Protein Kinase Cβ Isoform Inhibitors
A New Treatment for Diabetic Cardiovascular Diseases

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The center of the pandemic of diabetes is the life-threatening cardiovascular complications that can be categorized into macrovasculopathy, microvasculopathy, and diabetic cardiomyopathy. Macrovasculopathy affects large vessels and manifests as atherosclerosis and the subsequent coronary artery disease, peripheral artery disease, and cerebrovascular disease. Atherosclerosis and its related complications are responsible for most of the mortality in diabetic patients. Indeed, diabetes has been firmly established as an independent risk factor for atherosclerosis. This risk seems to precede the onset of type 2 diabetes but begins with hyperglycemia in patients with type 1 diabetes. Multiple factors in diabetes—including insulin resistance, dyslipidemia, elevated free fatty acid, and hypertension—increase the risk. Many clinical surveys, such as the Framingham Study, have shown that the incidence of carotid artery disease is increased 2- to 4-fold in patients with diabetes or insulin resistance. When intima-media thickness is used as the indicator for the severity of atherosclerosis, both type 1 and type 2 diabetes are associated with more advanced atherosclerosis when compared with age- and sex-matched controls. This association could also be related to hyperglycemia because intensive euglycemic control has been shown to reduce the progression of intima-media thickness in the Epidemiology of Diabetes Interventions and Complications study, which involves 1229 patients. These clinical data suggest that hyperglycemia itself, in addition to the aforementioned established risk factors such as dyslipidemia, hypertension, insulin resistance, and oxidative stress, might have an independent role in the acceleration of atherosclerosis.

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Diabetes or insulin resistance clearly alters the biology of the multiple cellular components that participate in atherosclerosis, including endothelial cells, monocytes/macrophages, lymphocytes, and vascular smooth muscle cells. In endothelial cells, both the expression and the activity of the endothelial cell nitric oxide synthase are decreased, leading to the reduction of antiatherogenic nitric oxide (NO) production and, furthermore, impairment of endothelium-dependent vasodilation. The expression of endothelial cell NO synthase is apparently regulated by insulin receptor–mediated phosphatidylinositol-3 kinase (PI-3K) signaling pathway. Inhibition of the PI-3K/Akt but not the Ras/Raf/MEK/Erk pathway of insulin actions in insulin-resistant states has been suggested to cause the downregulation of NO production. Therefore, selective inhibition of insulin action in vascular cells has been proposed as a likely mechanism for the pathogenesis of atherosclerosis. The activation of protein kinase C (PKC), especially the β isoform, has been shown to selectively suppress the insulin receptor–mediated PI 3K/Akt signaling cascade and might be responsible for the reduced NO production. In a clinical study, Beckman et al showed that LY333531 could normalize the reduced endothelium-dependent vasodilation induced by glucose infusion in healthy subjects. On the contrary, the expression of vasoconstrictors such as endothelin-1 (ET-1) is increased in diabetic states, in part also via a PKC activation–dependent pathway. Interaction of endothelial cells, monocytes/macrophages, and lymphocyte is increased in diabetes through the induction of cell adhesion molecules such as vascular endothelial cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule, E-selectin, and P-selectin. In addition, hyperglycemia has been reported to affect macrophages by increasing their uptake of oxidized LDL and secretion of proinflammatory factors and production of free radicals. These actions greatly enhance the macrophage incorporation of the accumulating oxidized LDL and glyco-oxidized LDL that are also modified by hyperglycemia. The activated macrophages will then transform into foam cells, a hallmark of atherosclerosis. Activation of vascular smooth muscle cells is also achieved in diabetes to facilitate migration into the nascent intimal lesion and subsequent proliferation, in part stimulated by PKC activation, advanced glycation end-product accumulation, oxidative stress, and a nuclear factor κB (NFκB)–mediated inflammatory program (Figure).

