Molecular mechanisms of diabetic vascular complications

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ABSTRACT
Diabetic complications are the major causes of morbidity and mortality in patients with diabetes. Microvascular complications include retinopathy, nephropathy, and neuropathy, which are leading causes of blindness, end-stage renal disease, and various painful neuropathies; whereas macrovascular complications involve atherosclerosis-related diseases, such as coronary artery disease, peripheral vascular disease, and stroke. Diabetic complications are the result of interactions among systemic metabolic changes, such as hyperglycemia, local tissue responses to toxic metabolites from glucose metabolism, and genetic and epigenetic modulators. Chronic hyperglycemia is recognized as a major initiator of diabetic complications. Multiple molecular mechanisms have been proposed to mediate hyperglycemia’s adverse effects on vascular tissues. These include increased polyol pathway, activation of the diacylglycerol/protein kinase C pathway, increased oxidative stress, overproduction and action of advanced glycation end products, and increased hexosamine pathway. In addition, the alterations of signal transduction pathways induced by hyperglycemia or toxic metabolites can also lead to cellular dysfunctions and damage vascular tissues by altering gene expression and protein function. Less studied than the toxic mechanisms, hyperglycemia might also inhibit the endogenous vascular protective factors such as insulin, vascular endothelial growth factor, platelet-derived growth factor, and activated protein C. Therefore, effective therapies for diabetic complications need to inhibit mechanisms induced by hyperglycemia’s toxic effects and also enhance the endogenous protective factors. The present review summarizes these multiple biochemical pathways activated by hyperglycemia and the potential therapeutic interventions that might prevent diabetic complications. (J Diabetes Invest, doi: 10.1111/j.2040-1124.2010.00018.x, 2010)

KEY WORDS: Diabetic complications, Diabetes mellitus, Endogenous protective factors

INTRODUCTION
According to the recent edition of International Diabetes Federation Atlas in 2009, the estimated diabetes prevalence for 2010 had risen to 285 million, representing 6.6% of the world’s adult population, with a prediction that by 2030 the number of people with diabetes in the world will have risen to 438 million, with the majority of the new diabetic population coming from Asia. Diabetes-induced vascular dysfunction and pathologies are the major causes of morbidity and mortality in diabetic patients. Microvascular complications include retinopathy, nephropathy, and neuropathy, which are the leading causes of blindness, renal failure, and nerve injuries that are associated with non-healing ulcers and non-traumatic amputation. Macrovascular complications involve atherosclerosis-related diseases, such as coronary artery disease, peripheral vascular disease, stroke, and possibly cognitive dysfunction.

Two large studies, the Diabetic Control and Complications Trial (DCCT) and the United Kingdom Prospective Diabetes Study (UKPDS) clearly showed that intensive treatment for hyperglycemia could reduce the progression of diabetic microvascular complications. Furthermore, the long-term follow-up studies of DCCT showed that patients who received intensive blood glucose control decreased the incidence of cardiovascular diseases involving atherosclerosis. These clinical observations indicate that hyperglycemia is a major responsible factor for the pathogenesis of diabetic complications. In contrast, it is known that multiple factors, such as fatty acid, lipid, insulin resistance, inflammatory cytokines and others also can increase the risk for atherosclerosis in diabetes.

Multiple potential molecular mechanisms have been proposed to explain hyperglycemia-induced diabetic complications. Some of the most studied mechanisms include increased polyol pathway, activation of the diacylglycerol/protein kinase C (PKC) pathway, increased oxidative stress, increased advanced glycation end products (AGE) formation and action, and increased hexosamine pathway. In addition, alterations of signal transduction pathways induced by hyperglycemia or toxic metabolites have been reported to cause multiple vascular and neurological dysfunctions, such as abnormal blood flow, increased rate of apoptosis, hyperpermeability and accumulation of extracellular matrix (ECM) in vasculature by alteration of

