

# Vascular Endothelial Growth Factor and Diabetes

## The Agonist Versus Antagonist Paradox

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Much of the morbidity and mortality associated with diabetes is primarily attributable to sequelae of microvascular and macrovascular disease. Over the past decade, dramatic progress has been achieved in elucidating the fundamental processes underlying the pathogenesis of these complications. Angiogenic factors in particular now appear to play a pivotal role in the development of microvascular complications as well as the response to macrovascular disease. Hyperglycemia, other growth factors, advanced glycation end products, oxidative stress, and ischemia can increase growth factor expression. In some microvascular tissues, the result is pathologic neovascularization and increased vascular permeability. These responses account for much of the visual loss associated with diabetic retinopathy and may, in addition, serve a significant role in nephropathy and neuropathy. In contrast, recent data suggest that vascular collateralization resulting from ischemia-induced growth factor release in tissues compromised by macrovascular disease may be important in reducing clinical symptoms and tissue damage. This angiogenic response, which may be beneficial in coronary artery and peripheral limb disease, appears to be reduced in patients with diabetes. Thus, two apparently diametrically opposed therapeutic paradigms are arising for the treatment of vascular complications in diabetes. Indeed, growth factor antagonists have been used successfully in diabetes-related animal models to block angiogenic and permeability complications in the retina and kidney. Conversely, growth factor agonists have been successfully used to stimulate collateral vessel formation and reduce ischemic symptoms from macrovascular disease in the coronary arteries and peripheral limbs. Both of these approaches are currently being evaluated in clinical trials for their respective indications. Thus, as these divergent therapeutic modalities begin to enter the

clinical arena, this apparent paradox necessitates careful consideration of the potential risks, benefits, and interactions of the opposing regimens. Using vascular endothelial growth factor as a classic example of growth factor involvement, we discuss the current preclinical and clinical data supporting these approaches and the implications arising from the probable coexistence of these two therapeutic modalities. *Diabetes* 48:1899–1906, 1999

Over the past several years, dramatic progress has been made in identifying many of the underlying etiologies for the microvascular and macrovascular complications associated with diabetes. Of particular note are investigations delineating the detrimental role of angiogenic growth factors in the pathogenesis of diabetic microvascular complications and, conversely, the potential therapeutic usefulness of these same molecules in ameliorating tissue ischemia associated with macrovascular disease. It is becoming evident that diabetes results in the increased expression of angiogenic growth factors in numerous tissues as a response to both hyperglycemia and tissue ischemia. Unfortunately, the increased angiogenesis and vascular permeability induced by these growth factors in microvascular tissues such as the retina often have serious detrimental effects. Angiogenic inhibitors would be expected to ameliorate these complications. Macrovascular disease, however, is also a major cause of morbidity and mortality in patients with diabetes. In these cases, the majority of complications result from a reduction in appropriate large artery blood flow to various tissues, as is particularly evident in diabetes-associated coronary artery disease and peripheral limb ischemia. Growth factor-induced angiogenesis under these conditions might increase vascular collateralization and reduce macrovascular morbidity.

Thus, research into the field of growth factor mediation of diabetes-associated vascular complications has suggested diametrically opposed therapeutic approaches and generated a perplexing paradox. On the one hand, the excess production of endogenous growth factors appear responsible for many microvascular complications, suggesting that inhibition of these factors would have significant clinical benefit. Indeed, animal studies have demonstrated remarkable effectiveness of anti-angiogenic therapies in reducing retinal and

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LCx, left circumflex coronary artery; MRI, magnetic resonance imaging; PKC, protein kinase C; MI, myocardial infarction; VEGF, vascular endothelial growth factor.

renal disease. Conversely, augmentation of growth factor action might induce vascular collateralization, reducing the ischemic tissue damage observed with diabetic macrovascular disease. This approach has also been effective in animal models. Each of these opposing approaches holds extraordinary promise for dramatic clinical benefit to large numbers of individuals worldwide. Indeed, clinical trials using both paradigms are currently underway, with promising early results. However, as these novel treatment modalities begin to enter the clinical arena, this apparent paradox necessitates careful consideration of the potential risks, benefits, and interactions arising from the probable future coexistence of these two therapeutic approaches.

