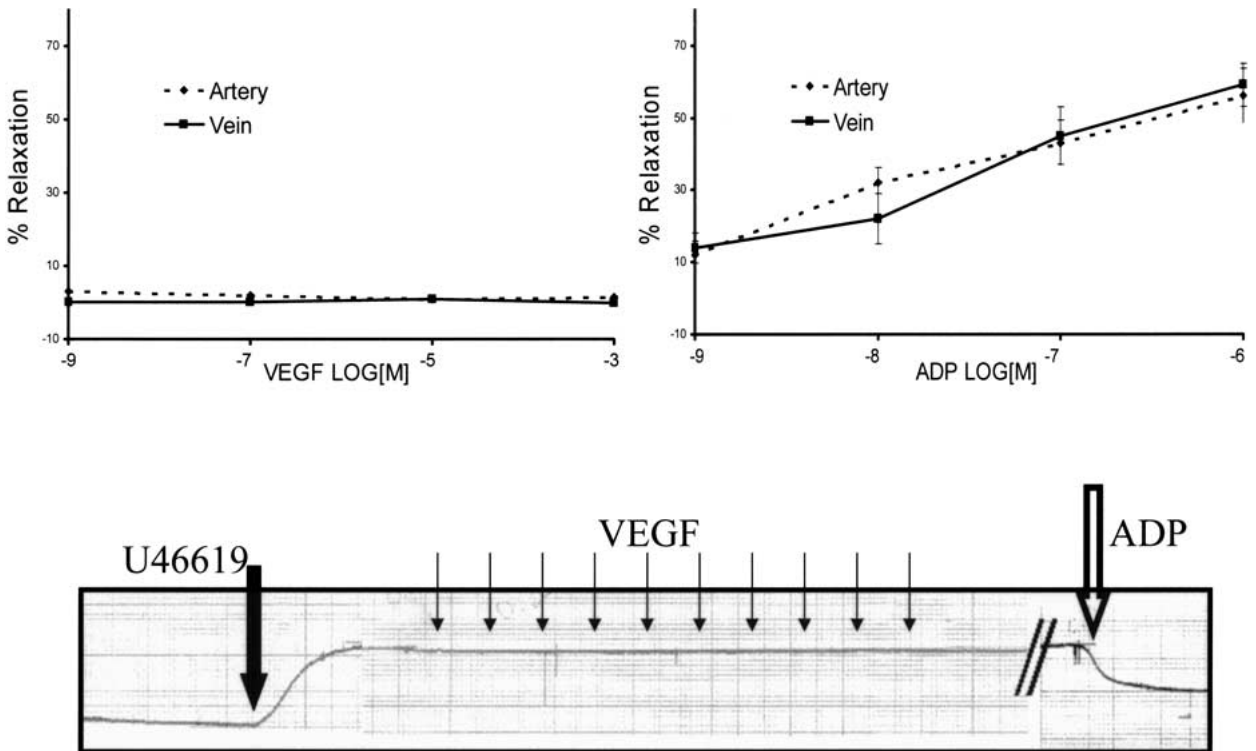
	Baseline	VEGF	NTG
IVUS CSA (cm <sup>2</sup> )	11.8 $\pm$ 5	12.3 $\pm$ 8	15.5 $\pm$ 0.8



**Fig. 3.** Effect of VEGF on the vasodilation of coronary arterioles and venules and role of NO blockade. There is a dose-dependent vasodilatory effect of VEGF on both arterioles and venules (solid line, diamond: venules, square: arterioles), partially inhibited by pretreatment with L-NNA (dotted line, L-NNA). Data presented as mean  $\pm$  standard deviation.

internal diameter) were dissected from the epicardial region of the myocardium and the quadriceps muscle. Microvessels were studied for vasodilatory response to VEGF before and after the administration of 0.1 mM L-NNA. Similar concentrations of U46619 were required in myocardial arterioles and

venules and systemic arterioles to attain similar (45  $\pm$  5% of baseline) degrees of precontraction. VEGF resulted in a dose-dependent relaxation that was similar in myocardial arterioles and venules and systemic arterioles (Fig. 3, relaxation of 55.1  $\pm$  7.6% in arterioles and 52.7  $\pm$  6.0% in venules in



