PKC-δ inhibition: a new therapeutic approach for diabetic complications?

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SUMMARY
PKC comprises a superfamily of isoenzymes that is activated in response to various stimuli. Hyperglycaemia induces the activation of different PKC isoforms. However, the PKC-β isoform appears to be preferentially activated by high glucose levels and has been shown to be associated with diabetic vascular complications. In vitro and in vivo animal studies have shown that ruboxistaurin mesylate, a novel selective inhibitor of PKC-β, ameliorates the biochemical and functional consequences of PKC activation and may have the potential to reduce the burden of vascular complications associated with diabetes. Results of the first phase-II and phase-III trials evaluating the efficacy of this compound on diabetic microvascular complications have been published recently. They confirm that this compound may favorably influence the evolution of diabetic microvascular complications.

Key-words: Proteine kinase C · Ruboxistaurin · Microvascular complications · Diabetes.

RÉSUMÉ
Inhibition de la protéine kinase C-δ : une nouvelle approche thérapeutique des complications du diabète ?
La protéine kinase C (PKC) est une famille enzymatique constituée d’au moins 11 isoformes activées en réponse à divers stimuli. Dans les tissus d’animaux diabétiques ainsi que dans les cellules cultivées en présence de concentrations élevées de glucose, plusieurs de ces isoformes sont activées. La PKC-β subit cependant une activation préférentielle dans la plupart des tissus et modèles cellulaires étudiés ainsi que dans les organes cibles des complications microvasculaires (œil et rein) ou macrovasculaires (artères et cœur) chez les patients diabétiques. La PKC-β a donc tout naturellement été invoquée comme potentiellement impliquée dans le développement des complications chroniques du diabète. Les études expérimentales in vitro ainsi que les données animales in vivo utilisant la ruboxistaurine, nouvel inhibiteur sélectif de la PKC-β, sont venues confirmer cette hypothèse et ont ainsi conduit à la réalisation d’essais cliniques chez l’homme.
Les résultats des premières études de phase-II et de phase-III qui ont évalué l’efficacité de cette molécule sur les complications microvasculaires du diabète viennent d’être publiés. Ils confirment l’intérêt potentiel de la ruboxistaurine sur l’évolution des complications microangiopathiques mais nécessitent d’être confirmés par des études à plus grande échelle.

Mots-clés : Protéine kinase C · Ruboxistaurine · Complications microvasculaires · Diabète.

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Introduction

Diabetes mellitus is a state of chronic hyperglycaemia due to an absolute or relative deficiency of insulin secretion that may or may not be associated with insulin resistance. The worldwide prevalence of diabetes was estimated to be 2.8% in 2000 and is projected to reach 4.4% by 2030 [1]. Patients who suffer from diabetes are prone to long-term complications, such as retinopathy, nephropathy, neuropathy, and atherosclerosis, thus reducing their life expectancy. Both the Diabetes Control and Complications Trial (DCCT) [2] and the United Kingdom Prospective Diabetes Study (UKPDS) [3] demonstrated that hyperglycaemia plays an important role in the pathogenesis of these long-term complications, although this process is influenced by individual susceptibility (i.e., genetic determinants), and by accelerating factors, such as hypertension and dyslipidemia, among others. Complications primarily affect organs whose cells do not require insulin for glucose uptake, such as those of the nervous system, kidneys, and blood vessels. Thus, these cells are subjected to high concentrations of intracellular glucose during hyperglycaemia, which leads to the activation of different intracellular metabolic pathways. As described by Brownlee in his 2004 Banting Medal Award Lecture [4], several attractive hypotheses have arisen recently to explain the role of hyperglycaemia in the pathogenesis of long-term complications. Such hypotheses include (i) increased polyol pathway flux, (ii) increased formation of advanced glycation end products, (iii) increased hexosamine pathway flux, and (iv) hyperglycaemia-induced activation of protein kinase C (PKC). The PKC pathway has received the most attention recently. Its activation is involved in the regulation of vascular permeability, contractility, extracellular matrix, cell growth, angiogenesis, cytokine action, and leukocyte adhesion, all of which are involved in the pathophysiology of diabetic complications [5]. Research regarding the PKC pathway has led to the discovery of a specific PKC-β inhibitor, ruboxistaurin (RBX), the first of a new class of compounds currently investigated for the treatment of diabetic microvascular complications. This compound is currently in development by the Eli-Lilly Company and a new drug application to the U.S. Food & Drug Administration (FDA) for the treatment of diabetic retinopathy is forthcoming. Thus, we are going to review the role of PKC in diabetic complications, as well as discuss the experimental and clinical evidence currently available on the potential role of PKC-β inhibitors in the treatment of diabetic complications.