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Recently, we have proposed that hyperglycemia can also inhibit endogenous protective factors in the vascular tissues, such as insulin, vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), and activated protein C (APC), which play important roles in maintaining vascular homeostasis and neutralizing hyperglycemia-induced toxic factors including oxidative stress, AGE or activation of nuclear factor-κB (NF-κB), resulting in the prevention and delaying of the progression of diabetic complications (Figure 1). Genetic factors also have been suggested as important risk markers for developing diabetic complications. It has been well established that merely 30–40% of type 1 diabetic patients develop chronic renal failure. The risk of developing renal failure in diabetic patients decreases after 25–30 years of disease duration. Recently, we have reported that the results of the 50-Year Medalist Study of the Joslin Diabetes Center (JDC), which was initiated to recognize JDC or non-JDC patients who survived at least 50 years with insulin-dependent diabetes or type 1 diabetes. The patients were questioned about the presence or absence of eye, kidney and peripheral neuropathies. The Medalist Study showed that significant numbers (40%) of diabetic patients could live with no or mild levels of microvascular complications, regardless of their HbA1c levels and other classical markers thought to be important and predictive markers for diabetic complications. These data suggest that they might possess endogenous protective factors that can neutralize the adverse effects of hyperglycemia. Epigenetic factors are also important. The DCCT and Epidemiology of Diabetes Interventions and Complications (EDIC) studies reported that patients from the original DCCT study continue to have discordance in the development of microvascular complications, even 10 years after maintaining the same levels of glycemic control as shown by HbA1c. These findings showed that hyperglycemia might induce epigenetic changes that are not reversed easily. Thus, diabetic complications are a result of interactions among systemic metabolic changes, such as hyperglycemia, differential local tissue responses to toxic metabolites of glucose metabolism, and genetic and epigenetic modulators.

**MOLECULAR MECHANISMS OF DIABETIC VASCULAR COMPLICATIONS**

Hyperglycemia is recognized as a major responsible factor for the development of diabetic complications, especially for microvascular diseases. For example, pathologies in the retina and renal glomeruli are specific to diabetes and not usually observed in elderly or insulin resistant people without diabetes. The most studied mechanisms include: (i) increased polyol pathway; (ii) increased DAG/activation of PKC pathway; (iii) increased oxidative stress; (iv) increased AGE formation and action; and (v) increased hexosamine pathway (Figure 1).

**Increased Flux Through the Polyol Pathway**

In the polyol pathway, intracellular glucose is converted to sorbitol by aldose reductase (AR), which is the rate-limiting enzyme, in a nicotinamide adenine dinucleotide phosphate (NADPH) dependent reaction. Sorbitol is then oxidized to fructose by sorbitol dehydrogenase (SDH). In a normal glucose condition, only a small fraction of glucose is metabolized through this pathway, because Michaelis–Menten kinetics of
AR for glucose is above normoglycemic levels. In diabetic states, elevation of intracellular glucose levels can cause an increased flux through AR\textsuperscript{13,14}. The activation of the polyol pathway has been suggested to cause vascular pathologies by osmotic damage and reduced Na\textsuperscript+–K\textsuperscript+–ATPase activity\textsuperscript{15}. AR and SDH use NADPH and NAD\textsuperscript{+} as a cofactor, respectively. Therefore, the decline in cellular NADPH and the increased NADH/NAD\textsuperscript{+} ratio changes the intracellular redox balance resulting in the reduced production of nitric oxide and increased oxidative stress\textsuperscript{16}. Lenses specific AR overexpressed transgenic mice with diabetes showed a significant decrease in glutathione (GSH) level, leading to enhanced oxidative stress. In the AR null mutant mice, diabetes did not lead to any decrease in the nerve GSH level\textsuperscript{17}.

Studies inhibiting the polyol pathway using aldose reductase inhibitors (ARI) in vivo have yielded inconsistent results. In animal studies, ARI have been shown to prevent some abnormalities in cataracts, retinopathy\textsuperscript{18,19}, nephropathy\textsuperscript{20}, neuropathy\textsuperscript{21,22} and cardiomyopathy. However, in a 5 year study in dogs, AR inhibition could prevent only neuropathy, but failed to prevent retinopathy and nephropathy\textsuperscript{23}. In clinical studies, ARI have not been shown to be clearly effective in patients with diabetic retinopathy (DR) and nephropathy\textsuperscript{24}. For diabetic retinopathy, some studies have suggested positive effects. In a double-blind placebo controlled study, fidarestat showed improved nerve conduction velocity and a variety of subjective symptoms, such as numbness and spontaneous pain\textsuperscript{25}. In addition, it has been reported that long-term treatment with epalrestat also can effectively delay the progression of diabetic neuropathy and ameliorate the associated symptoms of the disease\textsuperscript{25,26}. More large full studies in phase three trials are needed to show that ARI can clearly be effective for neuropathy.