**Fig. 4.** Effect of VEGF on large and medium-sized epicardial and systemic arteries and veins. VEGF failed to induce any vasodilation in arteries and veins (*upper left*) with normal vasodilatory response to ADP, an endothelium-dependent vasodilator (*upper right*). Typical strip

chart recording (*bottom*) from the arterial and venous ring experiments, shows maximal precontraction using acetylcholine, followed by no response to VEGF and significant response to ADP.

response to  $10^{-10}$  M VEGF). This effect was partially inhibited after pretreatment with L-NNA (Fig. 3, ANOVA  $p < 0.01$ ), confirming that VEGF vasodilatory effect is at least in part NO dependent.

To confirm the observed *in vivo* effects on epicardial coronary arteries and to investigate the cause of VEGF hypotensive effect, epicardial coronary arteries and veins as well as systemic medium-sized (femoral) arteries and veins were dissected from six normal animals and circular strips were maximally precontracted using U46619 and the response to increasing concentrations of VEGF was recorded (Fig. 4). VEGF at concentrations of up to  $10^{-4}$  M failed to produce any vasodilatory effects on coronary and systemic arteries and veins. We then measured the response to ADP to ensure normal endothelial function and the presence of endothelial-dependent vasodilation. ADP in concentration of  $10^{-6}$  M induced maximal relaxation of coronary and systemic arteries and veins.

### Immunocytochemical Analysis

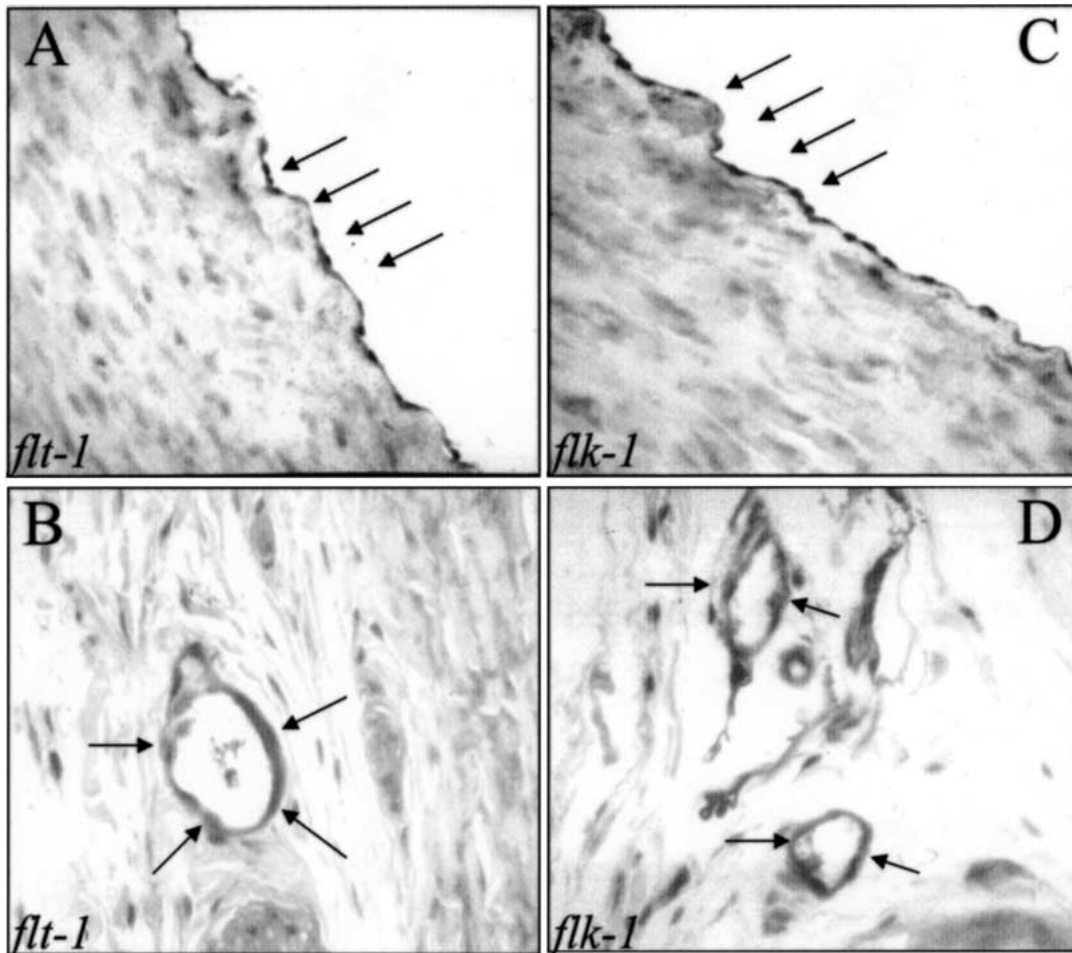
The lack of responsiveness of epicardial and systemic medium-sized arteries and veins may be due