PKC

PKC comprises a superfamily of isoenzymes, many of which are activated by 1,2-diacylglycerol (DAG) in the presence of phosphatidylserine. In order to be activated by DAG, PKC must first undergo phosphorylation at three conserved sites. PKC isoforms are serine/threonine kinases that act by catalyzing the transfer of a phosphate group from ATP to serine and threonine residues of their substrate proteins [6]. They phosphorylate a wide variety of intracellular target proteins and have multiple functions in signal transduction-mediated cellular regulation. Activation of one or more PKC isoforms leads to a variety of biological responses, including changes in cell proliferation and differentiation, transmembrane ion transport, glucose and lipid metabolism, smooth muscle contraction, and gene expression [7]. Individual PKC isoforms mediate unique cellular functions and phosphorylate unique protein substrates. These isoforms are classified into 3 subfamilies, according to the structure of the N-terminal regulatory domain, which determines sensitivity to both Ca$^{2+}$ and diacylglycerol (DAG), their main activators (figure 1). The classical isoforms (e.g., PKC-α, PKC-βI, PKC-βII, and PKC-γ) are regulated by Ca$^{2+}$, DAG, and phosphatidylserine; the novel isoforms (e.g., PKC-δ, PKC-ε, PKC-θ, and PKC-η) are insensitive to Ca$^{2+}$ and the atypical isoforms (e.g., PKC-ζ and PKC-ι/λ) are regulated neither by DAG nor by Ca$^{2+}$, but can be activated by phosphatidylserine. During activation, PKC itself undergoes phosphorylation, during which it is translocated from the cytosol to the cell membrane, allowing its colocalization with both activators and substrates [6].

PKC isoforms are widely distributed in mammalian tissues and have different patterns of tissue distribution. For example, PKC-γ is restricted primarily to the central nervous system and spinal cord, PKC-θ is predominantly expressed in skeletal muscle, and PKC-β is present in pancreatic islet cells, monocytes, brain, and many vascular tissues, including retina, kidney, and heart. This specific

![Figure 1](https://example.com/figure1.png)

**Figure 1**
Catalytic and regulatory domain of indicated PKC isoforms with conserved regions (C1-C4), variable regions (V1-V5), and binding sites for calcium, phosphatidylserine (PS), DAG, and ATP.
tissue distribution is associated with different isoform function [7]. Coordinated regulation of these isoforms is crucial for normal cellular function. Unusually persistent activation of PKC has long been recognized to be associated with uncontrolled growth and tumorigenesis [8]; in diabetes, the activation of different PKC isoforms has been involved in insulin signal transduction [9], insulin resistance [10], and in vascular complications [11]. Indeed, diabetes-induced PKC-β activation appears to be strongly implicated in the pathogenesis of both diabetic microangiopathy and macroangiopathy.

