Targeting Vascular Endothelial Cell Insulin Resistance in Type 2 Diabetes Mellitus
Is Protein Kinase Cβ the Bullseye for Reducing Vascular Risk in Diabetes?

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More than 25 million Americans, or 8.3% of the population, have diabetes mellitus, of which ≈95% of diagnosed cases are type 2 diabetes mellitus. Between 1990 and 2010, the number of new cases of diabetes mellitus has almost tripled in the United States, and the Centers for Disease Control projects that 1 in 3 Americans will have diabetes mellitus by 2050 if this trend continues. The presence of type 2 diabetes mellitus increases the risk of microvascular complications, such as retinopathy and nephropathy, and macrovascular disease, most notably atherosclerotic coronary artery disease. Indeed, coronary artery disease–related death remains the primary cause of mortality in patients with type 2 diabetes mellitus, even on standard glucose control. However, more intensive glucose control is not associated with reduced coronary artery disease risk and may even increase mortality; therefore there remains a critical need to determine novel adjunct therapy to standard glycemic control that will reduce vascular risk in patients with type 2 diabetes mellitus.

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In 1996, a seminal study by Ishii et al demonstrated that selective inhibition of the β isoform of protein kinase C (PKCβ), a serine/threonine protein kinase that becomes activated in nutrient excess states such as hyperglycemia, resulted in significant improvement in retinal and glomerular microvascular function in diabetic rats in the absence of any alterations in glycemia or blood pressure. This study elucidated PKCβ as a potential novel target in diabetes-related vascular complications and identified increased tissue concentrations of diacylglycerol, likely as a result of excess de novo synthesis in hyperglycemia, as the primary activator of PKCβ in rats. Consistent with this, diacylglycerol concentration and PKCβ activity are increased in aortas of obese insulin-resistant rats and are associated with reduced insulin-stimulated activation of protein kinase B (Akt). Akt is a serine/threonine kinase regulated by upstream phosphatidylinositol 3 kinase (PI3K), which directly phosphorylates endothelial nitric oxide synthase (eNOS) at serine 1177 (eNOSε1177), resulting in eNOS activation and nitric oxide (NO) synthesis (Figure).

Interestingly, the reduction in insulin-stimulated Akt phosphorylation and NO bioavailability in aortas of obese insulin-resistant rats are partly reversed after 2 weeks of PKCβ inhibition. Additionally, 2 weeks of PKCβ blockade improves NO-mediated endothelium-dependent vasodilation (EDV) of mesenteric arteries in streptozotocin-induced diabetic rats. Consistent with these animal data, overexpression of PKCβ1 or β2 in cultured endothelial cells demonstrates a decrease in insulin-mediated Akt phosphorylation and eNOS expression, and exposure of bovine aortic endothelial cells to high glucose for 24 hours results in reductions in insulin-stimulated tyrosine phosphorylation of insulin receptor substrate-1, phosphorylation of Akt and eNOS, and NO production. Taken together, these studies provide strong in vivo and in vitro evidence that increased endothelial PKCβ activity in diabetes mellitus or hyperglycemia results in a vascular endothelial insulin resistant phenotype characterized by impairments in the insulin-mediated insulin receptor substrate-1/Akt/eNOS pathway and EDV. Thus, these data support the idea that PKCβ inhibition may be a novel clinical strategy to reverse endothelial insulin resistance and dysfunction in humans with diabetes mellitus.

However, results from several large randomized clinical trials investigating the efficacy of the selective PKCβ inhibitor ruboxastaurin on diabetic retinopathy and nephropathy have been equivocal. Also, several small clinical studies in humans testing the efficacy of short-term PKCβ inhibition on peripheral vascular EDV in humans have been disappointing. Although Beckman et al demonstrated that 7 days of oral ruboxastaurin prevented a reduction in forearm resistance artery EDV after 6 hours of experimentally induced-hyperglycemia in healthy adults, 2 weeks of ruboxastaurin had no effect on forearm EDV in middle-aged adults with type 2 diabetes mellitus. In another study, 6 weeks of ruboxastaurin appeared to improve conduit artery EDV (brachial artery flow-mediated dilation) in middle-aged patients with type 2 diabetes mellitus. However, the differences between groups were largely the result of a decline in EDV in the placebo group rather than a treatment effect of ruboxastaurin in the patients with diabetes mellitus. Collectively, the available evidence in humans to date suggests a lack of efficacy of PKCβ inhibition on microvascular disease outcomes and vascular EDV in patients with type 2 diabetes mellitus.

In the current issue of Circulation, Tabit et al describe an interesting cross-sectional study where they compared basal and insulin-mediated alterations in eNOS activation before and after ex vivo PKCβ blockade in venous endothelial cells that were freshly isolated from patients with type 2 diabetes mellitus and healthy controls. They report a paradoxical higher basal expression of phosphorylated eNOSε1177 in endothelial cells from patients with type 2 diabetes mellitus compared with...
healthy controls. The finding of increased phosphorylation of eNOS Ser1177 in the absence of differences in total eNOS protein expression also has been observed in obese humans.12 This observation may represent a compensatory upregulation of eNOS phosphorylation in the setting of excess superoxide production possibly from increased eNOS uncoupling or nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity in diabetes mellitus and obesity that results in reduced NO bioavailability and accumulation of nitrotyrosine in the vascular wall (Figure). In a novel translational approach, the authors exposed the isolated endothelial cells to insulin before fixation, and this resulted in a reduction in phosphorylated eNOS Ser1177 in cells from the diabetic patients but an increase in phosphorylated eNOS Ser1177 in the healthy control cells, suggestive of insulin resistance in endothelial cells of diabetics.