to the absence of VEGF receptors (*flt-1* and *flk-1*) on endothelial cells in these vessels. To this end, immunocytochemical analysis was performed to identify the expression or lack thereof of these receptors in medium-sized vessels (Fig. 5). Both VEGF receptors (*flt-1* and *flk-1*) were identified on the endothelial cells of epicardial coronary arteries and veins. These receptors were also identified in myocardial arterioles (Fig. 5).

### DISCUSSION

In addition to its angiogenic potential, VEGF, a 46-kD heparin-binding growth factor, possesses a number of other biological activities that include, most notably, the ability to induce vascular permeability<sup>19</sup> and NO release. The latter effect is responsible for severe hemodynamic changes seen following one systemic administration of even small amounts of VEGF.<sup>13,17,20</sup>

The current study showed that in Yorkshire pigs, the intracoronary administration of 10  $\mu$ g of VEGF leads to a drop in systemic blood pressure accompanied by a marked increase in coronary



**Fig. 5.** Immunocytochemical staining for *flt-1* (A, B) and *flk-1* (C, D) receptors in epicardial arteries (A, C) and myocardial arterioles (B, C). Both VEGF receptors were identified on the endothelial cells of arteries and microvessels (arrows).

blood flow. Both effects were partially inhibited by pretreatment with L-NNA, indicating a NO-dependent pathway. Furthermore, the increase in coronary blood flow was not associated with a corresponding increase in epicardial coronary artery CSA (measured by IVUS), suggesting that medium-sized arteries were not affected by VEGF. This was confirmed in vitro using isolated microvascular (arterioles and venules) and epicardial and systemic arteries and veins. Epicardial coronary arteries and veins did not exhibit any vasodilatory effect to even high concentrations of VEGF ( $10^{-4}$  M), despite normal response to ADP, an endothelium-dependent vasodilator. Therefore, VEGF's coronary vasomotor effects are confined to the microvasculature, without any effects on the epicardial and systemic arteries and veins and VEGF's hypotensive effect is mediated largely by its microvascular effects, rather than vasodilation of the medium-sized arteries and capacitance veins. Accumulating evi-

dence suggests that *flk-1* is the main VEGF receptor involved in stimulation of NO release achieved by activation of endothelial nitric oxide synthase (eNOS),<sup>21,22</sup> although the *flt-1* role in this event has been suggested as well.<sup>23</sup> However, we have been able to show the presence of both receptors in medium-sized arteries and veins as well as in the microcirculation.

Our study suggests that despite the presence of VEGF receptors (*flt-1* and *flk-1*) in epicardial and systemic conduit arteries, VEGF fails to exhibit a vasodilatory effect, while ADP (an endothelium-dependent vasodilator) dilated these arteries and veins. This spatial heterogeneity of VEGF vasomotor effect suggests additional intermediary pathways in this vasodilatory cascade or differential patterns of signal transduction in the vascular tree.