**Increased DAG/Activation of PKC Pathway**

DAG and PKC are important intracellular signaling molecules that can regulate many vascular functions. Receptor-mediated physiological PKC activation is mediated mostly by the activation of phospholipase C, which leads to an increase in Ca\textsuperscript{2+} and DAG levels\textsuperscript{27}.

Intracellular hyperglycemia increases glycolytic pathway flux and leads to an elevation of glycolytic intermediate dihydroxyacetone phosphate. Increased levels of this intermediate can stimulate increases in the de novo synthesis of DAG through the reduction of the latter to glyceraldehydes-3-phospate and stepwise acylation\textsuperscript{28}. In diabetes, many studies have shown that DAG levels in various tissues, such as retina\textsuperscript{29}, glomeruli\textsuperscript{30,31}, aorta and heart\textsuperscript{32} are increased. Furthermore, various cell culture studies also show that DAG levels are increased by the elevation of glucose levels from low to high concentration in retinal and aortic endothelial cells\textsuperscript{29,32}, smooth muscle cells\textsuperscript{32}, mesangial cells\textsuperscript{33,34} and other vascular cells. These chronically elevated levels of DAG can activate PKC. In addition, several PKC isoforms are also activated through other mechanisms, such as reactive oxygen species\textsuperscript{35,36} and free fatty acids (FFA)\textsuperscript{37,38}.

Increased PKC activation has been associated with alterations in blood flow, basement membrane thickening, ECM expansion, increases in vascular permeability, abnormal angiogenesis, excessive apoptosis, increased leukocyte adhesion, and changes in enzymatic activity alterations, such as Na\textsuperscript+–K\textsuperscript+–ATPase, cPLA\textsubscript{2}, PI3K and mitogen activated protein kinase (MAPK)\textsuperscript{39}. These effects are probably mediated through the altered gene expression for vasoactive and growth factors, such as VEGF\textsuperscript{40–42}, endothelin-1 (ET-1)\textsuperscript{43,44}, transforming growth factor (TGF)-\textbeta\textsuperscript{45,46} and connective tissue growth factor (CTGF)\textsuperscript{41,46,47}. Furthermore, PKC activation contributes to the overexpression of plasminogen activator-1 (PAI-1)\textsuperscript{48,49}, the activation of NF-\textkappaB and the activation of NAPDH oxidase\textsuperscript{50,51} in many vascular cells including endothelial cells, smooth muscle cells, pericytes, mesangial cells, and others\textsuperscript{52,53}.

PKC is a family of enzymes composed of at least 12 members\textsuperscript{54}. Of the various PKC isoforms in vascular cells, PKC-\textalpha, -\beta and -\delta isoforms appear to be preferentially activated by immunoblottings studies in the aorta and heart of diabetic rodents, cultured aortic smooth muscle cells, and endothelial cells exposed to high levels of glucose\textsuperscript{55,56}. However, increases in other isoforms, such as PKC-\textepsilon, -\beta\textsubscript{2}, -\delta, and -\varepsilon in the retinal cells\textsuperscript{29,43} and PKC-\textalpha, -\beta\textsubscript{1/2}, -\delta, -\epsilon, and -\zeta in the glomerular cells\textsuperscript{45,56–59} exposed to high glucose or diabetes have also been shown to be activated. In animals with diabetes, ruboxistaurin mesylate (RBX), a PKC-\beta isoform selective inhibitor, has been shown to prevent many vascular abnormalities associated with retinopathy, nephropathy and neuropathy\textsuperscript{31,45,57–59}. Furthermore, we showed that PKC-\beta null mice with streptozotocin (STZ)-induced diabetes showed improvement in renal abnormalities including albuminuria, renal hypertrophy and mesangial expansion\textsuperscript{46}. Recent studies by Harja et al. have also suggested that PKC-\beta activation might also play a role in accelerating atherosclerosis\textsuperscript{60}. Clinical studies showed that RBX improved endothelial dysfunction\textsuperscript{61}, renal glomerular filtration rate\textsuperscript{62} and prevented loss of visual acuity\textsuperscript{63} in diabetic patients. However, RBX was not effective in patients with painful diabetic neuropathy. Thus, PKC activation involving several isoforms is likely to be responsible for some of the pathologies in DR (Figure 2), nephropathy and cardiovascular disease.