#### VASCULAR ENDOTHELIAL GROWTH FACTOR OVERVIEW

Vascular endothelial growth factor (VEGF), also known as vasopermeability factor and vasculotropin, is a 45-kDa homodimeric glycoprotein with potent vascular permeability (1,2) and angiogenic effects (3,4). VEGF is primarily mitogenic for endothelial cells (5,6), and its expression was initially found to be markedly increased in rapidly growing, highly vascularized tumors (7). VEGF expression is induced by hypoxia in tumors and glial myogenic tumor cell lines (8). VEGF binds several high-affinity transmembrane, autophosphorylating tyrosine kinase receptors with affinities in the low picomolar range. The two best characterized of these are fms-like tyrosine kinase (Flt) and fetal liver kinase 1 (Flk-1), also known as VEGF-R1 and VEGF-R2, respectively. VEGF and its receptors are critical for normal embryologic development; even heterozygous knockout of these genes results in lethal disruption of the macro- and microvasculature (9,10). Taken together, these characteristics strongly suggest that VEGF plays a central role in mediating diabetic microvascular complications, such as retinopathy, that are characterized by increased tissue ischemia, angiogenesis, and permeability.

#### ROLE OF VEGF IN DIABETIC OCULAR DISEASE

Nearly all patients with diabetes eventually develop some degree of diabetic retinopathy, which remains the leading cause of new-onset blindness among working-age Americans (11). The sight-threatening stages of this disease are characterized by progressive retinal vessel loss, increasing retinal ischemia, development of pathologic retinal neovascularization, and increased retinal vascular permeability. Subsequent bleeding, fibrosis, and tissue edema frequently result in visual loss (12). The clinical observations relating vascular loss to ocular neovascularization led to the hypothesis a half-century ago by Michaelson (13) that soluble factors induced by retinal ischemia may stimulate blood vessel growth (14,15). Subsequent extensive investigations to identify the molecules primarily involved in this response have suggested numerous candidate mediators including basic fibroblast growth factor, growth hormone, and the IGFs. More recently, VEGF has emerged as a major mediator of intraocular neovascularization, serving a pivotal role in the etiology of diabetic retinopathy. VEGF continues to fulfill all criteria predicted by the Michaelson hypothesis.

Many cell types within the eye produce VEGF, including retinal pigment epithelial cells, retinal capillary pericytes, endothelial cells, glial cells, Mueller cells, and ganglion cells (16–18). In these cell types, VEGF expression is increased

from 2.5- to more than 30-fold by hypoxia (16). Furthermore, retinal microvascular endothelial cells express large numbers of high-affinity VEGF receptors with affinity and molecular size similar to those of nonocular endothelial cells (19). Several animal models of ischemia-induced ocular neovascularization have demonstrated a temporal association between VEGF production and retinal neovascularization in neonatal mice (20), rats (21,22), cats (23), and dogs (24). VEGF production also correlates with the development of ischemia-induced iris neovascularization in the monkey (25).

Clinical studies have evaluated the correlation between diabetic retinopathy and intraocular VEGF concentrations. One study evaluated ocular fluid specimens from 164 patients undergoing intraocular surgery. VEGF concentrations were markedly elevated in both vitreous and aqueous fluids of patients with active proliferative diabetic retinopathy compared with samples from patients without diabetes, with nonproliferative diabetic retinopathy, or with quiescent proliferative diabetic retinopathy (26). Similar findings were observed in a study of vitreous VEGF concentrations in eight patients with proliferative diabetic retinopathy compared with patients without neovascularization (27) and in numerous other subsequent studies (28–31).

In addition to its potential role as a mediator of proliferative diabetic retinopathy, VEGF may also play an important role in the pathogenesis of diabetic macular edema. Macular edema arises from excessive permeability of retinal vessels in patients with diabetes and accounts for the greatest number of individuals with visual loss from this disease (12). In the dermal microvasculature, VEGF is 50,000 times more potent than histamine in inducing vasopermeability (2). VEGF can also increase retinal vascular permeability within the eye (32,33). Concentrations of VEGF have been reported to increase in the diabetic retina before extensive proliferation or retinal ischemia occurs (28,34), thus potentially accounting for the common clinical observation of macular edema at retinopathy stages prior to those involving vascular proliferation. In addition, the observation of retinal VEGF expression early in diabetic retinopathy and the finding in nondiabetic animals that exogenous intraocular VEGF administration can elicit retinal abnormalities resembling diabetic retinopathy (35,36) suggest that VEGF may also play a role in mediating the development and progression of the earliest stages of retinopathy.