An example of possible mediators is platelet-activating factor (PAF), which has been shown to play an integral part in VEGF-induced increase in

permeability.<sup>24</sup> The role of PAF in VEGF-dependent NO release, however, has not been defined. Alternatively, differences in intracellular signaling cascades among endothelial cells derived from large and small arteries may account for this result. The stimulation of eNOS activity can be achieved via increases in intracellular calcium levels and via direct activation by the enzyme Akt-1. Porcine resistance arteries express less eNOS and produce less NO than epicardial conduit arteries both basally and in response to an increase in intracellular calcium.<sup>25</sup> Yahima and colleagues studied the ERK and p38 MAPK pathways (two key signal transduction pathways for VEGF) in endothelial cell cultures of different origins (human aortic, microvascular, and umbilical vein) and found that VEGF activated ERK and p38 MAPK in all of three endothelial cell types.<sup>26</sup> However, GF109203X, a specific inhibitor of PKC, markedly inhibited VEGF-induced activation of ERK and p38 MAPK in cells of aortic and umbilical vein origin with little effects on cells of microvascular origin. This and other findings suggested that intracellular signal transduction pathways for VEGF-induced activation of MAPKs are heterogeneous and vary depending on the origin of endothelial cells.<sup>26</sup> This suggests an important role of signal transduction mechanisms in specific physiological responses that may underlie the observed spatial heterogeneity in VEGF's vasomotor effects.

## CONCLUSION

In a porcine model, intracoronary VEGF (10 µg) results in an increase in coronary blood flow and systemic hypotension, both NO-mediated processes. In vivo and in vitro isolated vessel studies showed that VEGF's hemodynamic effects are mediated by dilation of arterioles and venules without any effects on the epicardial and systemic conduit arteries and veins. This spatial heterogeneity of VEGF vasomotor effects cannot be explained by the absence of known vasomotor pathway mediators (VEGF receptors) and suggests differences in intracellular signaling among endothelial cells from different segments of the coronary arterial tree.

---

*This work was supported in part by NIH grants MO1-RR01032 and HL 63609 (R.J.L.), HL 46716 (F.W.S.).*

