D
dabetes mellitus (DM) is the seventh leading cause of
death in the United States with accelerated cardio-
vacular disease accounting for most (>75%) of the
mortality. Major complications include retinopathy, nephrop-
athy, 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 glu-
cose to delayed onset of complications. Other mechanisms
may also contribute because restoration of euglycemia does
not always abrogate progression of established disease. Thus,
alternative therapeutic approaches based on an under-
standing of the mechanisms of glucose-induced end-organ
damage are needed.

In the current issue of Circulation Research, Beckman et
al describe the reversal of hyperglycemia-induced endothe-

dial 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 ani-
imals. This study represents an important translational exten-
nion 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 accumu-
lation of NADPH. This reduces cellular uptake of myoinosi-
tol and decreases sodium-potassium ATPase activity. Block-
ing 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-prod-
ucts (AGEs). AGEs can directly alter protein function or
activate specific receptors with resultant changes in gene
expression. AGEs also stimulate production of reactive oxy-
gen 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 en-
zymes. The distribution of over 10 isoforms varies among
cell types with PKCα and β most prevalent in the vascula-
ture where hyperglycemia predominately activates the β
isoform. The effects of PKC activation are protean, includ-
ing alterations in cell signaling, production of vasoconstrictor
substances, and conversion of smooth muscle and endothelial
cells to a proliferative phenotype in the retinal microcircula-
tion 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 mole-
cules, inhibition of vascular smooth muscle cell apoptosis
that contributes to vascular remodeling in DM, and inhibi-
tion of gap junctions. The common denominator may be
reactive oxygen species (ROS) that are key in the patholog-
ical 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 complica-
tions 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 nonspe-
cific 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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Role of PKC in the Complications of Diabetes

Pathway of hyperglycemia-induced vascular dysfunction. Elevations in cellular glucose increase flux through the polyol pathway, forming sorbitol and NADH. Both glucose and NADH stimulate formation of DAG that activates PKC. AGEs produced by glycation of proteins can also stimulate PKC or cause ROS activation directly. ROS are a proposed final common pathway for the genesis of atherosclerosis. In addition to AGEs and endogenous DAG, PKC is activated by fatty acids and angiotensin II. Inhibition of membrane translocation and activation of PKC is conferred by vitamin E which accelerates breakdown of DAG, and PKC. In vascular tissue, the PKC/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. Available evidence in humans suggests that the nephropathy, retinopathy, and microvascular disease of DM may also be improved by PKC inhibition.

Five years later, in this issue of Circulation Research, Beckman et al.\(^{3}\) provide evidence in human subjects that the vascular dysfunction associated with hyperglycemia can be ameliorated with the same inhibitor of PKC\(^\beta\). In a placebo controlled randomized double-blind trial, they show that the hyperglycemia-induced reduction in endothelium-mediated vasodilation is improved by LY333531. The test compound had no effect on methacholine dilation in euglycemia but restored dilation during hyperglycemia. The endpoint of improved endothelial function is important given the critical role of the endothelium in preventing atherosclerosis.\(^{16,17}\)

This study paves the way for future clinical investigation of a novel targeted pharmacological inhibitor in the treatment of diabetic complications.

The present study examined peripheral microvascular function. Mortality in DM involves conduit vessel disease; thus, it will be important to determine whether treatment also preserves endothelial function in the aorta and coronary arteries. The impaired dilation imposed by hyperglycemia in this study was modest and may reflect the short duration of glucose stress. In most models, 6 hours of hyperglycemia is not sufficient to maximally stimulate ROS production or for generation of AGEs. It will be important to determine the effectiveness of PKC blockade in subjects with longer periods of poor glycemic control as occur in DM. Finally, it will be important to determine the mechanism of improvement by assessing endothelial-derived substances such including NO.

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.\(^{19}\) 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.\(^{19}\) In humans, LY333531 treatment for 1 month normalized retinal blood flow.\(^{20}\) 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. PKC\(^\beta\) is highly expressed in the brain.\(^{21}\) Mice lacking this isoform show impaired ability to learn.\(^{22}\) Reduction in PKC\(^\beta\) isoforms are present in mice with Huntington’s disease and in the caudate-putamen of patients with this condition suggesting an etiological role.\(^{21}\) 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.\(^{23}\) Vitamin E also ameliorates complications of DM by reducing PKC activity and by antioxidant properties.\(^{24}\) \(\alpha\)-Tocopherol treatment reduced PKC\(^\beta\) expression by over 50% and restored NO production in vascular smooth muscle cells grown in high glucose.\(^{24}\) 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.\(^{25}\) The benefits of ACE-I in DM are already well-established.\(^{26}\)

In summary, many of the vascular and other end-organ complications of diabetes can be ameliorated by inhibition of PKC. In vascular tissue, the PKC\(^\beta\) 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.

References


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