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Commentary

Glucose, VEGF-A, and Diabetic Complications

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Diabetes is a widespread disease with multiple complications that affect both the microvasculature and macrovasculature. In the past decade, studies of the underlying factors in diabetic complications have resulted in an interesting dilemma: both microvascular insufficiencies and microvascular proliferative diseases plague diabetic patients, sometimes simultaneously. Advances in therapeutic treatments of microvascular disease continue to show promise for the treatment of the variety of diabetic complications. Although many factors have been shown to contribute to these complications, the angiogenic growth and survival factor, vascular endothelial growth factor (VEGF)-A, is commonly mis-regulated in most microvascular disorders. The short communication by Pinter and colleagues[1] found in this issue of *The American Journal of Pathology* demonstrates this correlation yet another time, only for the first time these authors investigate the effects of hyperglycemia on VEGF-A function during embryonic vascular development.

One of the more studied microvascular complications in diabetes is proliferative retinopathy. The large number of studies regarding etiology and treatment of this blindness-causing disease have shown that during proliferative stages, plasma and vitreous levels of VEGF-A are high in patients.[2–7] Additionally, in specimens of diabetic retinas increased expression of VEGF-A and its three receptors has been extensively demonstrated.[8–10] Other growth factors, such as IGF-1 and its receptor, have been shown to collaborate with VEGF-A to increase retinal neovascularization.[11] Additionally, a study of diabetic patients that did not develop retinopathy showed that there was a correlation to impaired hypoxic induction of VEGF-A in these patients, again supporting the hypothesis that retinopathy involves hypoxic expression of VEGF-A as a fundamental aspect of its etiology.[12] And importantly, antagonists of VEGF and its receptors have been shown to reduce retinopathy in animal models.[13–16] VEGF-A function to induce permeability is also a likely contributor to the vascular leakage that greatly contributes to the morbidity of diabetic retinopathy.[17,18]

Other complications that have seen more progress in clinical studies are peripheral ischemia, marked by decreased microvascular function and subsequent circulation in the extremities, and neuropathy. Many diabetic patients suffer from both loss of circulation and neuropathy. The loss of feeling in their lower extremities further increases the likelihood of permanent tissue damage because of injury, and the poor circulation compromises wound healing and successful treatment of infections. Clinical trials to increase peripheral circulation by administering VEGF-A in one form or another have shown success in both increasing the circulation and reducing neuropathy.[19–22] In particular, animal models of diabetes were examined for their response to injury and VEGF-A therapy in models of hindlimb ischemia. It was found that the severity of ischemia was increased in NOD (nonobese diabetic) mice, and this could be reduced by VEGF-A treatment.[23] Samii and colleagues[24] hypothesized that peripheral nerves and dorsal root ganglia in diabetic animals up-regulate VEGF-A and made the hypothesis that VEGF-A may help restore nerve function. Another less direct correlation could relate to the spatial co-ordination of the vascular and nervous system. This coordination may reflect a dependence of the nerves on the factors supplied via close proximity to blood vessels, and microvascular damage starves the nearby nerves.

Another major complication of diabetes is renal dysfunction. Recently some attention has been paid to the possible involvement of VEGF-A in this pathology. In streptozotocin-induced diabetic rats, VEGF-A and its receptor, VEGFR-2, were up-regulated in the kidney after 3 weeks, but not after 32 weeks.[25] The transient increase seemed to be via VEGFR-2 expression in the glomerulus and may explain some of the renal changes in diabetic patients via VEGF-A permeability functions. VEGF-A-induced permeability alterations in the glomerulus could lead to the protein leakage into the urine of diabetic patients. Studies have shown that glucose-induced albumin permeation can be blocked by antagonism of VEGF-A function.[26,27]

The study presented in this issue of *The American Journal of Pathology* by Pinter and colleagues[1] adds to the understanding of VEGF-A's involvement in yet another complication of diabetes, that of vascular abnor-

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malities in the embryos and fetuses of diabetic mothers.[28] Fetuses of diabetic mothers have increased incidence of vascular abnormalities, some of which are diagnosed at birth and others are found after miscarriage or stillbirths.[29,30] Moreover, earlier abnormalities may account for the increased fetal resorption and difficulty in establishing pregnancy.[29,31–35] Pinter and colleagues[1] established an embryo culture system that mimics the plasma glucose levels of diabetic mothers and diabetic animals and found that these embryos had malformations in the earliest vascular beds resulting in arrested development. They looked at VEGF-A expression in two ways. First they used a LacZ knock-in construct that eliminates the 3′UTR and inserts an internal ribosome entry site LacZ after the VEGF stop codon.[36] In the heterozygous state this knock-in was fully viable and had a normal vasculature despite missing 50% of RNAs ability to respond to stabilization via the 3′UTR. In these animals, LacZ expression is a mark of VEGF-A transcription. Additionally, the authors looked at total VEGF-A protein on Western blots and observed that VEGF expression was reduced. In correlation with reduced VEGF expression, VEGF receptor signaling was reduced. The effects on VEGF-A signaling and the embryonic vasculopathy were eliminated by low levels of exogenous VEGF-A_{165} added to the culture medium. This result may be directly correlated to VEGFR-2 signaling because the related growth factor, PlGF, could not rescue these embryos. PlGF binds only to VEGR-1 and neuropilin, and thus partially distinguishes between VEGF receptor signaling.[37–42]