**Diabetes/glucose-induced PKC activation**

Generation of DAG is one of the major physiological pathways by which PKC is activated in the cell. DAG can be liberated from either membrane phospholipids and more specifically, from phosphatidylinositol by agonist-induced activation of phospholipase C, or by de novo synthesis from glycolytic intermediates. When hyperglycaemia is evident, glycerolaldehyde phosphate accumulates in cells where it is converted to phosphatidic acid, in turn leading to de novo synthesis of specific DAG species that are characterized by their unique fatty acid composition that can activate different PKC isoforms (figure 2). Most studies indicate that it is the de novo synthesis that is involved in glucose-induced DAG formation [11,12]. Increases in DAG levels with corresponding increases in PKC activity have been reported to result from an increase in glucose concentration in the media of cultured cells, such as aortic endothelial cells, smooth muscle cells, retinal endothelial cells, and renal mesangial cells. Similarly, diabetes-induced increases in DAG levels have been reported in different tissues, including retina, heart, glomeruli, liver, aorta, and granulation tissues, with parallel increases in PKC activity in the membranous fractions of most of these tissues [7]. These findings support the hypothesis that high glucose levels induce widespread activation and translocation of PKC.

However, the effects of high glucose appear to be tissue specific since no alteration in brain PKC activity in the presence of high glucose concentrations have been reported [11].

**Preferential activation of PKC-δ in diabetic tissues and its potential role in diabetic complications**

**Diabetic microangiopathy**

Many experimental studies, both in cultured cells and animal models, support the predominant role of PKC-βII in diabetic microvascular complications. Even though PKC isoforms α, βI, βII, and ε are all increased in both membrane and cytosolic fractions of diabetic rat retinas, the PKC-βII isoform shows the greatest increase in the membrane fraction [13]; in cultured bovine retinal endothelial cells, increasing glucose concentrations from 5 to 25 mM consequently increases both PKC-βII and δ levels relative to other isoforms examined [14].

Vascular endothelial growth factor (VEGF) is a potent cytokine that induces angiogenesis and markedly increases endothelial cell permeability. Elevated levels of VEGF have been reported in the ocular fluid of patients with proliferative retinopathy [15], in the retina of spontaneously non-obese diabetic rats prior to the appearance of observable retinopathy [16], and in the kidneys of preclinical models of diabetes [17]. Thus, VEGF has been implicated in both the development of vascular hyperpermeability during early diabetic retinopathy and in the neovascularization process of proliferative retinopathy, and may be potentially involved in the increased permeability of the glomerulus. Intravitreal injection of VEGF in vivo rapidly activates PKC in the retina, inducing membrane translocation of PKC-α, -βI, and -δ in parallel with a greater than 3-fold increase in retinal vasopermeability, suggesting that the VEGF-induced increase in intraocular vascular permeability can occur through activation of PKC, including PKC-βII [18]. Overexpression of the PKC-βII isoform in different mouse tissues, including the retina, increases the angiogenic response to oxygen-induced retinal ischaemia (a model for proliferative retinopathy), whereas this response is decreased in PKC-β null mice. The mitogenic action of VEGF is also increased by 2-fold in retinal endothelial cells of animals that overexpress PKC-βII or -βII isoforms and is significantly inhibited by the overexpression of a dominant-negative PKC-βII isoform; overexpression of the PKC-α, -δ, and -ζ isoforms do not, however, alter VEGF function [19].

The enhanced action of endothelin-1 (ET-1, the most potent vasoconstrictor released by the endothelium), associated with decreased blood flow in the retina of diabetic animals, may also be secondary to PKC activation.
Hyperglycaemia has been postulated to increase ET-1 secretion in endothelial cells [14]. Indeed, in both bovine retinal endothelial cells and bovine retinal pericytes, elevation of glucose from 5.5 to 25 mM increases membrane-associated PKC activity and ET-1 mRNA levels in parallel. Glucose-induced ET-1 overexpression is inhibited by a general PKC inhibitor. Results of immunoblot analysis show that in the membrane fraction, both the PKC-βI and -δ isoforms are predominantly increased. Overexpression of either one of these 2 isoforms, but not of PKC-ζ, enhances PKC activity and protein levels in parallel with basal and glucose-induced ET-1 mRNA expression by at least 2-fold. These results show that enhanced ET-1 expression, induced by hyperglycaemia, is partly due to activation of the PKC-β and -δ isoforms.