#### ROLE OF VEGF IN DIABETIC NEPHROPATHY AND NEUROPATHY

The evidence that VEGF is involved in diabetic renal disease is less extensive than the evidence for its involvement in retinopathy, but several investigations suggest such an association. VEGF expression and binding have been identified in the kidney of rats (37) and humans (38). VEGF has been detected in the epithelial cells of the glomerulus (38), podocyte (39), and collecting ducts (40). Angiotensin II increases VEGF in human mesangial cells through the AT1 receptor (41,42). Because recent studies have suggested that inhibition of the renin-angiotensin system can delay the development and progression of diabetic nephropathy and retinopathy (43,44), a potential role of VEGF in diabetic nephropathy is suspected (39). Elevated VEGF expression has also been observed by immunohistochemistry in the sciatic nerves and dorsal root ganglia of 12-week-old diabetic rats compared with those of control rats or rats treated with

insulin, suggesting a potential role for VEGF in diabetic neuropathy as well (45).

#### DIABETIC OCULAR DISEASE: THE CASE FOR VEGF ANTAGONISTS

Extensive data supporting a role of VEGF in promoting diabetic ocular disease has led to investigations of three different VEGF inhibitory agents in animal models of ischemic retinopathy. Soluble VEGF receptor chimeric proteins that bind VEGF (thus preventing VEGF binding to endogenous endothelial cell VEGF receptors) were injected into the vitreous bodies of mice undergoing ischemic retinal neovascularization (20,46). Treatment with the VEGF inhibitor reduced neovascularization compared with control eyes by up to 77%, with a mean reduction of 50%. VEGF-neutralizing antibodies have been used similarly in a primate model of ischemic iris neovascularization. Treatment resulted in dramatic inhibition of iris neovascularization (47). In vivo, VEGF inhibition has also been achieved using antisense phosphorothioate oligodeoxynucleotides to inhibit VEGF protein synthesis. Two different antisense molecules reduced retinal neovascularization in the ischemic mouse retina model by 25 and 31%, respectively (48).

In addition to directly inhibiting VEGF as described above, the intracellular signal transduction cascade initiated by VEGF has become a therapeutic target. VEGF binding to its most physiologically relevant receptor, VEGF-R2, results in the activation of phospholipase C $\gamma$  (PLC $\gamma$ ), which in turn converts phosphatidylinositol 4,5-bisphosphate to diacylglycerol and inositol triphosphate (49). Diacylglycerol then induces activation and translocation of protein kinase C (PKC). VEGF specifically activates the  $\alpha$  and  $\beta$  isoforms of PKC in vitro and the  $\alpha$ ,  $\beta$ , and  $\delta$  isoforms in vivo. Selective inhibition of the PKC- $\beta$  isoform inhibits VEGF-stimulated endothelial cell growth in vitro (49). LY333531, a PKC- $\beta$ -selective inhibitor with excellent absorption after oral ingestion and little evident toxicity, is now available (50,51). In animals, this inhibitor has proven effective in suppression of VEGF-induced alterations in retinal vascular leakage (32) and retinal blood flow (51) as well as ischemia-induced retinal neovascularization (52).

#### DIABETIC CARDIOVASCULAR DISEASE

Patients with diabetes have a two to five times greater risk of cardiovascular disease than nondiabetic patients (53–55). Although diabetes affects nearly 6% of the population in the U.S., >30% of patients hospitalized for acute coronary syndromes have diabetes. The age-adjusted prevalence of coronary artery disease in patients with diabetes in the U.S. is between 30 and 51% (56). The age-adjusted prevalence of diabetic peripheral vascular disease and stroke is 9 and 10%, respectively (56). Patients with diabetes have greater morbidity and mortality after myocardial infarction (MI) than nondiabetic patients (57,58). As recently as the mid-1960s, 40% of diabetic patients suffering an MI died (59). Although modern acute coronary care has dramatically improved survival rates following MI, patients with diabetes still have twice the mortality rate within 30 days after MI compared with nondiabetic patients. This twofold increase in mortality persists even when adjusted for the extent of coronary artery disease, presence of known cardiac risk factors, and other end-organ disease (60). Similarly, diabetic patients experience

more congestive heart failure than nondiabetic patients with the same size infarct (60). Revascularization procedures such as percutaneous transluminal coronary angioplasty, stenting, and coronary artery bypass grafting are less effective in patients with diabetes than in those without diabetes (61,62).

