Vascular Dysfunction in Hyperglycemia
Is Protein Kinase C the Culprit?

David D. Gutterman

Diabetes mellitus (DM) is the seventh leading cause of death in the United States with accelerated cardiovascular disease accounting for most (>75%) of the mortality. Major complications include retinopathy, nephropathy, neuropathy, and vasculopathy. Each has been linked to the severity of hyperglycemia; thus, the mainstay of treatment has been to aggressively control serum glucose levels. This practice is supported by the large NIH-sponsored Diabetes Complications and Control Trial that linked control of glucose to delayed onset of complications. Other mechanisms may also contribute because restoration of euglycemia does not always abrogate progression of established disease.

In the current issue of Circulation Research, Beckman et al describe the reversal of hyperglycemia-induced endothelial dysfunction in subjects treated with a novel, selective blocker of protein kinase C (PKC) β. The role of PKC in the vascular complications of DM has been established in animals. This study represents an important translational extension into the clinical arena supporting the possibility of drug trials. However, to appreciate the role of PKC in diabetic vascular disease, the complexity of mechanisms by which elevated levels of glucose causes tissue damage must be recognized. Three major mechanisms have been proposed.

First, glucose stimulates flux through the sorbitol pathway creating an intracellular reductive redox shift with accumulation of NADPH. This reduces cellular uptake of myoinositol and decreases sodium-potassium ATPase activity. Blocking the sorbitol pathway with aldose reductase inhibitors improves peripheral nerve conduction but has little effect on diabetic retinopathy, indicating that different mechanisms participate in the vasculopathy of DM.

Second, glucose can nonenzymatically glycosylate cellular proteins over time, yielding advanced glycosylated end-products (AGEs). AGEs can directly alter protein function or activate specific receptors with resultant changes in gene expression. AGEs also stimulate production of reactive oxygen species (ROS), common culprits in vascular pathology.

Third, through formation of diacylglycerol (DAG) and AGEs, glucose can activate and upregulate PKC. PKC is a ubiquitous family of serine-threonine phosphorylating enzymes. The distribution of over 10 isoforms varies among cell types with PKCα and β most prevalent in the vasculature where hyperglycemia predominately activates the β isoform. The effects of PKC activation are protean, including alterations in cell signaling, production of vasoconstrictor substances, and conversion of smooth muscle and endothelial cells to a proliferative phenotype in the retinal microcirculation and peripheral conduit vasculatures.

How does PKC produce such diverse abnormalities within the vascular bed? Several proposed mechanisms include PKC-stimulated expression of endothelial adhesion molecules, inhibition of vascular smooth muscle cell apoptosis that contributes to vascular remodeling in DM, and inhibition of gap junctions. The common denominator may be reactive oxygen species (ROS) that are key in the pathological changes of PKC activation (Figure).

Although the ability of PKC to induce ROS is well-established, the source of ROS is not clear. In cultured cells, glucose stimulates NADPH oxidase, whereas in intact tissues, such as aorta, nitric oxide synthase is involved. The net result is that activation of PKC generates ROS with subsequent impairment of endothelial function (Figure). These actions are responsible for the early and diffuse atherosclerosis in DM. A positive feedback loop exists whereby ROS generated from PKC activate phospholipase D, which hydrolyzes phosphatidylcholine to produce DAG and PKC activation again.

The specific PKC isoform activated by hyperglycemia varies across tissues and species. Thus, involvement of PKC in the vascular dysfunction of diabetes may depend on the bed studied.

The importance of PKC in the development of complications of DM is evident from studies in which inhibitors (staurosporine, H7, chelerythrine, calphostin C) or activators (PMA, overexpression) of PKC have been used to abrogate or reproduce the pathological changes associated with DM, respectively. Most pharmacological studies are restricted to in vitro models because common inhibitors of PKC are nonspecific and are associated with unacceptable toxicity. Recently, a novel compound has been developed, LY333531, that is a highly specific inhibitor of PKCβ2, the predominant isoform activated by hyperglycemia in the retina, heart, and aorta of diabetic rats. LY333531 has much less toxicity than other PKC inhibitors and has facilitated in vivo studies showing that oral administration to diabetic rats for 2 weeks improves albumin excretion and glomerular filtration rate and retinal...

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Use of PKC inhibitors in DM may confer broader benefit than described by Beckman et al. LY333531 prevents diabetic neuropathy in rats by preserving myoinositol levels in the tissue. VEGF mediates endothelial cell proliferation, angiogenesis, and increased permeability in retinal and peripheral tissues in diabetes. Each of these pathological changes is abrogated by LY333531. In humans, LY333531 treatment for 1 month normalized retinal blood flow. Thus, the nephropathy, retinopathy, and microvascular disease of DM may also be improved by PKC inhibition.

It is important to consider possible detrimental effects of long-term treatment with PKC inhibitors. PKCβ is highly expressed in the brain. Mice lacking this isoform show impaired ability to learn. Reduction in PKCβ isoforms are present in mice with Huntington’s disease and in the caudate-putamen of patients with this condition suggesting an etiological role. The duration of treatment in the present study was short, minimizing toxicity. Furthermore, the induced vascular abnormalities were of brief duration. Higher doses of the inhibitor and longer treatment regimens would likely be needed to improve vascular function in chronically diabetic subjects.

Although LY333531 is effective at reducing PKC activity, the study by Beckman et al raises the possibility that other more widely established treatments may be effective through a similar mechanism (Figure). HMG CoA reductase inhibitors can reduce DAG-mediated activation of PKC and the resultant migration of human vascular smooth muscle cells. Vitamin E also ameliorates complications of DM by reducing PKC activity and by antioxidant properties. α-Tocopherol treatment reduced PKCβ expression by over 50% and restored NO production in vascular smooth muscle cells grown in high glucose. Similar benefits were also observed in retina, aorta, and hearts of diabetic rats. Finally, ramipril, an angiotensin-converting enzyme inhibitor (ACE-I), prevented elevations in PKC in retina and mesenteric arteries from diabetic rats. The benefits of ACE-I in DM are already well-established.

In summary, many of the vascular and other end-organ complications of diabetes can be ameliorated by inhibition of PKC. In vascular tissue, the PKCβ isoform appears to be a prominent mediator of changes in cell proliferation, vascular endothelial function, microvascular permeability, and collagen formation. Thus, PKC is clearly situated as a key perpetrator of the vascular complications of diabetes. Availability of selective isoform antagonists (e.g., LY333531) may provide a novel mechanism for treating vascular and other complications of diabetes independent of glycemic control and insulin resistance.

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