**Increased Oxidative Stress**

Recent studies have suggested increases in oxidative stress as being a main metabolic abnormality involved in the development of diabetic complications\textsuperscript{14,64–66}. Oxidative stress occurs when the production of reactive oxygen species (ROS) exceeds the capability of antioxidant systems. There is substantial evidence showing that ROS production is increased in endothelial cells, kidney, retina either exposed to hyperglycemia or from diabetic animals\textsuperscript{51,67–69}. Likewise, diabetic patients have elevated levels of isoprostanes, 8-hydroxy-deoxyguanosine and lipid peroxides in the plasma or urine\textsuperscript{70,71}. The increased oxidative stress markers reflect increased production of ROS, decreased...
antioxidants, or both. Increased ROS production is a result of abnormal metabolism of glucose, FFA and other reactive metabolites in diabetes\textsuperscript{64,65}. Several processes are sources for increased ROS, including gluco-oxidants and AGE, which are created by non-enzymatic glycosylation and mitochondrial oxidative phosphorylation\textsuperscript{14,36}. Furthermore, byproducts of these processes can cause activation of certain signaling cascades, such as PKC, which can activate NADPH oxidase to increase ROS\textsuperscript{72}. Elevated FFA levels can also increase ROS production by β-oxidative phosphorylation through mitochondrial metabolism\textsuperscript{73}. Therefore, increased ROS production in diabetes can originate from the metabolism of both glucose and FFA through multiple pathways. This provides an explanation for the findings of increased oxidative stress in insulin resistant non-diabetic patients\textsuperscript{74}. In contrast, decreased antioxidants have been shown in diabetic animals and patients. For example, GSH level was decreased in kidneys and red blood cells from STZ-induced diabetic rats\textsuperscript{86,87}. Some studies showed that plasma vitamin C and E levels were decreased, whereas others showed no changes\textsuperscript{75,76}. However, it is possible that the plasma levels of antioxidants might not reflect those at the tissue levels.

Antioxidant therapies have been applied in animal experiments, such as vitamin C, vitamin E and α-lipoic acids. All of them have showed improved biological and pathological changes, and prevented or slowed the progression of diabetic complications\textsuperscript{64,65}. Overexpression of catalase or superoxide dismutase (SOD) protected the kidneys against hyperglycemia-induced damage in mice\textsuperscript{28,29}. However, large studies such as the Heart Outcomes Prevention Study using vitamin E and d-α-tocopherol, did not show the improvement of microvascular or cardiovascular damage\textsuperscript{80,81}. Therefore, the efficacy of antioxidants in humans is still inconclusive.

Increased AGE Formation and Action

Non-enzymatic reactions between glucose and proteins, known as the Maillard reaction, result in the formation of Schiff base. Over time, a series of chemical rearrangements lead to AGE\textsuperscript{82,83}. In the diabetic condition, elevated levels of AGE can be found in serum\textsuperscript{84}, glomerular tissue\textsuperscript{85} and retinal tissues\textsuperscript{86,87}. Some AGE are stable, irreversible products that can be formed intracellularly and extracellularly. AGE can cause vascular damage through several mechanisms. Intracellular proteins, such as basic fibroblast growth factor\textsuperscript{88} and mitochondrial electron related proteins\textsuperscript{89}, can be modified by AGE, which then alters their function. Glycation of ECM proteins, such as collagen I, IV and laminin\textsuperscript{90-92}, can change their function and alter cell/ECM interactions. Lipoproteins can also be glycated and altered in their metabolism\textsuperscript{93,94}.