Results on the effects of diabetes on PKC activity in the nervous system are more confusing since PKC activity has been reported to be decreased, increased, or unaltered [5]. Actually, decreases in neuronal DAG and PKC activity, secondary to a hyperglycaemia-induced increase in the polyol pathway and neuronal myo-inositol depletion have been reported [20,21]. The diminished activity of PKC reduces the phosphorylation of the Na-K-ATPase, which induces a nerve conduction deficit and further causes nerve degeneration [22]. However, a major factor that contributes to the development of diabetic neuropathy is ischaemia [23]. Since hyperglycaemia-induced activation of vascular PKC-βI has been reported to lead to both impairment in vasodilation and an increase in vasoconstriction, resulting in a decrease in both endoneurial blood flow and in neural dysfunction, the activation of PKC-β may still be involved in the development of diabetic neuropathy.

The role of PKC hyperactivity has also been thoroughly investigated in pain generation. Increases in PKC-βII activity have been reported to participate in hyperalgesia caused by adjuvant-induced inflammation in the rat hind paw [22,24]. This important contribution of PKC to hyperalgesia has also been reported in diabetic animals. Phorbol esters, well known PKC activators, also enhance thermal hyperalgesia in diabetic mice [25].

**Diabetic macroangiopathy and cardiopathy**

Even though early reports mention the relationship between PKC-β and diabetic complications, suggesting the specific activation of this isoform in both the aorta and heart of diabetic rats [26], most studies focused on microvascular complications. However, there is now increasing evidence to support that PKC-β, as well as other isoforms, plays a significant role in many mechanisms that promote atherosclerosis and macrovascular complications. These mechanisms have been extensively reviewed [27]. Table I summarizes the mechanisms that are potentially related to PKC-β activation. They include activation of vascular NAD(P)H oxidase, dysfunction of endothelial nitric oxide synthase (eNOS), and induction of ET-1, all leading to endothelial dysfunction. Additionally, vascular remodeling by vascular smooth muscle cells apoptosis [28], induction of adhesion molecules [29], and foam cell formation [30] are also regulated by PKC-β.

It has also been suggested that activation of PKC-β, in response to increased glucose levels, is involved in the signaling mechanisms that underlie diabetes-induced coronary microvascular dysfunction. In the coronary system, increased microvascular permeability may, in large part, contribute to myocardial insufficiency and ventricular dysfunction, as frequently seen in diabetic heart disease. Yuan et al. [31] showed that perfusion of high concentrations of glucose in porcine coronary venules induced a dose-dependent increase in the apparent permeability coefficient of albumin, which was prevented by PKC blockade. An elevated basal permeability to albumin was also observed in coronary venules during early onset of streptozotocin-induced diabetes and was corrected by β-specific (e.g., hispidin) PKC inhibitors. Immunoblot analysis of diabetic hearts showed a significant subcellular translocation of both PKC-βI and -ε isoforms from the cytosol to the membrane [31]. It has also been demonstrated that overexpression of PKC-βII in mice myocardia induces molecular and biochemical alterations similar to those observed in diabetic cardiomyopathy, including increased expression of c-fos, tumor growth factor β1 (TGF-β1), and collagen types IV and VI, in association with decreased systolic and diastolic functions [32]. These results demonstrate that PKC-β is potentially involved in the early alterations observed in diabetic heart disease.

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<td>Principal mechanisms that are potentially associated with PKC-β activation in diabetes.</td>
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<td>Induction of NAD(P)H oxidase</td>
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<tr>
<td>eNos upregulation and dysfunction</td>
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<tr>
<td>eNos downregulation (in response to insulin)</td>
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<td>ET-1 expression</td>
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<td>Apoptosis of VSMC</td>
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<td>Induction of VCAM-1</td>
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<td>Macrophage LOX-1 expression</td>
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- eNos: endothelial nitric oxide synthase; ET-1: endothelin 1; VSMC: vascular smooth muscle cell; VCAM-1: vascular cell adhesion molecule; LOX-1: Oxidized Low-Density Lipoprotein Receptor-1.

