EDITORIAL (SEE TUTTLE ET AL., P. 2686)

Can Protein Kinase C β–Selective Inhibitor, Ruboxistaurin, Stop Vascular Complications in Diabetic Patients?

The life span and quality of life for diabetic patients are adversely affected mostly by systemic vascular injuries leading to nephropathy, retinopathy, neuropathy, and cardiovascular pathologies. Both the Diabetes Control and Complications Trial and the U.K. Prospective Diabetes Study have established that intensive glycemic control can delay the onset and progression of vascular complications (1,2). However, maintaining euglycemia in diabetic patients with present therapeutic agents has been challenging. In addition, recent reports using patients included in the Diabetes Control and Complications Trial suggested that previous history of hyperglycemia exposure will cause persistent vascular damage, even years after the resumption of intensive glucose management (3). Thus, it is of great clinical importance to develop therapeutic agents that can prevent vascular damage in diabetic patients, even in the presence of hyperglycemia. Over the last 20 years, there have been numerous studies on the molecular pathogenesis of diabetic vasculopathy. These theories can be separated into two categories, which are focused either on the formation of glucotoxins or on changes in cellular signalings induced by the glucotoxins. Theories on the formation of glucotoxins include *1*) increased flux via the aldose-reductase pathway (4), *2*) accelerated formation of advanced glycation end products (5), *3*) elevated systemic and vascular-derived oxidative stress (4), and *4*) enhanced flux via the hexosamine pathway (6). Furthermore, glucotoxin can also induce cellular signaling alterations, for example, the activation of protein kinase C (PKC), mitogen-activated protein kinase, and inflammatory signaling cascades such as the nuclear factor-κB pathway to cause vascular diseases (7). Studies using inhibitors to all of these diabetes-altered pathways have shown encouraging results either preventing or inhibiting the progression of vasculopathies in animal models of diabetes. However, clinical studies using inhibitors to many of these pathways have not yielded robust positive results (7).

Among the proposed theories, the activation of PKC pathway, especially the PKCβ isoform, has been shown extensively to cause diabetic vascular dysfunctions in rodent models of diabetes. Thus, PKC inhibitors have been developed, and a PKCβ isoform–selective inhibitor, ruboxistaurin, has shown promising effects on delaying the progression of clinical parameters of diabetic nephropathy in type 2 diabetic patients, as reported in this issue (8). PKC is a family of serine/threonine kinases that consist of 12 isoforms. They are separated into three categories based on their structure and regulation, including conventional PKCs (α, β1/2, and γ), novel PKCs (δ, ε, θ, and η), and atypical PKCs (ζ and λ). Conventional PKCs have response elements to phorbol ester, diacylglycerol, and Ca^{2+}. Novel PKCs can be activated by phospholipids but are independent of Ca^{2+} for their activation, whereas atypical PKC is not responsive to these activators but can be activated by insulin. Both conventional and novel PKC isoforms can translocate to the membranous compartment of the cells to elicit biological actions in the presence of diacylglycerol, of which the de novo synthesis is increased by hyperglycemia (7). Being vital enzymes, general inhibition of all PKC isoforms will cause severe consequences that can possibly endanger the survival of animals. Therefore, isoform specificity is a key element in the development of a clinically useful therapeutic PKC inhibitor. Since we originally proposed that hyperglycemia can induce PKC activation, especially the PKCβ1/2 isoforms (8), a large number of studies have confirmed that PKCβ1/2 isoforms are activated chronically in vascular tissues, including the retina, heart, aorta, renal glomeruli, and circulating monocytes in diabetic patients and animals. In addition, recent studies have shown that oxidants and advanced glycation end products derived from hyperglycemia can also activate PKC. The activation of PKCβ1/2 and other isoforms has now been associated with multiple retinal, renal, and cardiovascular abnormalities, including increases in capillary permeability, retinal neovascularization, thickening of basal membrane, renal hyperfiltration, glomerulosclerosis, endothelial dysfunction, and decreases in cardiac contractility, all of which have been shown to be abnormal in diabetic patients (9).

To validate the role of PKCβ isoforms in the development of diabetic vascular pathologies, our group, in collaboration with Lilly Laboratories, reported in 1996 that ruboxistaurin, a PKCβ isoform–selective inhibitor, displayed a 50-fold higher selectivity for PKCβ1/2 over other isoforms tested (10). In rodent models of diabetes, ruboxistaurin has been shown to prevent or even reverse vascular dysfunctions, such as basal membrane thickening and mesangial expansion, elevated expression of profibrotic factors transforming growth factor β1, and extracellular matrix proteins (11). In the retina of diabetic animals, ruboxistaurin can reduce vascular endothelial growth factor–induced angiogenesis and permeability and normalize retinal blood flow (12,13). PKC inhibitors have also been shown to decrease cardiac and endothelial cell dysfunction and systemic oxidative stress in animal models of diabetes or insulin resistance (13,14).