AGE might also interact with cellular receptors, one of which is called receptor for AGE (RAGE), a transmembrane receptor that is a member of the immunogloblin superfamily of proteins\textsuperscript{95}. The AGE/RAGE interactions have been reported in the development of diabetic complications. It is reported that the expression of RAGE is increased in glomeruli in diabetic patients compared with healthy control subjects\textsuperscript{96,97}. Furthermore, detailed studies by Yamamoto \textit{et al}. clearly showed AGE/RAGE interactions, leading to diabetic nephropathy\textsuperscript{97} in animal studies. They overexpressed RAGE in vascular endothelial cells in mice and induced diabetes by crossbreeding them with mice overexpressing inducible nitric oxide synthase (iNOS) under the

![Figure 2](image-url) Outline for mechanisms of diabetic retinopathy. (A) Activation of protein kinase C (PKC)-β in retinal endothelial cells contributes to increased vascular permeability and formation of microaneurisms. (B) Activation of PKC-δ induce retinal pericytes apoptosis through activation of nuclear factor-κB (NF-κB) by oxidative stress and activating Src homology-2 domain containing phosphatase-1 (SHP-1) to inhibit platelet-derived growth factor’s (PDGF) survival actions. Akt, protein kinase B; ERK, extracellular signal regulated kinase; ET-1, endothelin-1; MAPK, mitogen activated nuclear factor-κB (NF-κB) by oxidative stress and activating Src homology-2 domain containing phosphatase-1 (SHP-1) to inhibit platelet-derived growth factor’s (PDGF) survival actions.
control of the insulin promotor. These mice consistently developed hypoinsulinemic diabetes as a result of NO-mediated selective destruction of insulin producing pancreatic β cells. The double transgenic mice showed the characteristics of diabetic nephropathy and progressive renal insufficiency, such as exacerbation of nephromegaly, mesangial expansion, albuminuria, glomerular hypertrophy and sclerosis.

Binding of AGE-modified proteins to RAGE induces activation of cellular signaling cascades including NF-κB98–100, ET-1, vascular cell adhesion molecule-1, intercellular adhesion molecule-1, E-selectin, VEGF, and proinflammatory cytokines including IL-1β, IL-6 and TNF-α are induced by NF-κB101,102. AGE/RAGE interactions have been also shown to induce vascular oxidative stress through the activation of NADPH oxidase103. In addition, other receptors, such as the macrophage scavenger receptor, p60, p90 and galectin-3, have also been reported to bind AGE104,105.

An inhibitor of AGE formation, aminoguanidine, can prevent the development of diabetic complications, such as retinopathy and nephropathy in animal models.106,107 However clinical trials using aminoguanidine have been inconclusive as a result of the presence of limiting toxicity. The cross-link breakers, including ALT-711 and N-phenyl-thiazolium bromide, improved arterial compliance and cardiac function, atherosclerosis and diabetic nephropathy.111,112 Blockade of the AGE/RAGE interaction by soluble RAGE has been shown to suppress atherosclerosis and neointimal formation and nephropathy in diabetic animals.116

Increased Flux Through the Hexosamine Pathway

In a normal glucose condition, only a small fraction (approximately 1–3%) of glucose is metabolized through the hexosamine pathway. Elevation of intracellular glucose levels can cause an increased flux through the hexosamine pathway. Fructose-6-phosphate, a glycolysis intermediate, is converted to glucosamine-6-phosphate by the rate-limiting enzyme, glutamine:fructose-6-phosphate aminotransferase (GFAT).117 The major end-product is uridine diphosphate N-acetylglucosamine (UDP-GlcNAc), which is a substrate for the subsequent O-linked GlcNAc modification of target proteins at serine and threonine residues. Functional importance of O-GlcNAc modification has been reported for several transcription factors, such as Sp-1118–120. Some reports have showed that glucosamine or overexpression of GFAT increased the promoter activity and expression of PAI-1 through the increased O-GlcNAc modification of Sp-1 in vascular endothelial cells121, smooth muscle cells122 and mesangial cells123. Furthermore, it is reported that glucosamine or GFAT overexpression stimulates the overexpression of TGF-β1 through increased expression upstream stimulatory factors 1 and 2 (USF1 and 2), but not increased O-GlcNAc modification of those transcription factors in mesangial cells.124 In addition, hyperglycemia might inhibit endothelial nitric oxide synthase (eNOS) activity by the O-GlcNAc modification at serine 1177 in endothelial cells125.