Recently, the role of PKC activation has received substantial attention since a PKCβ-selective inhibitor, ruboxistaurin (RBX, LY333531), has been shown to prevent diabetic microvascular complications both in culture and in vivo. PKC is a family of serine/threonine kinases that consist of at least 12 structurally related members that can be classified into conventional PKC (cPKC), novel PKC (nPKC), and atypical PKC (aPKC). The activation of cPKC including PKCβ required the phosphorylation of threonine-500 in their activation loop as the priming process for subsequent translocation to the membrane. The subsequent intramolecular transphosphorylation of two other key residues, threonine-641 in the turn motif and serine-660 in the hydrophobic motif, is important for the stability of this enzyme and its interaction.
with the substrates and ATP. Cardiovascular tissues showed the upregulation of PKC activities in diabetic states. This is probably caused by the de novo synthesis of diacylglycerol (DAG) in response to the overflow of the glycolysis pathway in hyperglycemic conditions. Accumulation of the metabolites in the glycolysis pathway, such as glyceraldehydes 3-phosphate, will drive the synthesis of DAG, which in turn recruits the primed PKC into the plasma membrane to render a competent kinase, a key event in its activation. Vascular cells display increased DAG contents and PKC activity both in culture and in vivo in hyperglycemic conditions. This mainly involves PKC-β2 and is suggestive of its central role in the development of complications. This hypothesis has been validated by multiple studies showing the following: (1) Overexpression or activation of PKC-β2 causes pathological changes similar to those observed in diabetes. Targeted overexpression of PKCβ2 in the cardiomyocyte in mice results in reduced cardiac contractility, cell death, and severe fibrosis. These pathological changes are induced through the phosphorylation of troponin I and the increased expression of transforming growth factor (TGF)-β1, connective tissue growth factor, and basement membrane proteins, similar to that of diabetic cardiomyopathy. (2) Inhibition of PKCβ2 activation can prevent some of the pathological changes induced by diabetes or high glucose. It has been shown that inhibition of PKCβ activation, using β isoform–selective inhibitor ruboxistaurin or dominant negative adenoviral vectors, could prevent part of the vascular pathologies in diabetes. The PKCβ inhibitor ruboxistaurin is currently being evaluated in phase II/III clinical trials for its efficacy in treating diabetic retinopathy, neuropathy, and nephropathy.

Endothelial cells have long been used as a model to study the impact of hyperglycemia on vascular cell biology. For example, bovine aortic endothelial cells cultured in media containing 25 mmol/L glucose showed translocation of PKC-β2 and -δ isoforms to plasma membrane that are associated with the increased expression of ET-1. In addition, expressions of profibrotic factors such as plasminogen activator inhibitor-1 and TGF-β are also increased by hyperglycemia, accompanied by the upregulation of extracellular matrix protein components. Although mounting evidence is available showing that hyperglycemia induces endothelial cell dysfunction, the role of PKC-β phosphorylation and activation as the direct link between hyperglycemia and vascular inflammation and endothelial dysfunction remains elusive. Recently, Cosentino et al have used human aortic endothelial cells to investigate the role of PKC activation in the regulation of inflammation, oxidative stress, and cell adhesion in hyperglycemic conditions. In this system, hyperglycemia alone can induce the expression of key proinflammatory genes such as COX-2 and the production of free radicals. This regulation apparently depends on the activation of PKC, given that calphostin, a PKC inhibitor, can abolish these responses. In the present issue of Circulation, the same group of investigators advance their findings and report that hyperglycemia-induced VCAM-1 expression is mediated through a PKCβ2–activation–dependent pathway. Using PKCβ2–specific inhibitor CGP53352, the authors provide evidence suggesting that PKCβ2 activation downregulates the protein expression of IkBα, the inhibitory subunit of NFκB, and results in the enhanced activity of this nuclear transcription factor and the expression of VCAM-1, of which the promoter contains an NFκB–responsive element. Furthermore, they report that hyperglycemia-induced activation of PKCβ2 is associated with the phosphorylation of serine residue 660, which is essential for VCAM-1 expression, whereas phosphorylation of threonine-641 is not altered by hyperglycemia. This interesting finding provides evidence suggesting that the activation of PKCβ2 by high glucose is associated with the phosphorylation of certain key residues and also provides a potential mechanism for the upregulation of VCAM-1 expression in hyperglycemic conditions.