The success in animal studies has led to the evaluation of ruboxistaurin in several clinical studies for its efficacy on dia-

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betic nephropathy, retinopathy, and neuropathy. In this issue, Tuttle et al. (8) have reported encouraging results of a pilot study regarding the effects of ruboxistaurin on nephropathy in type 2 diabetic patients with intensive glycemic control and blood pressure regulation by ACE inhibitor or angiotensin receptor blocker (8). In this multicenter, double-masked, placebo-controlled study, 123 type 2 diabetic patients with proteinuria (mean albumin-to-creatinine ratio [ACR] 764 mg/g) and near-normal serum creatinine received either 32 mg/day ruboxistaurin or placebo for up to 1 year. Changes in ACR were used as the primary end point, and estimated glomular filtration rate was also evaluated as an index of renal function. These investigators reported that the ruboxistaurin-treated group showed a 24% reduction of ACR and a slower decline of renal function when compared with the baseline. These results are exciting and significant since this is the first clinical study using ruboxistaurin that has achieved primary end point. A previous clinical study showed that healthy human subjects taking ruboxistaurin (32 mg/day) for 7 days improved glucose infusion–impaired forearm vasodilation (15). This study, however, was performed in healthy subjects but not patients with diabetes. Another recently released study evaluated ruboxistaurin's effect on severe nonproliferative diabetic retinopathy. Oral application of 32 mg/day ruboxistaurin for as long as 46 months failed to prevent the progression to proliferative diabetic retinopathy, although it may have prevented the loss of visual acuity in these diabetic patients (16). In a neuropathy study, ruboxistaurin appeared to decrease symptoms in a subset of diabetic patients but did not prevent the progression of diabetic peripheral neuropathy as measured by nerve conduction velocity (17).

The study reported by Tuttle et al. is also very exciting for other reasons. The beneficial effect of ruboxistaurin is additive to intensive euglycemic control plus the inhibition of angiotensin actions (1,2). This addictive effect of ruboxistaurin suggests that PKCβ inhibition may suppress part of hyperglycemia-induced adverse effects not mediated through angiotensin's action. Interestingly, angiotensin and PKCβ activation have been shown to share similar vascular pathways and actions such as cell migration, permeability, oxidative stress, inflammation, and fibrosis. It is not surprising that PKC activation and angiotensin may have overlapping actions since many of angiotensin's action are mediated via PKC activation (18). However, results of this study provided clinical evidence suggesting that activation of PKCβ isoform and angiotensin may have significantly different renal effects despite their similarity in vascular actions. Further comparison of gene expression profile in vascular tissues between placebo and ruboxistaurin-treated patients may shed light on the different effects between ruboxistaurin and ACE inhibition and angiotensin receptor blocker. Lastly, this report and others using ruboxistaurin also demonstrated the importance of selectivity in PKC isoform inhibition. An earlier trial using a nonselective PKC inhibitor (PKC412), although it exhibited some beneficial actions in diabetic macular edema, reported serious toxicity that excludes its clinical applications in diabetic patients (19). These toxic effects are not surprising since PKC activation is essential for vital tissue functions in the heart and kidneys. Tuttle et al. have therefore provided the first clinical demonstration that a PKC isoform–selective inhibitor can be used for chronic clinical treatment with minimal side effects.

Although this study showed promising results and initiated the first step toward clinical application of ruboxistaurin for diabetic nephropathy, it should be noted that the piloting nature of this investigation generally serves as a proof of principle only. As we have stated, this study is underpowered to evaluate the therapeutic effects of ruboxistaurin on diabetic nephropathy. Whereas the ruboxistaurin-treated group showed a significant reduction of ACR when compared with baseline levels, the changes are not statistically different from those achieved by placebo during intergroup comparison. Another weakness is the short duration of the study (1 year), which may not be able to translate into long-term clinical end points such as progression to end-stage renal failure. Lastly, side effects of ruboxistaurin require further evaluation in chronic studies even though this drug is well tolerated at 32 mg/day for as long as 3–4 years. Thus, long-term studies are needed to evaluate ruboxistaurin with respect to its efficacy and side effects since it is the first of its class in clinical use.

Overall, it is exciting to witness the development of a pharmacological agent based on ideas from cell culture and animal models of diabetes complications. Over the next few years, we should have several new therapeutic compounds for diabetes complications that are derived from a basic understanding of vascular biology of diabetes complications. As for ruboxistaurin, we are cautiously optimistic, but clearly, more large studies are needed to establish its efficacy for treatment of nephropathy and other vascular complications in diabetic patients.

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