Activation of Protein Kinase C Isoforms and Its Impact on Diabetic Complications

Pedro Geraldes, George L. King

Abstract: Both cardio- and microvascular complications adversely affect the life quality of patients with diabetes and have been the leading cause of mortality and morbidity in this population. Cardiovascular pathologies of diabetes have an effect on microvenules, arteries, and myocardium. It is believed that hyperglycemia is one of the most important metabolic factors in the development of both micro- and macrovascular complications in diabetic patients. Several prominent hypotheses exist to explain the adverse effect of hyperglycemia. One of them is the chronic activation by hyperglycemia of protein kinase (PK)C, a family of enzymes that are involved in controlling the function of other proteins. PKC has been associated with vascular alterations such as increases in permeability, contractility, extracellular matrix synthesis, cell growth and apoptosis, angiogenesis, leukocyte adhesion, and cytokine activation and inhibition. These perturbations in vascular cell homeostasis caused by different PKC isoforms (PKC-α, -β1/2, and PKC-δ) are linked to the development of pathologies affecting large vessel (atherosclerosis, cardiomyopathy) and small vessel (retinopathy, nephropathy and neuropathy) complications. Clinical trials using a PKC-β isoform inhibitor have been conducted, with some positive results for diabetic nonproliferative retinopathy, nephropathy, and endothelial dysfunction. This article reviews present understanding of how PKC isoforms cause vascular dysfunctions and pathologies in diabetes. (Circ Res. 2010;106:1319-1331.)

Key Words: protein kinase C ■ vascular cell biology ■ diabetes ■ cardiovascular diseases
Non-standard Abbreviations and Acronyms

ACE  angiotensin-converting enzyme
AGE  advanced glycation end product
CTGF  connective tissue growth factor
DAG  diacylglycerol
EC  endothelial cell
eNOS  endothelial nitric oxide synthase
ERK  extracellular signal–regulated kinase
ET-1  endothelin-1
MAPK  mitogen-activated protein kinase
MI  myocardial infarction
NTSS-6  neuropathy total symptoms score-6
PDGF  platelet-derived growth factor
PI3K  phosphoinositide 3-kinase
PKC  protein kinase C
PMA  phorbol 12-myristate 13-acetate
RBF  retinal blood flow
RBX  ruboxistaurin
SMC  smooth muscle cells
TGF  transforming growth factor
VEGF  vascular endothelial growth factor

Activation of DAG–PKC Pathway in Diabetes

DAG levels are elevated chronically in the hyperglycemic or diabetic environment because of an increase in the glycolytic intermediate dihydroxyacetone phosphate. This intermediate is reduced to glycerol-3-phosphate, which subsequently increases de novo synthesis of DAG. In diabetes, total DAG levels are elevated in vascular tissues, such as the retina, aorta, heart, and renal glomeruli, and in nonvascular tissues, such as liver and skeletal muscles. However, there is no consistent change in DAG levels in the central nervous system and peripheral nerves. Various cell culture studies have shown that DAG levels increase in a time-dependent manner as glucose levels elevate from 5.5 to 22 mmol/L in aortic endothelial cells, retinal pericytes, smooth muscle cells, and renal mesangial cells.