Despite these significant findings, some key issues are needed to validate this working model: (1) The authors conclude that the phosphorylation of serine-660 in PKCβ2 is a selective regulatory mechanism for glucose-induced
VCAM-1 expression. This is based mainly on the finding that CGP53353 could inhibit the phosphorylation of this site and, concomitantly, the expression of VCAM-1 mRNA expression. Caution should be taken in the interpretation of this result. The hyperglycemia-induced phosphorylation of serine-660 in PKCβ2 could be correlative to the upregulation of VCAM-1 expression instead of being a cause–effect relationship. The fact that CGP53353 can suppress both responses does not support the claim that serine-660 phosphorylation has any relevance to the downstream effects mediated by hyperglycemia, such as NFκB activation or VCAM-1 expression. Further mutagenesis study using a phosphorylation-incompetent mutant of PKCβ2 at serine-660 is required to establish this relationship. (2) Although cultured endothelial cells serve as a good model for the reductionist’s approach to dissect pathophysiological mechanisms, this finding needs to be tested in vivo. The interesting finding by Kouroedov et al16 should be extrapolated into in vivo situations. Although mice with endothelial-specific ablation of PKCβ2 are still not available, genetic deletion of PKCβ2 in mice has been established.17 It will be of particular interest to study whether hyperglycemia failed to induce VCAM-1 expression in these mice.

Nevertheless, this study clearly shows that PKCβ2 may be a key molecular link between hyperglycemia and endothelial cell dysfunction, especially on the expression of adhesion molecules such as VCAM-1 that recruit macrophages to the sites prone to atherosclerosis. In addition, the authors also suggest the potential development and application of inhibitors that selectively inhibit PKCβ2’s regulatory mechanisms. This is of great clinical importance for the application of inhibitors targeted to vital enzymes such as PKCβ2. Although multiple PKC inhibitors have been developed,18 few have displayed isoform selectivity except for ruboxistaurin, PKC translocation inhibitors,19 and the CGP53353. The work of Kouroedov et al suggests that development of inhibitors neutralizing the specific phosphorylation state of PKC might be a promising approach to yield response-specific inhibitors that are of great clinical importance. In addition, it is also essential to develop tissue-specific delivery of these inhibitors because the same PKC isoform could have distinctive roles in different tissues. For example, the expression of vascular endothelial cell growth factor (VEGF) is increased in retinal tissues in diabetic or insulin-resistant states, whereas this expression is decreased in the myocardium.20 Because a PKCβ2-dependent pathway is essential for VEGF-mediated endothelial cell growth and angiogenesis, the application of PKCβ2 inhibitor might be detrimental to myocardium vascularization, although it can reverse VEGF-mediated pathologies in diabetic retinopathy. Therefore, selective inhibitor and tissue-specific delivery are equally important in the clinical application of these compounds targeted to key enzymes such as PKCβ2; the action is vital for physiological processes, yet abnormal activation is central to many pathological conditions. This article and many recent studies of cardiovascular cells have established the importance of PKC activation, especially the α, β2, and δ isoforms in cardiovascular pathologies in diabetic and nondiabetic conditions. The synthesis of isoform-specific inhibitors for PKCβ2 activation will provide important insight into cardiovascular diseases and, it is hoped, a new class of therapeutic compounds for cardiomyopathy, atherosclerosis, and the restenosis process.

References

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Circulation. 2004;110:7-9
doi: 10.1161/01.CIR.0000133428.02295.6C
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2004 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

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