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 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.

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 (eNOSser1177), 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 dilatation) 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 eNOSser1177 in endothelial cells from patients with type 2 diabetes mellitus compared with...
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 PKC\(\beta\) inhibition in endothelial cells from diabetic patients resulted in increased IxB\(\alpha\) expression consistent with reduced NF\(\kappa\)B activation. However, ICAM-1 was not measured again after treatment so it is unknown whether transcription of NF\(\kappa\)B-regulated proteins was altered by PKC\(\beta\) blockade. Taken together, these data indicate a clear link between elevated PKC\(\beta\) activity and NF\(\kappa\)B activation in endothelial cells exposed to chronic hyperglycemia, suggesting the intriguing possibility that endothelial PKC\(\beta\) activity directly modulates NF\(\kappa\)B-related inflammation in the vascular endothelium of humans with type 2 diabetes.

The integrative scheme demonstrating increased protein kinase \(C\beta\) activity in type 2 diabetes mellitus that mediates a decrease in insulin-mediated phosphorylation of endothelial nitric oxide synthase at serine 1177, nitric oxide bioavailability and endothelial function, as well as increased nuclear factor kappa B activation and expression of intercellular adhesion molecule-1. Akt indicates protein kinase B; DAG, diacylglycerol; eNOS, endothelial nitric oxide synthase; ICAM-1, intercellular adhesion molecule-1; IxB\(\alpha\), I kappa B alpha; IKK\(\beta\), I kappa kinase beta; IRS-1, insulin receptor substrate-1; NADPH, nicotinamide adenine dinucleotide phosphate; NO, nitric oxide; O\(2^-\), superoxide anion radical; ONOO\(^-\), peroxynitrite anion; PDK-1, phosphoinositide-dependent kinase-1; PI3K, phosphatidylinositol 3 kinase; PIP\(3\), phosphatidylinositol trisphosphate; PKC\(\beta\), protein kinase C beta; PTEN, phosphatase and tensin homolog; and Ser1177, serine 1177.

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 excessive 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 PKC\(\beta\) 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 PKC\(\beta\) 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 (NF\(\kappa\)B), a key proinflammatory transcription factor that regulates hundreds of inflammatory genes, that is attenuated after PKC\(\beta\) blockade.\(^{13,14}\) Consistent with these in vitro findings, the study by Tabit et al\(^{11}\) reports that endothelial cells from diabetic patients compared with control subjects demonstrate a trend for higher expression of NF\(\kappa\)B p65 and intracellular adhesion molecule-1 (ICAM-1), and reduced expression of the inhibitor protein of NF\(\kappa\)B (IxB\(\alpha\)) (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 PKC\(\beta\) or NF\(\kappa\)B blockade.\(^{13,14}\) In the current study, acute ex vivo PKC\(\beta\) inhibition in endothelial cells from diabetic patients resulted in increased IxB\(\alpha\) expression consistent with reduced NF\(\kappa\)B activation. However, ICAM-1 was not measured again after treatment so it is unknown whether transcription of NF\(\kappa\)B-regulated proteins was altered by PKC\(\beta\) blockade. Taken together, these data indicate a clear link between elevated PKC\(\beta\) activity and NF\(\kappa\)B activation in endothelial cells exposed to chronic hyperglycemia, suggesting the intriguing possibility that endothelial PKC\(\beta\) activity directly modulates NF\(\kappa\)B-related inflammation in the vascular endothelium of humans with type 2 diabetes.
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 NFκB 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 NFκB signaling in glucose- and cytokine-exposed endothelial cells, suggesting a mechanism to explain the clinical benefits of metformin not attributable to glucose control. Nonacetylated salicylates, such as salsalate, have the clinical benefits of metformin not attributable to glucose on vascular cell adhesion molecule-1 expression in human endothelial cells.

The effects of salsalate on glycemic control in patients with type 2 diabetes mellitus because they have been shown to inhibit NFκB activation in cytokine-stimulated endothelial cells, increase insulin signaling in muscle and liver of obese/insulin resistant rodents, as well as improve glycemic control in humans with diabetes mellitus and restore vascular EDV in older obese adults. Although the effects of salsalate on PKCβ are unknown, in vitro and in vivo studies also strongly implicate NFκB in endothelial insulin resistance in hyperglycemia. Moreover, functional NFκB inhibition of the endothelium in transgenic rodents has a profound modulatory effect in the prevention of both endothelial and systemic insulin resistance in diet- and genetic-induced obesity. Taken together, these studies support the general concept that targeted NFκB inhibition may be a logical approach to treat endothelial insulin resistance and dysfunction in humans with type 2 diabetes mellitus.

In summary, the study by Tabit et al demonstrates for the first time in humans that PKCβ and NFκB 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 NFκB with salsalate or newly developed more selective NFκB inhibitors may be the new therapeutic bullseye for reducing vascular risk in patients with type 2 diabetes mellitus.

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Disclosures

None.

References


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