PKC, a group of enzyme members of the AGC (cAMP-dependent protein kinase/PKG/PKC) family, is a serine/threonine-related protein kinase that plays a key role in many cellular functions and affects many signal transduction pathways. There are multiple isoforms of PKC that function in a wide variety of biological systems. The conventional PKC (ePKC) isoforms (PKC-α, -β1, -β2, and -γ) are activated by phosophatidylserine, calcium, and DAG or phorbol esters such as phorbol 12-myristate 13-acetate (PMA), whereas novel PKCs (nPKCs) (PKC-δ, -ε, -θ, and -η) are activated by phosophatidylserine, DAG or PMA, but not by calcium. The atypical PKCs (aPKCs) (PKC-ζ and -δ/A) are not activated by calcium, DAG or PMA (Figure 1). Extensive and excellent reviews concerning PKC structural basic activation have been published. Given the present breadth of knowledge in this area, we focus our attention on how hyperglycemia modulates PKC activation. PKCs can also be activated by oxidants such as H2O2 in a manner unrelated to lipid second messengers and by mitochondrial superoxide induced by elevated glucose levels. Many abnormal vascular and cellular processes and deregulations, including endothelial dysfunction, vascular permeability, angiogenesis, cell growth and apoptosis, changes in vessel dilation, basement membrane thickening, and extracellular matrix (ECM) expansion; enzymatic activity alterations, such as in mitogen-activated protein kinase (MAPK), cytosolic phospholipase A2, Na+/ K+–ATPase; and alterations in several transcription factors (Figure 2) are attributed to multiple PKC isoforms that are changed by diabetes (Table 1). These PKC-induced vascular and tissue pathologies are discussed in detail in the following paragraphs.

PKC Activation: Cell Culture Studies

Endothelial Cells

Endothelial cells (ECs) regulate both vasodilator and vasoconstrictor substances mediating coagulation, platelet adhesion and immune function and control volume and electrolyte content of the intravascular and extravascular spaces. Tight junctions between ECs form a vascular barrier, which in the diabetic state becomes more vulnerable and permeable as a result of hyperglycemia.
result of EC abnormalities. PKC activation directly increases the permeability of albumin and other macromolecules through barriers formed by ECs. Moreover, Inoguchi et al reported that hyperglycemia or PMA inhibits gap junction intercellular communication in bovine aortic ECs. Stauroporine, a serine/threonine kinase inhibitor, prevented these effects of high glucose. High glucose exposure induced translocation of PKC-α, -β1, -β2, and PKC-δ but not PKC-ε or PKC-ζ in retinal ECs. A previous study showed that overexpression of PKC-β1 in human dermal microvascular ECs enhances phorbol ester–induced increased permeability to albumin. Hempel et al also showed that PMA increases permeability via translocation of PKC-α similar to that caused by high glucose levels. This permeability is prevented by stauroporine. PKC activation also results in endothelium-dependent vasodilator dysfunction by altering the bioavailability of nitric oxide (NO), affecting vascular endothelial growth factor (VEGF) expression and actions and decreasing production of prostacyclin, as well as increasing the production of thromboxane, other cyclooxygenase-dependent vasoconstrictors and endothelin (ET)-1 (Figure 3). A previous study demonstrated that endothelial nitric oxide synthase (eNOS) expression is decreased in aortic ECs cultured in high glucose concentrations, resulting in a reduction of NO. Treatment with calphostin C, an inhibitor of c- and nPKC (novel PKC), prevented high glucose levels from reducing NO production. Another potential pathway by which high concentrations of glucose reduce NO bioavailability is by increasing superoxide production from NADPH oxidase in aortic ECs. The induction of several subunits of NADPH oxidase by constant or intermittent high glucose concentrations is decreased with treatment of a PKC-β inhibitor (LY379196). PKC activation also modulates vascular endothelial permeability and neovascularization via the expression of growth factors, such as VEGF/vascular permeability factor. One study showed that both VEGF/vascular permeability factor mitogenic and permeability actions are, in part, the result of the membrane expression of PKC-α and -β through tyrosine phosphorylation of phospholipase-C, which is reduced by a PKC-β inhibitor, LY333531. Moreover, PKC-β is required for VEGF-induced retinal EC permeability by altering occluding phosphorylation. One
Hyperglycemia regulates the LOX-1 (leptin-like oxidized lipoprotein receptor-1) and enhanced membrane PKC-β2 expression in human monocyte-derived macrophages. These effects of high glucose levels in LOX-1 expression are prevented by treatment with calphostin C or PKC-β inhibitor (LY379196). Ceolotto et al reported that monocytes isolated from diabetic patients showed higher membrane PKC activity compared to those from control subjects. This study also demonstrated that membrane PKC-β activity, but not -α, isoform, is increased in diabetic monocytes. Furthermore, Osto et al reported that inhibition of PKC-β reduced low-density lipoprotein uptake by macrophages. This study also showed that treatment with LY333531 significantly attenuates macrophage activation by reducing intercellular adhesion molecules-1 and monocyte chemotactic protein-1 protein expression. A recent study from Dasu et al reported that elevation of glucose levels in the media increases the expression of toll-like receptors 2/4 in the human acute monocytic leukemia cell line, whereas inhibition of PKC-α and PKC-δ prevents this effect. Overall, these data suggest that PKC signaling could be in part responsible for macrophage activation/attachment and foam cell formation induced in the hyperglycemic state (Figure 3).