## REFERENCES

- Ladoux A, Frelin C. Hypoxia is a strong inducer of vascular endothelial growth factor mRNA expression in the heart. *Biochem Biophys Res Commun* 1993;195:1005-1010.
- Lopez JJ, Laham RJ, Stamler A, et al. VEGF administration in chronic myocardial ischemia in pigs. *Cardiovasc Res* 1998;40:272-281.
- Pearlman JD, Hibberd MG, Chuang ML, et al. Magnetic resonance mapping demonstrates benefits of VEGF-induced myocardial angiogenesis. *Nat Med* 1995;1:1085-1089.
- Folkman J. Angiogenic therapy of the human heart. *Circulation* 1998;97:628-629.
- Ferrara N. Vascular endothelial growth factor. *Eur J Cancer* 1996;32A:2413-2422.
- Banai S, Jaklitsch MT, Shou M, et al. Angiogenic-induced enhancement of collateral blood flow to ischemic myocardium by vascular endothelial growth factor in dogs. *Circulation* 1994;89:2183-2189.
- Harada K, Friedman M, Lopez JJ, et al. Vascular endothelial growth factor administration in chronic myocardial ischemia. *Am J Physiol* 1996;270 Pt 2:H1791-H1802.
- Takeshita S, Zheng LP, Brogi E, et al. Therapeutic angiogenesis. A single intraarterial bolus of vascular endothelial growth factor augments revascularization in a rabbit ischemic hind limb model. *J Clin Invest* 1994;93:662-670.
- Takeshita S, Weir L, Chen D, et al. Therapeutic angiogenesis following arterial gene transfer of vascular endothelial growth factor in a rabbit model of hindlimb ischemia. *Biochem Biophys Res Commun* 1996;227:628-635.
- Mack CA, Patel SR, Schwarz EA, et al. Biologic bypass with the use of adenovirus-mediated gene transfer of the complementary deoxyribonucleic acid for vascular endothelial growth factor 121 improves myocardial perfusion and function in the ischemic porcine heart. *J Thorac Cardiovasc Surg* 1998;115:168-176; discussion 176-177.
- Simons M, Laham RJ. Therapeutic angiogenesis in myocardial ischemia. In: Ware J, Simons M, eds. *Angiogenesis and Cardiovascular Disease*. New York: Oxford University Press, 1999; pp 289-320.
- Henry T, Annex B, Azrin M, et al. Double-blind placebo controlled trial of recombinant human vascular endothelial growth factor—the VIVA trial. *J Am Coll Cardiol* 33:384A.
- Lopez J, Laham RJ, Carrozza JC, et al. Hemodynamic effects of intracoronary VEGF delivery: evidence of tachyphylaxis and NO dependence of response. *Am J Physiol* 1997; 273:H1317-H1323.
- Hariawala MD, Horowitz JJ, Esakof D, et al. VEGF improves myocardial blood flow but produces EDRF-mediated hypotension in porcine hearts. *J Surg Res* 1996;63:77-82.
- Sellke FW, Tofukuji M, Laham RJ, et al. Comparison of VEGF delivery techniques on collateral-dependent microvascular reactivity. *Microvasc Res* 1998;55:175-178.
- Sellke FW, Wang SY, Stamler A, et al. Enhanced microvascular relaxations to VEGF and bFGF in chronically ischemic porcine myocardium. *Am J Physiol* 1996;271: H713-H720.
- Yang R, Thomas G, Bunting S, et al. Effects of vascular endothelial growth factor on hemodynamic and cardiac performance. *J Cardiovasc Pharmacol* 1996;27:838-844.
- Doucette JW, Corl PD, Payne HM, et al. Validation of a Doppler guide wire for intravascular measurement of coronary artery flow velocity. *Circulation* 1992;85:1899-1911.
- Mukhopadhyay D, Nagy JA, Manseau EJ, Dvorak HF. Vascular permeability factor/vascular endothelial growth factor-mediated signaling in mouse mesentery vascular endothelium. *Cancer Res* 1998;58:1278-1284.

20. Ku DD, Zaleski JK, Liu S, Brock TA. Vascular endothelial growth factor induces EDRF-dependent relaxation in coronary arteries. *Am J Physiol* 1993;265: Pt 2:H586-H592.
21. Malavaud B, Tack I, Jonca F, et al. Activation of Flk-1/KDR mediates angiogenesis but not hypotension. *Cardiovasc Res* 1997;36:276-281.
22. Kroll M, Margottin F, Kohl A, et al. Inducible degradation of  $\text{I}\kappa\text{B}\alpha$  by the proteasome requires interaction with the F-box protein h- $\beta$ TrCP. *J Biol Chem* 1999;274:7941-7945.
23. Shizukuda Y, Tang S, Yokota R, Ware JA. Vascular endothelial growth factor-induced endothelial cell migration and proliferation depend on a nitric oxide-mediated decrease in protein kinase C $\delta$  activity. *Circ Res* 1999;85:247-256.
24. Sirois MG, Edelman ER. VEGF effect on vascular permeability is mediated by synthesis of platelet-activating factor. *Am J Physiol* 1997;272:H2746-H2756.
25. Xu XP, Liu Y, Tanner MA, Sturek M, Myers PR. Differences in nitric oxide production in porcine resistance arteries and epicardial conduit coronary arteries. *J Cell Physiol* 1996;168:539-548.
26. Yashima R, Abe M, Tanaka K, et al. Heterogeneity of the signal transduction pathways for VEGF-induced MAPKs activation in human vascular endothelial cells. *J Cell Physiol* 2001;188:201-210.