Cardiovascular complications are the leading cause of morbidity and mortality in patients with diabetes mellitus; up to 80% of deaths in patients with diabetes are closely associated with vascular disease. The ability of the organism to form a collateral network of blood vessels constitutes an important response to vascular occlusive disease and determines to a large part the clinical consequences and severity of tissue ischemia. The development of new vessels is significantly reduced in diabetic patients with coronary or peripheral artery disease.1,2 This probably contributes to the severe course of limb ischemia in diabetic patients, in which peripheral artery disease often results in foot ulceration and lower extremity amputation.

Diabetic retinopathy remains one of the major causes of acquired blindness in developed nations. This is true despite the development of laser treatment, which can prevent blindness in the majority of those who develop macular edema or proliferative retinal detachment. The factors that stimulate retinal neovascularization that may ultimately cause severe vitreous cavity bleeding and/or retinal detachment. The factors that stimulate retinal blood vessel growth have not been fully defined, but there is accumulating evidence that the renin-angiotensin-bradykinin system (RAKS) may be involved in a number of retinal vascular disorders, including retinopathy of prematurity and proliferative diabetic retinopathy.3,4 Only a few studies have specifically evaluated the effect of diabetes on angiogenesis in ischemic vascular disease and in the retina. Moreover, the mechanisms by which diabetes could both limit the formation of new blood vessels in most organs and simultaneously induce proliferative diabetic retinopathy remain largely undefined.

**Main Molecular Mechanisms of Ischemia-Induced Neovascularization**

After acute ischemia, hypoxia and inflammation are believed to be the major stimuli causing neovascularization.5 Cells exposed to hypoxia respond by increasing the level of hypoxia-inducible factor-1 (HIF-1). This factor then activates a number of genes by binding to hypoxia response elements in their promoter regions. A second hypoxia-responsive factor, HIF-2, can activate many of the same genes as HIF-1, such as the vascular endothelial growth factor (VEGF).6 VEGF is a major proangiogenic factor activating phosphatidylinositol-3'kinase/Akt and thus the cell survival, migration, and proliferation.7 Akt has been shown to phosphorylate endothelial nitric oxide synthase (eNOS) leading to a persistent calcium-independent enzyme activation and enhanced endothelial NO synthesis and thereby influences the long-term regulation of vessel growth. A large body of literature indicates an essential role of endothelial NO for postnatal neovascularization.8 The downstream effector pathways, by which NO mediates its effects, are less clear but may involve integrin-linked signal transduction processes.

Inflammatory processes are also necessary in the ischemia-induced neovascularization process. Activated monocytes and macrophages have been evidenced in ischemic tissues; these cells adhere to the vascular wall during angiogenesis and activate the production of both proangiogenic cytokines such as basic fibroblast growth factor (bFGF), VEGF, IL-1β binding protein, tumor necrosis factor (TNF)-α, and matrix metalloproteinases (MMPs), a family of enzymes that proteolytically degrade various components of the extracellular matrix.9–12

Ischemia-induced neovascularization also involves circulating vascular progenitor stem cells. After tissue ischemia, progenitor endothelial cells are mobilized from the bone marrow to the blood stream, and then home to ischemic tissues where they promote neovascularization through the paracrine production of growth factors and possible also by incorporation into neovessels.13–15

**Impaired Ischemia-Induced Neovascularization in Diabetes**

In diabetic patients, collateralization and angiogenesis are insufficient to overcome the loss of blood flow through narrowed or occluded arteries leading to ischemia and often nontraumatic limb amputation. However, only few studies have focused on the identification of factors that may affect neovascularization in the setting of ischemia in diabetes. It has been suggested that alteration in VEGF expression and signalization participate to neovascularization abnormalities in diabetes mellitus.2 Attenuation of the ability of monocytes to migrate has also been reported in diabetic patients because of a downstream signal transduction defect. The abrogation of the resulting inflammatory reaction might be critical to the formation of new blood vessels in this context.16 Increased formation of advanced glycation end-products (AGEs) is also regarded as one of the main mechanisms responsible for vascular damage in patients with diabetes. Glycation of extracellular matrix is a consequence of prolonged elevated glucose levels that react with proteins by a nonenzymatic posttranslational modification process called nonenzymatic
glycation. This process is purely adventitious and therefore is likely to be more important in proteins possessing a long biological half-life such as collagen. In experimental models of diabetes, the ability to inhibit these pathways prevented diabetic retinopathy. AGE formation has also been shown to reduce the proteolysis of the glycated proteins and therefore may affect the angiogenic reaction. Hence, pharmacological inhibition of AGE formation is able to restore the ischemia-induced revascularization process in mice hindlimb. Finally, reduction in the mobilization, differentiation, and incorporation of endothelial progenitor cells at the level of the neo-vessels might participate to the angiogenic deficit in a diabetic context. In a diabetic type-1 mouse model, it has been reported that the ability of bone marrow mononuclear cells to differentiate into endothelial progenitor cells was strongly affected, resulting in reduced proangiogenic potential. In the same way, EPCs from type-1 or -2 diabetic patients evidenced a impaired proliferation and adhesion and incorporation into vascular structures, supporting the hypothesis of altered EPCs in diabetes.

Tanii et al in this issue of Circulation Research used a model of severe hind limb ischemia to further investigate the mechanisms of microangiopathy in streptozotocin-induced diabetic mice (STZ-DM). Diabetic mice frequently lost their hind limbs at various levels after ischemia, whereas the nondiabetic mice did not. The authors showed a disturbance of the PDGF-BB/PKC axis, but not of impaired expression or efficiency of angiogenic factors. Hence, VEGF-A, VEGF-C, HGF, FGF-1, PDGF-A, and their receptors flk-1, flt-1, PDGFRα, and -β gene expression were unaffected in STZ-DM. In addition, FGF-2 gene transfer resulted in the upregulation of endogenous VEGF and HGF, prevented limb amputation, and restored limb blood flow, suggesting that angiogenic responses were minimally impaired in the STZ-DM model. In contrast, the PDGF-B expression was reduced in the STZ-DM in correlation with AGEs accumulation and morphological abnormalities of newly formed capillaries (dissociation of pericytes from the capillaries in ischemic muscles). Supplementation of the PDGF-B gene expression and PKC inhibitors restored the tissue levels of PDGF-B and were effective in preventing auto-amputation (Figure).

Many questions remain unanswered. Is PKC/PDGF-BB axis affected in other models of diabetes; how does a 50% reduction in PDGF-BB expression lead to pericytes dissociation; and what is the nature of the intracellular signaling involved in PKC-induced downregulation of PDGF-BB gene expression? How does an alteration in the expression of a single growth factor can induced abnormalities in neovascularization? With a disease as complex as diabetes, other factors are likely to be involved as well. However, these results strongly suggest that defects and impairments in the main proangiogenic factors are not obligatorily involved in the complication of severe ischemia occurring in this model of STZ-induced diabetes. In addition, the PKC/PDGF-BB axis could be a new molecular target for treating severe ischemic peripheral lesions in patients with diabetic complications.

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References


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