**Mesangial Cells**

The hallmark of diabetic kidney disease is the thickening of glomerular basement membrane and accumulation of ECM in glomerular mesangium and tubulointerstitium. Histological analysis showed increases in type IV and VI collagen, fibronectin, and laminin and decreases in proteoglycans in the mesangium of diabetic patients with nephropathy, and probably in the vascular endothelium in general. In vitro experiments reported that hyperglycemia or PMA increases type IV collagen and fibronectin expression in mesangial cells, which is prevented by calphostin C. Kapor-Drezgic et al reported that total PKC activity measured by in situ 32P-phosphorylation of epidermal growth factor substrate is increased by hyperglycemia in rat mesangial cells. Length of exposure to high glucose also influences PKC isoform translocation in these cells. Previous studies reported that low-density lipoprotein receptor-1 and enhanced membrane PKC-β2 expression in human monocyte-derived macrophages. These effects of high glucose levels in LOX-1 expression are prevented by treatment with calphostin C or PKC-β inhibitor (LY379196).
exposure to high glucose levels or PMA stimulation increases PKC-α, -β1, -β2, PKC-δ, and -ε membrane phosphorylated fraction in mesangial cells.\textsuperscript{53-57} AGEs also play a significant role in the pathogenesis of vascular and renal complications associated with diabetes. Two groups indicated that AGEs modulate, directly or indirectly via oxidative stress, particulate and membrane localization of PKC-β in mesangial cells.\textsuperscript{58,59} In addition, the development of mesangial expansion and basement membrane thickening in diabetes correlates with increased expression of transforming growth factor (TGF)-β. The implication of PKC causing elevated production of ECM and TGF-β is further supported by several reports showing that LY333531 prevents hyperglycemia-increased ECM production and TGF-β expression in mesangial cells.\textsuperscript{60} Another group demonstrated that high glucose concentrations stimulate PKC-ζ activity, as measured by immune complex kinase assay and immunofluorescence confocal microscopy. This activity is suppressed by a TGF-β receptor inhibitor (LY364947).\textsuperscript{61}

**PKC Activation: Animal Model Studies**

**Atherosclerosis**

Diabetes macrovasculopathy is associated with structural and functional changes in large vessels that lead to blood flow obstruction, hypertension, myocardial infarction (MI) and possibly death. Atherosclerosis, characterized by endothelial dysfunction, cytokine expression, monocyte infiltration, SMC proliferation, impaired fibrinolysis combined with increased thrombosis, and chronic inflammation, causes ischemic heart diseases, MI, stroke, and peripheral arterial diseases.\textsuperscript{62-64} Hyperglycemia is believed to participate in atherosclerotic plaque formation. Intensive therapy treatment (Diabetes Control and Complications Trial study) showed a significant decrease in carotid artery intima/media thickness.\textsuperscript{65} However, the disease state associated with insulin resistance can also accelerate atherosclerosis which suggests that metabolic abnormalities associated with diabetes, and not hyperglycemia per se, can increase the risk for macrovascular complications. Nevertheless, activation of PKC isoforms, especially the β isoform, has been the subject of recent investigation. Schmidt, Yan, and colleagues recently published supportive data on the potential role of PKC-β in atherosclerotic plaque formation. Depletion of PKC-β gene or treatment with LY333531 in apolipoprotein e-deficient mice decreased atherosclerosis by inhibiting the Erg-1 (early growth response-1) protein, which regulates vascular cell adhesion molecule expression and matrix metalloproteinase-2 activity preferentially in ECs.\textsuperscript{66} However, additional isoforms may be involved in other steps of atherosclerosis. Novel PKC-δ participates in SMC apoptosis and deletion of this isoform, leading to formation of arteriosclerosis.\textsuperscript{67} Therefore, whether it will be beneficial to inhibit multiple PKC isoforms to halt or prevent complex mechanisms related to atherosclerosis remains to be resolved.

