

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- κ B

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 PKC β isoform, has been shown extensively to cause diabetic vascular dysfunctions in rodent models of diabetes. Thus, PKC inhibitors have been developed, and a PKC β 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 , β 1/2, 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 PKC β 1/2 isoforms (8), a large number of studies have confirmed that PKC β 1/2 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 PKC β 1/2 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 PKC β isoforms in the development of diabetic vascular pathologies, our group, in collaboration with Lilly Laboratories, reported in 1996 that ruboxistaurin, a PKC β isoform-selective inhibitor, displayed a 50-fold higher selectivity for PKC β 1/2 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 β 1, 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 glomerular 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 additive effect of ruboxistaurin suggests that PKC β inhibition may suppress part of hyperglycemia-induced adverse effects not mediated through angiotensin's action. Interestingly, angiotensin and PKC β 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 PKC β 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 ani-

mal 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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References

1. The Diabetes Control and Complications Trial Research Group: The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med* 329:977–986, 1993
2. UK Prospective Diabetes Study (UKPDS) Group: Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). *Lancet* 352:837–853, 1998
3. The Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Research Group: Sustained effect of intensive treatment of type 1 diabetes mellitus on development and progression of diabetic nephropathy. *JAMA* 290:2159–2167, 2003
4. Brownlee M: Biochemistry and molecular cell biology of diabetic complications. *Nature* 414:813–820, 2001
5. Naka Y, Bucciarelli LG, Wendt T, Lee LK, Rong LL, Ramasamy R, Yan SF, Schmidt AM: RAGE axis: animal models and novel insights into the vascular complications of diabetes. *Arterioscler Thromb Vasc Biol* 24: 1342–1349, 2004
6. McClain DA: Hexosamines as mediators of nutrient sensing and regulation in diabetes. *J Diabetes Complications* 16:72–80, 2002
7. Sheetz MJ, King GL: Molecular understanding of hyperglycemia's adverse effects for diabetic complications. *JAMA* 288:2579–2588, 2002
8. Tuttle KR, Bakris GL, Toto RD, McGill JB, Hu K, Anderson PW: The effect of ruboxistaurin on nephropathy in type 2 diabetes. *Diabetes Care* 28:2686–2690, 2005
9. Inoguchi T, Battan R, Handler E, Sportsman JR, Heath W, King GL: Preferential elevation of protein kinase C isoform beta II and diacylglycerol levels in the aorta and heart of diabetic rats: differential reversibility to glycemic control by islet cell transplantation. *Proc Natl Acad Sci U S A* 89:11059–11063, 1992

10. Ziyadeh FN, Sharma K: Overview: combating diabetic nephropathy. *J Am Soc Nephrol* 14:1355–1357, 2003
11. Ishii H, Jirousek MR, Koya D, Takagi C, Xia P, Clermont A, Bursell SE, Kern TS, Ballas LM, Heath WF, Stramm LE, Feener EP, King GL: Amelioration of vascular dysfunctions in diabetic rats by an oral PKC beta inhibitor. *Science* 272:728–731, 1996
12. Koya D, Haneda M, Nakagawa H, Isshiki K, Sato H, Maeda S, Sugimoto T, Yasuda H, Kashiwagi A, Ways DK, King GL, Kikkawa R: Amelioration of accelerated diabetic mesangial expansion by treatment with a PKC beta inhibitor in diabetic db/db mice, a rodent model for type 2 diabetes. *FASEB J* 14:439–447, 2000
13. Xia P, Aiello LP, Ishii H, Jiang ZY, Park DJ, Robinson GS, Takagi H, Newsome WP, Jirousek MR, King GL: Characterization of vascular endothelial growth factor's effect on the activation of protein kinase C, its isoforms, and endothelial cell growth. *J Clin Invest* 98:2018–2026, 1996
14. Abiko T, Abiko A, Clermont AC, Shoelson B, Horio N, Takahashi J, Adamis AP, King GL, Bursell SE: Characterization of retinal leukostasis and hemodynamics in insulin resistance and diabetes: role of oxidants and protein kinase-C activation. *Diabetes* 52:829–837, 2003
15. Kuboki K, Jiang ZY, Takahara N, Ha SW, Igarashi M, Yamauchi T, Feener EP, Herbert TP, Rhodes CJ, King GL: Regulation of endothelial constitutive nitric oxide synthase gene expression in endothelial cells and in vivo: a specific vascular action of insulin. *Circulation* 101:676–681, 2000
16. Beckman JA, Goldfine AB, Gordon MB, Garrett LA, Creager MA: Inhibition of protein kinase C β prevents impaired endothelium-dependent vasodilation caused by hyperglycemia in humans. *Circ Res* 90:107–111, 2002
17. The PKC-DRS Study Group: The effect of ruboxistaurin on visual loss in patients with moderately severe to very severe nonproliferative diabetic retinopathy: initial results of the Protein Kinase C β Inhibitor Diabetic Retinopathy Study (PKC-DRS) multicenter randomized clinical trial. *Diabetes* 54:2188–2197, 2005
18. Bastyr III E, Price K, Skljarevski V, Lledo A, Vignati L: Ruboxistaurin (RBX) mesylate treatment in patients with diabetic peripheral neuropathy (DPN) improves clinical global impression (CGI) and correlates with change in patient symptoms and signs (Abstract). *Diabetes* 52 (Suppl. 1):A191, 2003
19. Kelly DJ, Zhang Y, Hepper C, Gow RM, Jaworski K, Kemp BE, Wilkinson-Berka JL, Gilbert RE: Protein kinase C β inhibition attenuates the progression of experimental diabetic nephropathy in the presence of continued hypertension. *Diabetes* 52:512–518, 2003
20. Campochiaro PA: Reduction of diabetic macular edema by oral administration of the kinase inhibitor PKC412. *Invest Ophthalmol Vis Sci* 45:922–931, 2004