Numerous heart-specific conditions in diabetes affect cardiac response. Previous silent MI is detectable in 40% of diabetic patients when they present with their first clinically recognized MI (63,64). Cardiac autonomic neuropathy is present in 50% of diabetic patients with coronary artery disease and can result in diastolic and systolic dysfunction (65). Cardiomyopathy from diabetes is often subclinical (66). Hypertension combined with diabetes results in more cardiac fibrosis than either condition alone (67). Many patients with type 1 or type 2 diabetes have diminished vagal activity and thus a relative increase in sympathetic activity. Increased sympathetic activity is associated with increased cardiac ischemic events and sudden death (68,69). Endothelial dysfunction in diabetes can impair coronary perfusion and result in ischemia (70).

The results of two large, rigorously controlled, randomized studies suggest that factors other than serum glucose may play a major role in diabetes-associated cardiac morbidity. The U.K. Prospective Diabetes Study was an 11-year-long multicenter randomized controlled trial of different therapies for type 2 diabetes designed to determine whether improved glucose control in patients with newly diagnosed type 2 diabetes was effective in reducing the incidence of clinical complications. After 3 months of initial diet therapy, 3,867 asymptomatic newly diagnosed patients with type 2 diabetes who remained hyperglycemic (fasting plasma glucose levels 6.0–15.0 mmol/l) were randomly assigned to either conventional therapy (primarily with diet alone) or intensive therapy (aiming for fasting plasma glucose levels <6.0 mmol/l) with assignment to primary therapy with a sulfonylurea (chlorpropamide, glibenclamide, or glipizide), insulin, or metformin (71). The study recently reported that intensive glycemic control did not decrease the risk of cardiovascular disease despite a clear decrease in microvascular complications (43).

In contrast, the Diabetes Complications and Control Trial was a multicenter randomized clinical study designed to determine whether an intensive treatment regimen directed at maintaining blood glucose concentrations as close to normal as possible would affect the appearance or progression of early vascular complications in patients with type 1 diabetes (72). This study found a trend toward decreasing cardiovascular disease with intensive glycemic control in the 1,441 type 1 diabetic patients evaluated. The result did not reach statistical significance, however ( $P = 0.08$ ) (73,74).

The effect of diabetes on the large vessels mostly results from the acceleration of atherosclerosis and an increase in thrombosis, particularly in patients with type 2 disease (75). It has been proposed that atherogenesis is induced by hyperlipidemia, hypertension, hyperinsulinemia, and insulin resistance even before the clinical diagnosis of diabetes (76). This tendency toward atherogenesis is further exacerbated by the hypercoagulable state associated with diabetes. Decreased plasma concentration of plasminogen activator inhibitor 1 observed with hyperinsulinemia, insulin resistance, and type 2 diabetes may be responsible for a decrease in fibrinolysis, and endothelial dysfunction associated with

these states may reduce the anticoagulant properties of the endothelium (75).

#### ROLE OF VEGF IN DIABETIC CARDIOVASCULAR DISEASE

Coronary collateral blood vessel formation helps preserve myocardial function during coronary arterial obstruction. The presence of coronary collateral vessels reduces the degree of myocardial ischemia and functional deficit following acute coronary occlusive episodes (77,78). A correlation exists between collateral coronary blood flow and myocardial viability in patients with recent MI (78). In dogs, collateral blood vessels form during gradual occlusion of a major coronary artery, suggesting that development of coronary collaterals is a dynamic process that may precede acute coronary occlusion (79).

Several observations suggest that VEGF may play a significant role in this adaptive process. Heart tissue from several species expresses VEGF mRNA, and hypoxia stimulates VEGF mRNA expression in cultured rat cardiac cells (80,81). VEGF receptors are expressed in the normal rat heart (82). Normal human heart expresses VEGF mRNA in cardiomyocytes; after MI, however, expression is much greater and is observed in arteriole smooth muscle cells and infiltrating macrophages (83). In a rat model of cardiac infarction, VEGF, VEGF-R1, and VEGF-R2 mRNA expression in the heart are increased 275, 400, and 375%, respectively (84). Myocardial VEGF and VEGF-R2 expression are increased four- and six-fold, respectively, after cardiopulmonary bypass in pigs (85). When VEGF is included in cardioplegic solution of isolated rat hearts, there is significant improvement in cardiac output recovery, coronary flow, and stroke work. Coronary resistance is also lowered, and less creatinine kinase release occurs (86). In 45 patients with acute MI, serum VEGF concentrations were elevated at day 7 and were independently associated with the peak creatinine kinase concentration (87). Another study of 19 patients with acute MI showed similar results, with return of VEGF concentrations to the normal range after reperfusion (88).