**Cardiomyopathy**

Myocardial pathologies in diabetic patients include diastolic dysfunction, microvascular diseases, and interstitial fibrosis, the last of which could be the result of active tissue remodeling contributing to reduction of cardiac contractility.\textsuperscript{68} Independent of the severity of coronary artery disease, hypertension and other risk factors, diabetic patients have elevated risk of congestive heart failure, inadequate collateral vascular formation in response to ischemia\textsuperscript{69} and greater likelihood of developing heart failure and reinfarction after MI.\textsuperscript{70} Quantitative immunoblotting revealed a significant increase in membrane fraction expression of PKC-β1 and -β2 in human failed hearts compared to nonfailed.\textsuperscript{71} This study also showed that treatment with LY333531 decreases PKC activation in human heart.\textsuperscript{72} Previously, animal studies reported that PKC-α, -β1, and -β2 isoform mRNA and protein expression in the membrane fraction are increased in the heart and aorta in the hyperglycemic condition.\textsuperscript{12,72} Genetically modified mice have been developed to better examine the role of PKC in heart diseases. Transgenic mice overexpressing PKC-β2 develop cardiomyopathy, supporting the role of PKC-β2 in myocardial hypertrophy and fibrosis.\textsuperscript{73} Both angiotensin-converting enzyme (ACE) and PKC-β inhibitors ameliorate the metabolic gene profile and affect PKC activity in diabetic hearts without altering circulating metabolites.\textsuperscript{74} In cardiomyocytes, free fatty acids increased metabolic genes such as PDK4 and UCP3 and mediated the inhibition of basal and insulin-stimulated glucose oxidation, all of which are prevented by treatment with LY333531. Furthermore, treatment with islet cell transplantation, LY333531 and ACE inhibitor (captopril) improved diastolic function, increased glucose utilization by 36\%\textsuperscript{74} and attenuated myocyte hypertrophy and collagen deposition\textsuperscript{75} in diabetic rat hearts. Together, these results suggest that both ACE and PKC-β inhibitors regulate fuel metabolic gene expression directly in the myocardium and consequently improve cardiac function and metabolism in diabetes. Other PKC isoforms may also play a role in cardiomyopathy. A recent study reported that PKC-α-deficient mice exhibit increased cardiac contractility and are less susceptible to heart failure following long-term pressure-overload stimulation.\textsuperscript{76}

Connective tissue growth factor (CTGF) expression may contribute to cardiac fibrosis. Interestingly, transgenic PKC-β2 mice showed increased CTGF expression in the myocardium, suggesting that CTGF may act directly or indirectly with other cytokines to induce cardiac fibrosis.\textsuperscript{73} By opposition, inhibition of PKC-δ exacerbated CTGF expression and potentially cardiac fibrosis in the presence of angiotensin II.\textsuperscript{77} However, careful attention should be given to PKC-δ activation because this isoform regulates proapoptotic signaling in cardiomyocytes during myocardial ischemia/reperfusion injury.\textsuperscript{78} Furthermore, cardiac-specific activation of PKC-ε prevented diabetes-induced pathogenetic changes in the heart, including ventricular function\textsuperscript{79} (Figure 4).