ALTERED EXPRESSION AND ACTIONS OF ENDOGENOUS PROTECTIVE FACTORS

The discussion of the present review has focused on the mechanisms by which hyperglycemia could be mediating its toxic effects. However, very few studies have focused on the endogenous protective factors that might exist to neutralize hyperglycemia’s toxic actions. One clear example of endogenous protective factors is the antioxidative enzymes, which are generally activated by an elevated state of increased oxidant production.126 Clinically, the pulmonary system appears to be protected from the toxic actions of hyperglycemia because patients with type 1 diabetes are relatively free from vascular pathologies of the pulmonary system. Furthermore, the 50-Year Medalist Study shows that some protective factors might exist and can neutralize high glucose-induced adverse effects in many diabetic patients. In the following, we will propose that many of the changes in the elevation of cytokines are as a result of the body’s responses to protect itself from injury. Hyperglycemia, through several mechanisms, might be deactivating these cytokines and causing resistance to them.

Systemic metabolic changes in patients with diabetes lead to altered expression or action of several factors, such as insulin, PDGF, VEGF or APC, which are physiologically important factors for keeping the homeostasis of vasculatures. In this part, we will focus on these endogenous protective factors against diabetes-induced vascular injuries and potential mechanisms induced by hyperglycemia, which are deactivating their actions.

Insulin

Insulin resistance is observed in patients with not only type 2 diabetes and obesity, but also type 1 diabetes. In addition to its important role for maintaining glycemic control, insulin has many vasotropics actions. Insulin resistance in vascular tissues is associated with endothelial dysfunction, leading to cardiovascular diseases including atherosclerosis. Furthermore, microalbuminuria, which is known as not only the predictive marker for nephropathy but also as the independent risk factor for cardiovascular diseases, is also associated with endothelial dysfunction. Physiologically, insulin has an important role in the maintenance of blood vessels through the activation of endothelium-derived NO. Insulin increases endothelial NO production by rapid post-translational mechanisms, which are mediated by the PI3K/protein kinase B (Akt) signaling pathway or slowly by increases in its transcription process. In insulin resistance states, the PI3K/Akt pathway is selectively inhibited, but another major pathway of insulin signaling, MAPK, is not inhibited. This selective insulin resistance has been shown in skeletal muscle from obese people and patients with type 2 diabetes, and in the vasculature and myocardium of obese Zucker rats, which are the animal models of insulin resistance. These are likely multiple mechanisms for inducing selective insulin resistance on the PI3K/Akt pathway. We have reported that PI3K activity is inhibited by PKC activation in endothelial cells and vascular tissues of obese Zucker rats. Furthermore, we found that RBX
can improve insulin signaling on NO production in the vasculature and myocardium of Zucker rats. Insulin stimulates not only NO production from endothelial cells but also the expression of eNOS. The vascular endothelial cells specific insulin receptor knockout (VENIRKO) mice showed that eNOS expression in the aorta was decreased by 62%. Thus, insulin regulation of NO might be an important factor for vascular homeostasis, which is reduced in diabetes or insulin resistance.

Because intensive glycemic treatment in clinical trials using insulin can delay the progression of retinopathy and other microvascular pathologies in type 1 diabetic patients, the loss of direct vasotropin actions of insulin might increase the risks of developing retinal disease in type 1 diabetic patients. Recently, we found that insulin can inhibit oxidative stress-induced retinal pericyte apoptosis through the induction of hemeoxygenase-1 (HO-1), which is a representative mediator of antioxidants and cytoprotectors against various stress stimuli, including oxidants in vascular tissues. Furthermore, we showed that insulin induced the expression of HO-1 through the PI3K/Akt pathway, but not through the MAPK pathway. Thus, insulin might exert vascular protective effects through the production of NO or the induction of HO-1. Therefore, impairment of insulin action in vascular tissue might contribute to diabetic vascular complications.