A large literature on the regulation of VEGF-A has demonstrated that VEGF-A levels are exquisitely sensitive to multiple ischemic agents, including oxygen, iron, and glucose.[43–47] This regulation exists at multiple levels: 1)ischemia increases VEGF-A mRNA stability, in part via sequences in the 3′UTR and in association with the von Hippel Lindau protein.[48,49] 2) Ischemia increases transcription via hypoxia-inducible transcription factors.[50] Null animals in one of the hypoxia-inducible transcription factors, ARNT, make less hypoxia-induced VEGF-A and die with vascular anomalies in the yolk sac similar to those of the VEGF-A knock-out and hyperglycemic embryos.[51,52] 3) Ischemia increases translation efficiency via an endogenous internal ribosome entry site.[53] On the counter side, increased oxygen (hyperoxia)[54–57] and increased glucose (hyperglycemia)[58,59] have both been shown to reduce VEGF-A RNA levels, likely via the same mechanisms of RNA stability and transcription. Under the hyperglycemic conditions of the embryos cultured as reported by Pinter and colleagues,[1] it is unclear whether VEGF-A levels are reduced via VEGF-A mRNA stability and transcriptional reductions. However, in their experiments using a LacZ reporter knock-in to the VEGF-A 3′UTR, at least the mRNA stability reported in association with that sequence (and interacting von Hippel Lindau protein) was nonfunctional suggesting that the LacZ expression changes seen in hyperglycemic cultures were transcriptional.

The regulation of VEGF-A by glucose has not been as extensively investigated as the oxygen regulation, but elegant studies of VEGF-A RNA expression in spheroids clearly demonstrated a similar regulation by hypoglycemia as hypoxia.[45] *In vitro* experiments supporting this glucose regulation have been performed on tumor cells, glial cells, retinal Muller cells, and vascular smooth muscle cells.[58–62] Moreover, similar to hypoglycemia, *in vitro*, acute insulin treatment induced VEGF-A expression.[63] Insulin has also been reported to regulate transcription via the hypoxia-inducible transcription factors.[64] One report suggesting a contrary effect of glucose on VEGF-A is specific to mesangial cells of the kidney.[65] These studies suggested that hyperglycemia induced, rather than reduced VEGF-A expression. Should this result be correct *in vivo*, it would suggest that hyperglycemic incidents would transiently increase VEGF-A specifically in the kidney. In this organ such a response would lead to increased permeability in the glomerulus, a common symptom of diabetes.

Can glucose regulation of VEGF-A explain all of the microvascular complications in diabetes? The results of the 10-year diabetes control and complications trial conducted by the National Institute of Diabetes and Digestive and Kidney diseases showed that intensive treatment aimed at keeping blood sugar levels as close to normal as possible significantly reduced the onset and progression of retinopathy, nephropathy, and neuropathy.[66] Thus it seems that oscillations in glucose should be kept to a minimum. It may be that low glucose (high VEGF-A) levels can account for both sporadic proliferative events (such as retinopathy); and high glucose levels (low VEGF-A) can account for microvascular insufficiencies (loss of microvessels). It was widely believed that VEGF-A fluctuations in a stable and mature vasculature could not cause alterations in the vasculature without pre-existing vascular injury to allow greater responsiveness to VEGF-A. However, recent studies reporting the administration of adenovirus expressing VEGF-A to a fully mature vascular bed demonstrated massive vascular proliferation and associated edema.[67] Whether decreased VEGF-A in an adult vascular bed can lead to regression has not been clearly established. Nonetheless, it seems that increased VEGF-A in diabetic patients because of too much insulin, for example, could initiate a proliferative situation accompanied by edema and set off the initial vascular instabilities that are then more sensitive to further glucose/VEGF-A fluctuations. Thus, in the same patient episodic gross fluctuations can either lead to microvascular proliferation or loss. As for macrovascular complications of diabetes, these may be partially independent of the microvascular,[68] but it is clear that these complications and their treatments are made more complex in diabetic patients because of suboptimal microvascular function.

One potential problem for the diabetic patient that has not been solved is how to treat one vascular complication without exacerbating another. For retinopathy decreased angiogenesis is desired, whereas for peripheral ischemia increased angiogenesis is desired. For example, would a systemic treatment for peripheral ischemia designed to stimulate microvessel proliferation aggravate proliferative retinopathy? Or *vice versa*, would VEGF-A antagonists designed to treat retinopathy accelerate the onset of

cardiovascular or peripheral vascular disease? To date very little has been done to determine the answer to such questions. One difficulty in addressing this issue is the paucity of diabetic animal models that acquire multiple complications the way humans do. Unlike the current anti-angiogenic treatments in clinical trials for cancer, treatment of diabetic vascular diseases may need to be organ-specific. Thus it seems likely that alternate treatments for vascular disease that are either local or independent of systemic factors such as VEGF-A are needed to maintain a healthy balance in diabetic patients with multiple complications.

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