Among the processes induced by hyperglycemia, activation of PKC may contribute to cardiomyopathy by inhibiting insulin’s metabolic actions, perhaps by phosphorylation of serine/threonine residues on the insulin receptors or their substrates.\textsuperscript{80} Cardiomyocytes isolated from nondiabetic animals and cultured in high glucose concentrations exhibited impaired insulin-stimulated glucose uptake compared to myocytes in normal glucose levels.\textsuperscript{81} Loss of insulin action in myocardium is associated with lower basal expression of...
hyoxia-inducible factor-1α, which affects VEGF expression in the myocardium.82 In contrast to nondiabetic patients, insulin-resistant and diabetic patients showed a downregulation of VEGF and its receptors.83 Moreover, several studies of diabetic animals confirmed this finding, showing a reduction of mRNA and protein expression of VEGF and its receptors, cardiac VEGF signaling, and coronary capillary density in the myocardium.84,85 Interestingly, another group reported that diabetic animals, blood flow is decreased in the retina but elevated in glomeruli.90–92 In streptozotocin-induced diabetic rats, treatment with LY333531 (0.1 to 10 mg/kg) improved retinal hemodynamic abnormalities.13 Interestingly, systemic oral administration for 2 weeks of LY333531 to diabetic rats from the onset of the disease normalized the retinal blood flow (RBF)13 and prevented the induction of ET-1 mRNA expression.93 Furthermore, local intravitreous injection of LY333531 (5 nmol/L) reduced retinal PKC activation and retinal circulation time and restored RBF in diabetic rats.94 Alterations in NO production and eNOS expression directly influence vascular hemodynamics such as contraction and relaxation, which may affect RBF. In vessels isolated from diabetic patients and animals, acetylcholine stimulation, which induces vessel relaxation, appears to be delayed.95,96 PKC agonist PMA provokes vascular relaxation impairment in normal arteries.97

How diabetes regulates and increases proapoptotic factors has been researched for many years. However, the impact of hyperglycemia on reducing survival factors has been much less studied. We recently reported that both PKC-β and PKC-δ translocate to the membrane fraction in total retinal lysates of diabetic mice, but the consequences of these 2 PKC isoform activations were very different: PKC-δ induced cellular apoptosis,17 whereas PKC-β enhanced cellular growth.98 Accordingly, an increase in membrane PKC-δ levels for several months of diabetes correlated with the appearance of retinal pericyte apoptosis in vitro and acellular capillaries in vivo. In vivo studies showed that induction of retinal PKC-δ of diabetic mice leads to PDGF resistance, which is not observed in Prkcd−/− mice. Furthermore, specific inhibition of PKC-δ in pericytes in vitro or disruption of Prkcd−/− mice prevented NF-κB activation and restored signal transduction of PDGF. Our study found that hyperglycemia through PKC-δ actions promotes 2 distinct and equally important pathways by (1) increasing reactive oxygen species production and NF-κB activity and (2) decreasing the important survival signaling pathway of PDGF by upregulating the expression of a protein tyrosine phosphatase, SHP-1. These findings identify a pivotal role for PKC-δ in causing retinal cell apoptosis and the formation of acellular capillaries (Figure 5).17