PDGF-B

PDGF-B is essential for the recruitment of mural cells, such as pericytes, to the blood vessels. PDGF-B or PDGFR-β deficient mice show a loss of retinal pericytes that resemble the early changes of DR. Pericyte loss is known as a hallmark of human DR and might be causally involved in its pathogenesis. These animal studies suggest that PDGF-B deficiency might trigger the development for DR. However, paradoxically, it has been shown that the expression of PDGF-B is increased in retinal tissues of diabetic rats. Recently, we found that hyperglycemia persistently activates PKC-δ and p38 MAPK to increase Src homology-2 domain containing phosphatase-1 (SHP-1), which is a representative mediator of antioxidants and cytoprotectors against various stress stimuli, including oxidants in vascular tissues. Other studies have shown also that the expression of VEGF-A was decreased in sclerotic lesions of nephropathy and is associated with the severity of proliferative DR (PDR). Anti-VEGF treatment, including intravitreal injection, can inhibit the progression of PDR. However, it is likely that retinal VEGF levels are initially elevated as a result of a reaction against retinal hypoxia or ischemia in diabetes to maintain endothelial function and circulation, as a result of pericytes loss and acellular capillaries. This increase in VEGF is probably a tissue response to increase survival. Thus, the use of chronic anti-VEGF therapies might have beneficial effects on the vasculature in the short-term. Clinically, the loss of VEGF without good glycemic control or a decrease of metabolic demands, as by photocoagulation, might cause complications in the neural retina.

Increased concentration of VEGF-A has been reported in the ocular fluids and retinal tissues of diabetic patients and is associated with the severity of proliferative DR (PDR). Anti-VEGF treatment, including intravitreal injection, can inhibit the progression of PDR. However, it is likely that retinal VEGF levels are initially elevated as a result of a reaction against retinal hypoxia or ischemia in diabetes to maintain endothelial function and circulation, as a result of pericytes loss and acellular capillaries. This increase in VEGF is probably a tissue response to increase survival. Thus, the use of chronic anti-VEGF therapies might have beneficial effects on the vasculature in the short-term. Clinically, the loss of VEGF without good glycemic control or a decrease of metabolic demands, as by photocoagulation, might cause complications in the neural retina.

In early stage diabetic nephropathy, many reports have shown that the expression of VEGF-A is increased in glomeruli of diabetic animals and proposed that inhibition of VEGF-A might have beneficial effects against diabetic renal injuries. Treatment with VEGF-A antibodies in STZ-induced diabetic rats ameliorated renal changes, such as albuminuria, hyperfiltration, and glomerular hypertrophy. Furthermore, in db/db mice also, administration of antibodies to VEGF improved renal abnormalities including kidney weight, glomerular volume, basement membrane thickness and albuminuria. However, another study showed that treatment with VEGF-A antibodies did not improve diabetic renal abnormalities in G-K rats. At the early stage of human diabetic nephropathy, increased expression of VEGF accompanied glomerular endothelial cell proliferation and extra small vessel formations in the vascular pole. At the late stage of nephropathy, the expression of VEGF-A is decreased. Baelde et al. showed that the glomerular VEGF-A expression was decreased by 2.5-fold and coincided with endothelial cells and the reduction of podocyte makers in the moderate-severe stage of type 2 diabetic nephropathy. Other studies have shown also that the expression of VEGF-A was decreased in sclerotic lesions of nephropathy.

What is the physiological role of VEGF-A in the kidney, especially glomeruli? It is reported that treatment with anti-VEGF antibodies to patients with cancers or within patients with preeclampsia causes proteinuria and endothelial damage, suggesting that VEGF-A plays an important role in maintaining endothelial cell function and the glomerular filtration barrier. Supporting this, detailed reports by Quaggin et al. clearly show that VEGF-A is necessary for forming and maintaining the glomerular filtration barrier. In their reports, using a conditional Cre-loxP targeting system, podocyte-specific VEGF null mice failed to form a glomerular filtration barrier as a result of defects in endothelial cell migration, survival and differentiation; resulting in perinatal lethality. Loss of a single VEGF-A allele in podocytes leads to endotheliosis, a nephrotic syndrome accompanied by glomerulosclerosis, renal failure and death at 9–12 weeks-of-age. Furthermore, they reported that adult mice with inducible podocyte-specific knockout of VEGF