#### DIABETIC CARDIOVASCULAR DISEASE: THE CASE FOR VEGF AGONISTS

The clinically observed protective effect of preexisting collateral blood vessels has prompted investigations into the pharmacologic enhancement of collateral vessel growth in ischemic myocardium and peripheral limb. This approach has been termed "therapeutic angiogenesis." The angiogenic and physiologic properties of VEGF make it an ideal candidate for study in this regard. In dogs subjected to gradual occlusion of the left circumflex coronary artery (LCx), daily administration of exogenous VEGF through an indwelling catheter at a point just distal to the occlusion induced a 40% increase in collateral blood flow and an 89% increase in the numeric density of intramyocardial vessels in the collateral zone after 28 days (79). In pigs with mechanically induced constriction of the proximal LCx, the delivery of VEGF to the myocardium beyond the site of occlusion resulted in a reduced area of ischemia and improvement in ventricular function as assessed by magnetic resonance imaging (MRI) and image processing (89,90). Indeed, a single intracoronary bolus of VEGF is effective in stimulating physiologically significant angiogenesis in pigs with chronic myocardial ischemia (91).

In pigs transduced with a VEGF-expressing plasmid using carbon dioxide-transmyocardial laser revascularization, 100% (five of five animals) displayed no evidence of wall motion abnormality after 6 weeks, a statistically significant difference from ischemic control animals ( $P = 0.004$ ) (92).

A phase I clinical study evaluated direct VEGF myocardial gene transfer as the sole treatment in five patients with symptomatic myocardial ischemia who failed conventional therapy (93). Naked plasmid encoding human VEGF was injected directly into the ischemic myocardium by surgical thoracotomy. All patients experienced a significant reduction in angina and nitroglycerin use ( $P < 0.03$ ). Left ventricular ejection fraction was either unchanged or improved, and reduced ischemia was confirmed using computed tomography imaging techniques and coronary angiography.

In addition to studies of VEGF for the treatment of coronary heart disease, VEGF-induced therapeutic angiogenesis has been investigated in the setting of peripheral vascular disease. Nonobese diabetic (NOD) mice demonstrated reduced hindlimb neovascularization in response to femoral artery ligation compared with control animals (94). This reduced collateral formation was associated with attenuated VEGF mRNA and protein expression. Intramuscular injection of an adenoviral vector expressing VEGF restored normal neovascularization in the diabetic animals (94). In rabbits whose femoral artery was excised to induce severe hindlimb ischemia, VEGF administered as a single intra-arterial bolus to the ipsilateral internal iliac artery caused significant augmentation of collateral vessel development and improved hemodynamic function in the ischemic limb (95).

Therapeutic angiogenesis with VEGF has been evaluated in 10 limbs of 9 patients with severe peripheral arterial disease in whom rest pain (10 of 10) or nonhealing ischemic ulcers (7 of 10) were present (96). Naked plasmid DNA encoding human VEGF was directly injected into the ischemic limb muscles. Peripheral flow improved ( $P = 0.02$ ), new collateral vessels were observed by angiography (7 of 10), flow improved by MRI angiography (8 of 10), ischemic ulcers improved or healed (4 of 7), and 3 patients avoided previously recommended below-knee amputation. Transient lower-extremity edema, consistent with VEGF's vasopermeability activity, was the principle side effect. In another study, the administration during angioplasty of DNA encoding VEGF in a patient with severe ischemic limb disease increased collateral vessel formation in the limb (97).