Retinal Tissues
The pathogenesis of diabetic retinopathy is a complex process involving multiple factors.87 The early stage of diabetic retinopathy is characterized by loss of pericytes around capillaries in the retina. Indeed, pericyte loss occurs before clinically discernible retinopathy. This is followed by development of weakness in the capillary wall, leading to capillary aneurysm formation (microaneurysms) and fluid leakage from capillaries as their walls become more permeable, with increased adhesion of leukocytes and monocytes to the endothelium.87 Hyperglycemia activates several PKC isoforms in retinal tissues, including PKC-α, -β, -δ, and -ε.12,88 PKC activation causes retinal vascular dysfunction by altering enzyme activities in ECs (NO, ET-1, VEGF) and peri-cytes (platelet-derived growth factor [PDGF], reactive oxygen species, nuclear factor κB, and MAPK).89 In diabetic animals, blood flow is decreased in the retina but elevated in glomeruli.90–92 In streptozotocin-induced diabetic rats, treatment with LY333531 (0.1 to 10 mg/kg) improved retinal hemodynamic abnormalities.13
LY333531 protects against diabetes by causing increases in renal hypertrophy, glomerular hyperfiltration, ECM production, expression of CTGF, and production of TGF-β1 and reactive oxygen species (Figure 6). Even in nondiabetic kidney disease, LY333531 treatment attenuated the impairment in glomerular filtration rate and reduced the extent of both glomerulosclerosis and tubulointerstitial fibrosis in subtotally nephrectomized rats. Interestingly, analysis of the renal phenotype in Prkce−/− mice showed elevated albuminuria at 6 and 16 weeks of age, with increased tubulointerstitial fibrosis and mesangial cell expansion. These data suggest that not all PKC isoforms have adverse effects on kidney pathologies.

Neuronal Tissues

The contribution of PKC activation to diabetic neuropathy still requires clarification. PKC contributes to diabetic neuropathy by a neurovascular mechanism such as blood flow and conduction velocity. Immunochemical analysis demonstrated the presence of PKC-α, -β1, -β2, -γ, PKC-δ, and -ε isoforms in nerve. A previous report demonstrated a reduction of PKC activity by direct measurement of sciatic nerve tissues in streptozotocin diabetic rats. These results contrast with more recent studies showing that treatment with nonselective PKC inhibitor improve neural function in diabetic animals. Initial evidence suggested that PKC is involved in the mechanism leading to reduced Na+/K+-ATPase activity, resulting in decreased nerve conduction and nerve regeneration. Membrane-associated PKC activity was reduced in diabetic mice treated with nonselective PKC inhibitors that restored or maintained Na+/K+-ATPase activity. Furthermore, previous studies examined the effect of diabetes in rat sciatic nerve and demonstrated that LY333531 treatment prevents the development of diabetic nerve dysfunction. Indeed, Cameron et al showed that treatment with LY333531 at low dose improves motor nerve conduction velocity, normalizes nerve blood flow and restores Na+/K+-ATPase activity in diabetic rats. However, in this study, diabetes did not affect nerve PKC activity or DAG levels. Overall, these studies suggest that PKC inhibition on nerve conduction improves nerve blood flow rather than nerve Na+/K+-ATPase activity.

Insulin Resistance

Although the principal function of insulin is to regulate and maintain glycemic control, this hormone has many vasotropic actions. Insulin normally stimulates vasorelaxation through a direct effect on blood vessels mediated by endothelium-derived NO. Insulin also promotes endothelial NO production by rapid posttranslational mechanisms, as well as eNOS gene expression. Therefore, insulin resistance, diabetes or reduction of insulin-stimulated regulation of endothelium-derived NO may be an important factor for vascular homeostasis. Insulin signaling in vasculature of obese Zucker rats showed inhibition of the phosphoinositide 3-kinases (PI3Ks) pathway but no change in the extracellular signal-regulated kinases (ERK)1/2 cascade, suggesting a selective insulin resistance phenomenon. This observation was subsequently reported in skeletal muscle from obese people and patients with type 2 diabetes and in the myocardium of obese Zucker rats. Vasculature of obese Zucker rats also exhibited elevated PKC activity and reduced insulin-stimulated insulin receptor substrate tyrosine phosphorylation. The question of which PKC isoform's elevation is involved in this process has generated much interest. Treatment with LY333531 normalized the reduction of insulin-stimulated NO production in the aorta of obese Zucker rats. Transgenic mice overexpressing PKC-β2 exhibited decreased Akt activation in vascular cells after insulin stimulation. Although strong evidence corroborates PKC-induced insulin signaling inhibition, the complex mechanisms involved are not fully understood. PKC activation prevents PI3K pathway at the insulin receptor substrate level, which affects several signaling molecules related to this pathway (see elegant review by Sampson and Cooper).
Insulin actions mediating the ERK1/2 pathway are accentuated by PKC actions. Thus, selective insulin resistance, caused by PKC activation, enhances insulin’s proatherosclerotic mechanisms via ERK1/2 signaling or inhibits its antiatherosclerotic mechanisms by inhibiting the PI3K/Akt pathway.