As expected, brachial artery EDV was lower in the patients with diabetes mellitus compared with controls, but a new finding was that a greater increase in insulin-mediated phosphorylation of eNOS Ser1177 was associated with higher brachial EDV, consistent with the idea that abnormal insulin-mediated eNOS regulation is associated with impaired endothelial function in patients with type 2 diabetes mellitus. Perhaps a more intriguing finding was that coincubating the insulin-treated cells from diabetics with a selective PKCβ inhibitor reversed the abnormal decrease in insulin-stimulated phosphorylation of eNOS Ser1177 but had no effect on phosphorylation of inhibitory eNOS Thr495 in diabetics. Consistent with animal models of insulin resistance and cultured endothelial cells exposed to experimental high-glucose,4 these data support the notion that enhanced PKCβ activity negatively modulates eNOS regulation in the human diabetic endothelium.

Interestingly, endothelial cells exposed to high glucose in vitro also demonstrate enhanced activity of nuclear factor kappa B (NFκB), a key proinflammatory transcription factor that regulates hundreds of inflammatory genes, that is attenuated after PKCβ blockade.13,14 Consistent with these in vitro findings, the study by Tabit et al11 reports that endothelial cells from diabetic patients compared with control subjects demonstrate a trend for higher expression of NFκB p65 and intracellular adhesion molecule-1 (ICAM-1), and reduced expression of the inhibitor protein of NFκB (IκBα) (Figure). This is significant because ICAM-1 is responsible for leukocyte adhesion to the endothelium during the development of atherosclerosis and endothelial dysfunction. Indeed, endothelial cells exposed to high glucose in vitro demonstrate a rapid increase in expression of several proinflammatory adhesion molecules and adhesion of leukocytes that can be attenuated by PKCβ or NFκB blockade.13,14 In the current study, acute ex vivo PKCβ inhibition in endothelial cells from diabetic patients resulted in increased IκBα expression consistent with reduced NFκB activation. However, ICAM-1 was not measured again after treatment so it is unknown whether transcription of NFκB-regulated proteins was altered by PKCβ blockade. Taken together, these data indicate a clear link between elevated PKCβ activity and NFκB activation in endothelial cells exposed to chronic hyperglycemia, suggesting the intriguing possibility that endothelial PKCβ activity directly modulates NFκB-related inflammation in the vascular endothelium of humans with type 2 diabetes.

The findings of this study should be interpreted in the context of several limitations. A significant limitation of the study is that patients were not treated with systemic PKCβ inhibition in a randomized controlled study, therefore it is unknown whether brachial artery EDV would have improved after chronic PKCβ inhibition with ruboxistaurin. Also, because insulin-mediated phosphorylation of eNOS Ser1177 was the primary readout of endothelial insulin resistance, it remains uncertain whether insulin-mediated NO bioavailability was truly increased after PKCβ blockade because eNOS enzyme activity or NO were not directly measured in the isolated cells, although this would have been technically difficult given the small number of cells obtained from the patients with this technique. Lastly, without quantifying nuclear localization or DNA binding activity of p65 after ex vivo PKCβ inhibition, it
remains unknown whether PKCβ blockade abrogates NFκB-signaling in endothelial cells of diabetic humans.

The study has several strengths, including novel assessments of protein expression from freshly isolated endothelial cells and assessments of vascular endothelial function in patients with type 2 diabetes mellitus and healthy adults. This allowed for unique molecular insight into the integrative regulation of PKCβ, eNOS, and NFkB activity in the endothelium of diabetic versus healthy humans. Importantly, results of insulin-stimulated eNOS regulation were not different among patients taking versus not taking insulin and other medications. However, despite the unique findings of the current study, it should be emphasized that chronic PKCβ blockade has not as of yet translated into improved vascular outcomes in patients with type 2 diabetes mellitus. Therefore, the need to develop new antidiabetic drugs or investigate old drugs that have pleiotropic effects on the vasculature beyond glucose lowering remains an important focus of investigation in diabetes mellitus.

One such old drug is metformin, which is already widely used in patients with type 2 diabetes mellitus. In addition to its established effects on glycemic control, metformin also inhibits PKC-β and NFkB signaling in glucose- and cytokine-exposed endothelial cells, suggesting a mechanism to explain the clinical benefits of metformin not attributable to glucose control.14 Nonacetylated salicylates, such as salsalate, have gained interest in recent years in type 2 diabetes mellitus,15 and NFkappaB inhibition of the endothelium in transgenic genetic-induced obesity.20 Taken together, these studies support the general concept that targeted NFκB inhibition in the endothelium prevents acute effects of high glucose on vascular cell adhesion molecule-1 expression in human endothelial cells. Circulation. 2003;107:86–95. Silver AE, Beske SD, Christou DD, Donato AJ, Moreau KL, Eskurza I, Gates PE, Seals DR. Overall weight and obese humans demonstrate increased vascular endothelial NAD(P)H oxidase-p47(ephx) expression and evidence of endothelial oxidative stress. Circulation. 2007;115:627–637.

In summary, the study by Tabit et al1 demonstrates for the first time in humans that PKCβ and NFkB are key integrative mechanisms involved in mediating endothelial cell insulin resistance in patients with type 2 diabetes mellitus. Given the disappointing results of clinical studies using PKCβ inhibitors, targeting NFkB with salsalate or newly developed more selective NFkB inhibitors may be the new therapeutic bullseye for reducing vascular risk in patients with type 2 diabetes mellitus.

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Disclosures
None.

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