#### APPROACHES CURRENTLY IN CLINICAL TRIAL

Clinical trials to determine the effectiveness of angiogenic agonists and antagonists are currently underway. VEGF and VEGF agonists are continuing to be evaluated for their usefulness in treating myocardial and peripheral limb ischemia. Although many pharmaceutical and biotechnology companies currently have active programs devoted to the identification, development, and testing of novel anti-angiogenic approaches, most are initially being evaluated for their effect on vascular neoplasms. However, of the more than 20 anti-angiogenic agents that are in (or soon entering) clinical trial (98), several are being evaluated for ophthalmic indications. With specific regard to diabetes and the PKC- $\beta$ -selective inhibitor LY333531 (discussed above), patients are being enrolled in a multinational randomized double-masked placebo-controlled clinical trial to assess the molecule's

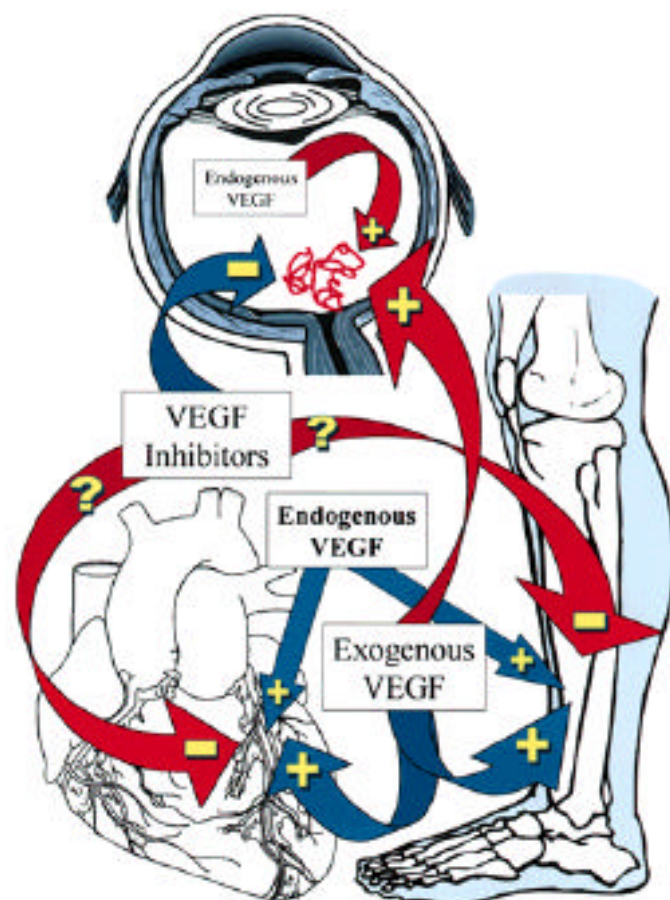
potential benefit for proliferative diabetic retinopathy and diabetic macular edema. Integrin and growth hormone antagonists are nearing clinical trial for the treatment of diabetic retinopathy as well. Other agents are also being evaluated that might have eventual usefulness in diabetic retinopathy. Thalidomide has been in clinical trials for several years to assess its effectiveness in suppressing the choroidal neovascularization associated with age-related macular degeneration (99). Similarly, VEGF-neutralizing antibodies and VEGF aptamers are likely to be entering clinical trials soon for this disorder (100). The first demonstration that pharmacologic intervention may be efficacious in diabetic retinopathy will need to await the results of these clinical investigations.

### RECONCILIATION OF OPPOSING PARADIGMS

The recent rapid evolution in our understanding of VEGF and its relationship to diabetic complications has fueled the development of therapeutic strategies aimed at ameliorating both microvascular and macrovascular manifestations of the disease. Both strategies are currently in clinical trial. These advances, however, have generated what may initially appear to be a perplexing paradox in our approach to the management of diabetic complications. While anti-VEGF therapies appear very promising as a means of reducing microvascular complications such as diabetic retinopathy, pro-VEGF therapies appear to hold equally exciting potential to increase collateral vascular formation and reduce macrovascular complications associated with MI and peripheral limb ischemia. Thus, systemic treatment aimed at one complication of diabetes may theoretically exacerbate another complication (Fig. 1).

Upon closer inspection, however, these approaches may not necessarily be mutually exclusive. Although excessive concentrations of VEGF in the retina will undoubtedly lead to ocular complications (26,36), VEGF agonist approaches for cardiac and peripheral limb ischemia to date have apparently been well localized. VEGF or its agonists have been administered directly to the coronary vasculature (79,91), the myocardium (93), and the muscle of the ischemic limb (94,96). Direct injection into muscle tissues appears to result in localized expression without significant distant effects. Even where intravascular administration is performed, the bolus is delivered near the desired site of action. Since VEGF is rapidly and efficiently degraded in the blood, significant activity in distant organs such as the eye is unlikely. Finally, since a finite extent of vascular collateralization is required to reduce or prevent tissue damage, the duration of required VEGF stimulation is likely to be limited as well.