**PKC Inhibitors and Human Clinical Trials**

General PKC isoform inhibitors exist. However, these non–isoform-specific inhibitors interact with other ATP binding kinases and therefore display toxic and severe side effects in vivo. Suitable specific PKC isoform inhibitors for therapeutic or clinical studies should target the phospholipid or phorbol ester binding site of the PKC structure, called regulatory domain, or bind to the substrate or ATP binding site of the catalytic domain. For example, indolcarbazole or bisindolylmaleimide are selective inhibitors that target the ATP-binding site of the PKC structure, called regulatory domain, or bind to the substrate or ATP binding site of the catalytic domain. The PKC-β inhibitor ruboxistaurin (RBX) (also known as LY333531 or Arxxant, Eli Lilly, Indianapolis, Ind) is a class of bisindolylmaleimide. Rottlerin (mallotoxin), a natural product derived from *Mal- lotus philippensis*, has higher affinity for PKC-δ (IC₅₀, 3 to 6 μmol/L) but also inhibits other isoforms of PKC (IC₅₀, >30 μmol/L). In contrast, RBX shows selective inhibition for PKC-β1 and PKC-β2 with IC₅₀ values at 4.5 and 5.9 nmol/L, respectively.

RBX is the most studied PKC inhibitor in cellular, animal and especially human studies (Table 2). Phase I studies using RBX included patients who had diabetes for less than 10 years and no evidence of clinical retinopathy. This study determined the dose needed to normalize RBF, an early marker of diabetic retinopathy. The dose–response curve showed that a minimum dosage of 32 mg/d orally is required to prevent decreases in RBF in diabetic patients. Low doses did not completely normalize RBF. Few side effects were found during clinical trials that lasted up to 4 years. Phase II and phase III clinical trials were conducted in late stages of nonproliferative diabetic retinopathy, with the loss of visual acuity as the primary end point. The first 2 clinical trials, PKC-Diabetic Retinopathy Study (PKC-DRS) and PKC-Diabetic Macular Edema Study (PKC-DMES), failed to reach primary outcomes because of multiple factors (unpowered, 3 treatment arms of differing dosages, high dropout rate of patients). However, there was a significant reduction in the secondary end point of the progression of diabetic macular edema to a certain stage. A much larger clinical trial, PKC-DRS2, was undertaken using a single oral dose (32 mg versus placebo), with the primary end point of visual acuity rather than diabetic retinopathy progression. The results showed that RBX significantly prevents reduction of visual acuity in diabetic patients with moderate visual loss and decreases the onset of diabetic macular edema. These clinical results suggest that PKC activation, especially of the β isoform, participates in the development of diabetic retinopathy. However, because treatment with RBX preserves visual acuity by decreasing capillary permeability or targeting the neural retina but cannot significantly delay the progression of diabetic retinopathy, these data suggest that inhibition of the PKC-β isoform alone is not enough to stop the early metabolic changes that are likely driving the progression of preproliferative diabetic retinopathy.

The role of PKC-β was also evaluated in patients with diabetic nephropathy. In kidney biopsies of diabetic patients, quantitative real-time PCR analysis showed a 9.9-fold increase in PKC-β mRNA expression as compared to control subjects. A phase II clinical trial was conducted using RBX (32 mg/d) to determine whether PKC-β inhibition can be effective in type 1 and 2 diabetic patients with high proteinuria (>300 mg/d) treated with ACE inhibitors or angiotensin-receptor blockers. Results suggested that 1 year of treatment with RBX in addition to angiotensin II inhibitors or receptor blockers decreases the loss of glomerular filtration rate and proteinuria in diabetic patients. However, more data and phase III clinical trials are needed to confirm the role of PKC and its subsequent signaling pathways in diabetic nephropathy, especially in very early histopathologic manifestations.