Vascular Endothelial Growth Factor A

VEGF includes a family of growth factors that act on endothelial cells regulated by hypoxia and promote angiogenesis, increase permeability in vasculature, and is also known as a major regulator of endothelial proliferation, migration, and survival.
developed proteinuria, hypertension with swollen endothelial cells and intracapillary thrombus in glomeruli after 4–5 weeks after induction of conditional knockout. The results from these mice were similar to renal abnormalities of patients with thrombotic microangiopathy as a result of treatment with anti-VEGF, bevacizumab\textsuperscript{164}. Furthermore, in several other glomerular diseases, a beneficial role of VEGF has been shown through the prevention of progressive capillary rarefaction, promotion of capillary repair/regeneration, improvement of glomerulosclerosis, and renal scarring\textsuperscript{165–168}. Quaggin et al. have also reported that overexpression of VEGF-A to mRNA levels 15–20-fold higher than in wild-type mice, leads to collapse of the glomerular tuft, proteinuria and death from renal failure within the first week of life\textsuperscript{162,163}. Therefore, it is thought that tight regulation of VEGF-A signaling is required for development and maintenance of the glomerular filtration barrier.

Is VEGF-A a bad or good player for the progression of diabetic nephropathy? Hohenstein et al. determined VEGF expression and its bioactivity in glomeruli of type 2 diabetic patients using specific antibodies for VEGF-A and VEGF-VEGFR complex\textsuperscript{154}. Although VEGF expression of glomeruli is upregulated during all stages (mild, moderate and severe) of nephropathy, VEGF bioactivity in endothelial cells is only increased in mildly injured glomeruli and decreased in moderate or severe lesions. Furthermore, they showed that glomerular capillary rarefaction was linked to the degree of glomerulosclerosis and endothelial cell proliferation, showing capillary repair was markedly increased only in mildly/moderately injured glomeruli, even if apoptosis was detected in all stages. They suggest that diabetic nephropathy is associated with glomerular capillary rarefaction by an imbalance of endothelial cell proliferation, repair and apoptosis, and injury; and reduced VEGF activity might be an indicator of an insufficient capillary repair reaction\textsuperscript{154}. Therefore, if increased VEGF expression occurs as a reaction of compensation for the damage of glomerular endothelial cells, inhibition of VEGF should not be given as a treatment for diabetic nephropathy. However, further studies are needed to conclude whether VEGF-A is or is not an endogenous protective factor for diabetic nephropathy.

**Activated Protein C**

APC is also an endogenous protective factor for endothelial cells. The production of APC is dependent on binding between thrombomodulin and thrombin, which occurs on the surface of the endothelial cells. The thrombin/thrombomodulin complex catalyses the conversion of protein C to its activation form, APC. APC acts directly on cells to exert multiple cytoprotective effects including anti-inflammation, anti-apoptotic activities and protection of endothelial barrier function through the endothelial protein C receptor, protease-activated receptor-1 or sphingosine-1 receptor\textsuperscript{169}.

It has been reported that plasma thrombomodulin levels, which are thought to reflect loss of thrombomodulin from the endothelium and reduced levels of APC, are elevated in patients with diabetes, and the impairment of thrombomodulin/protein C system is associated with diabetic complications, such as nephropathy and neuropathy\textsuperscript{170,171}. Recently, Isermann et al. showed that impaired APC formation as a result of reduced thrombomodulin expression is associated with diabetic nephropathy; and the increased levels of APC can prevent diabetic nephropathy through anti-apoptotic effects against diabetes-induced endothelial cells and podocytes\textsuperscript{172}.

Recent studies described in the present review identify various mechanisms by which hyperglycemia can induce adverse effects to cause diabetic complications. Inhibition of AR, PKC, AGE/RAGE interaction or oxidative stress should provide useful targets for treatment. Treatments for these targets have been successful in animal models with diabetes; however, many clinical trials using agents directly against these targets have not shown a robust effort to prevent or stop the various diabetic complications. The lack of efficacy of these agents suggests that other mechanisms are involved in the development of diabetic complications.

We proposed that the function of endogenous protective factors, including insulin, VEGF, PDGF and APC, are important for vascular homeostasis and might also be impaired by diabetes (Figure 3). Therefore, further studies are needed to understand the loss of protective factors in the development of diabetic complications. New therapies need to inhibit hyperglycemia’s toxic effect and enhance endogenous protective factors in order to be effective.

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