In contrast, anti-VEGF therapy for the manifestations of diabetic retinopathy will likely require longer treatment duration, since the retinal ischemic stimulus may persist despite effective VEGF inhibition. However, the natural course of diabetic retinopathy is to eventually become inactive and quiescent, a condition that persists for well over 15 years (101). If such quiescence still develops in the presence of VEGF-antagonist therapy, the possibility of eventually discontinuing treatment would remain. Local delivery of VEGF inhibitors to the eye should have fewer side effects than systemic administration, but achieving adequate concentrations of these agents at the retina by this approach is difficult. Intravitreal injections allow for delivery of high intraocular concentrations of an agent, but repeated injections for the treatment of a chronic dis-



**FIG. 1.** Schematic representation of the VEGF agonist/antagonist paradox. Principal organs affected by microvascular (ocular) and macrovascular (cardiac and peripheral vascular) complications in diabetes are shown in relation to their predicted response to VEGF inhibitors and VEGF agonists from either endogenous or exogenous sources. Blue arrows represent potential beneficial effects. Red arrows reflect possible adverse actions. +, stimulation of angiogenesis at the site; -, inhibition of angiogenesis at the site; ?, interactions where our understanding is particularly incomplete.

ease such as diabetic retinopathy are suboptimal because of the repetitive risks of endophthalmitis and retinal detachment.

Even if systemic therapy is used, it is uncertain that inhibition of VEGF in the normal adult would lead to significant side effects. Although some mature tissues express low basal levels of VEGF, it has not been demonstrated that reducing VEGF expression in this situation is detrimental. In the case of myocardial and peripheral limb ischemia, all studies showing a benefit from VEGF agonists are the result of iatrogenically increasing VEGF expression. A deficit of VEGF has not been shown to be the underlying cause of morbidity. Thus, it is possible that VEGF antagonists may be well tolerated under basal conditions. There are likely to be situations, however, such as after MI, extensive wounds, or peripheral arterial insufficiency, when a normal neovascular response would be desired in the patient. If local VEGF agonist therapy cannot overcome the VEGF inhibitory effect at the location required, then anti-angiogenic agents that can be rapidly reversed or have short half-lives would be desirable. These could be discontinued during the period of therapeutic angiogenesis and reinstated as required after such therapy. A more

aggressive and speculative approach might be to provide VEGF agonist therapy to improve collateralization at a point when a patient approaches, but before, the need for VEGF antagonist therapy. In this manner, established collateral vessels could be formed to protect the patient when they are eventually treated with VEGF inhibitors.

Thus, at present, it may be most important to ensure that VEGF agonist therapies maintain a localized effect so as to prevent near-certain complications if VEGF activity is increased in microvascular tissues such as the eye. In contrast, it is not currently known if VEGF antagonists will increase the risk of macrovascular disease. Once significant macrovascular disease occurs, localized VEGF augmentation could be performed either locally or after cessation of VEGF antagonist therapy. Thus, the apparent paradox may not be insurmountable. Future VEGF agonists and antagonists may be able to coexist, allowing diabetic patients to benefit from both therapeutic approaches.

#### CONCLUSIONS AND IMPLICATIONS FOR THE CARE OF PATIENTS WITH DIABETES

Numerous recent findings regarding the role of VEGF in diabetic microvascular and macrovascular disease have spurred efforts to develop effective VEGF-related therapies for these complications. Clinical trials are currently underway. Ironically, the findings have led to diametrically opposed therapeutic approaches. VEGF antagonists are being investigated for treatment of microvascular complications such as diabetic retinopathy, whereas VEGF agonists are being used to treat macrovascular complications such as MI and peripheral limb ischemia. Although these approaches may initially appear paradoxical, there are reasons to believe that the modalities may not necessarily be mutually exclusive.

The medical management of patients with diabetes has traditionally focused on optimizing control of blood glucose and other systemic risk factors. However, current research suggests that diabetes management may eventually need to include treatments targeted at specific complications of the disease. This new therapeutic armamentarium may include both agonists and antagonists of VEGF. If these treatment modalities can be shown to be efficacious and safe, and the apparent paradox resolved, then our ability to reduce morbidity and mortality in our patients with diabetes will have been greatly enhanced.

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