Vinik et al conducted a 1-year trial of RBX treatment, using a standardized clinical neurological examination to
measure changes in neuropathy sensory symptoms by the neuropathy total symptoms score-6 (NTSS-6) and quantitative sensory testing by the vibration detection threshold. Patients with diabetic peripheral neuropathy taking placebo versus either 32 or 64 mg of RBX were evaluated at baseline and at 1, 3, 6, and 12 months in each group. RBX had no significant effect on the overall patient population. However, results showed a significant change in the NTSS-6 at 6 months and 12 months in patients who had clinically significant neuropathy sensory symptoms (NTSS-6≥6) at baseline when treated with RBX 64 mg compared with placebo. There were no treatment-related differences in change from baseline to end point for vibration detection threshold among all symptomatic patients. However, in a subset of patients with less severe symptomatic diabetic peripheral neuropathy, as defined by a measurable sural nerve action potential, RBX (32 mg and 64 mg) statistically improved vibration detection threshold compared with placebo.

Abnormal endothelial function has been demonstrated in type 1 and type 2 diabetes, as well as in obese, insulin-resistant patients whose insulin sensitivity correlated with the magnitude of endothelium-dependent vasodilation. Vascular permeability is increased as early as 4 to 6 weeks’ duration of diabetes in patients, supporting the concept of EC dysfunction. A previous study also reported that infusion of glucose for 6 hours decreases endothelium-dependent vasodilation in nondiabetic and healthy people. In the natural history of type 2 diabetes, the onset of hyperglycemia precedes endothelial dysfunction, whereas onset of endothelial dysfunction coincides with the presence of hyperglycemia in type 1 diabetes. Treatment with RBX (32 mg/d for 7 days) prevented endothelium-dependent vasodilatation abnormalities induced by hyperglycemia. Moreover, a recent small, double-masked, placebo-controlled study in type 2 diabetes showed that RBX (32 mg/d) after 6 weeks improves femoral-mediated dilatation at 1 and 5 minutes after cuff deflation as compared to placebo. These data suggest that PKC activation, especially the β isoform induced by hyperglycemia, may be responsible for the endothelial dysfunction observed in diabetic patients. Clearly, further studies are needed to determine whether RBX can effectively improve endothelial function in type 1 and 2 diabetic patients.

Summary
In summary, many years of human and animal data confirm the long-held belief that hyperglycemia perturbs arteries and vascular cell function. The source of such complications in diabetes is certainly multifactorial, with key roles identified for oxidants and glycation metabolites. However, a large body of literature supports hypothesis that hyperglycemia or diabetes leads to vascular DAG accumulation and ensuing PKC activation, causing a variety of cardiovascular defects. Indeed, modulation of PKC signaling transduction pathways affects the pathogenesis of various diabetic vascular complications. Activation of specific PKC isoforms by hyperglycemia and lipid metabolites is likely to be responsible for specific vascular pathologies such as EC and SMC dysfunction, ECM synthesis and fibrosis, monocyte activation, deregulation of cytokines, and vascular insulin dysfunction.

These intermediate cellular alterations contribute significantly to the development of macrovascular complications such as cardiomyopathy and acceleration of atherosclerosis, and microvascular complications such as retinopathy and nephropathy. Clinical trials have shown potential for PKC-β inhibitor as a therapy for diabetic vascular complications. However, not all clinical trials demonstrated positive effects of PKC-β inhibition. For example, RBX trials did not exhibit robust effects on the improvement of painful neuropathy or sexual dysfunction in diabetic patients. Therefore, more clinical trials targeting multiple PKC isoforms are urgently needed to test the effectiveness in delaying, stopping and even reversing diabetic vascular complications.

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Disclosures
None.

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