**Specific PKC-δ inhibition by ruboxistaurin and diabetic complications: experimental data**

The PKC family is so large and widely distributed throughout the body that the systemic administration of a
non-specific inhibitor is likely to have serious, perhaps fatal, side effects. However, the effects of inhibitors that are specific for one or more PKC isoforms may be limited to specific organs or biochemical pathways, and hopefully, to those that are activated in diabetes. Following their observations that the PKC-β II isoform was preferentially activated in various vascular tissues of diabetic rats, including the retina, heart, and aorta, King’s group synthesized a selective and orally active inhibitor of this isoform [33]. On the basis of known PKC inhibitor structures, they performed extensive screening of inhibitors that would ideally be exempt of side effects and that could prevent and/or treat diabetes-induced alterations. Thus, the macrocyclic bis(indolyl)maleimide structure, LY333531 or RBX, was synthesized and shown to be a competitive, reversible inhibitor of both PKC-βI and -βII, with a half-maximal inhibitory concentration (IC50) of approximately 5 nM. This value is one-fiftieth of other PKC isoform IC50β and one-thousandth of non-PKC kinase IC50β (figure 3) [33]. Both animal and in vitro studies have confirmed the potential ability of RBX to prevent and/or revert diabetes-induced alterations in kidneys, retina, nerves, heart, and blood vessels.

**Diabetic retinopathy**

Initial studies on the vascular effects of RBX demonstrated that it was able to normalize retinal circulation time in diabetic rats in a dose-responsive manner, in parallel with its ability to inhibit PKC activity [34]. As described above, VEGF was extensively studied in terms of its role in the development of proliferative diabetic retinopathy. Intravitreal injection of RBX, in association with VEGF, prevents the increased retinal vascular permeability that is associated with VEGF injection alone. These effects are also prevented by the oral administration of RBX, suggesting that oral pharmacological therapies involving PKC-β isoform-selective inhibitors may prove efficacious for the treatment of VEGF-associated ocular disorders observed in diabetic retinopathy [18]. VEGF is a well-known mitogenic factor in vascular endothelial cells. However, it is also a “permeability factor” and, as such, it could play a major role in the development of diabetic macular edema in addition to proliferative diabetic retinopathy. Hence, the therapeutic inhibition of VEGF production and/or action by RBX should be able to inhibit the development and/or progression of both proliferative diabetic retinopathy and of diabetic macular edema, and even of early-stage diabetic retinopathy [35]. In agreement with its known effects on growth factors, RBX was demonstrated to inhibit both preretinal and optic nerve head neovascularization in a pig model of preretinal neovascularization, caused by retinal branch vein occlusion, thus demonstrating its effectiveness in preventing intraocular neovascularization after an ischemic stimulus [36]. In addition to VEGF inhibition, RBX can also reverse the alterations of the Na⁺-K⁺ATPase and calcium ATPase activities observed in diabetic retinas [37]. It can also attenuate the increase in leukocyte entrapment in the retinal microcirculation during the period of early diabetes, which may contribute to an improvement of abnormal retinal blood flow [38].

**Diabetic nephropathy**

The oral administration of RBX to diabetic rats specifically inhibits the activation of the PKC-βI isoform without affecting PKC-α isoform activation and prevents the increased mRNA expression of TGF-β1 and extracellular matrix components, such as fibronectin and alpha1(IV) collagen in the glomeruli [39,40]. RBX also improves the glomerular filtration and albumin excretion rates [34]. In cultured glomerular mesangial cells, RBX inhibits glucose-induced PKC activity in parallel with a block of glucose-induced increases in both arachidonic acid release and prostaglandin E2 production and a decrease in Na⁺-K⁺ ATPase activity.

RBX can abrogate the glucose-induced oxidative stress in the glomeruli of streptozocin-induced diabetic rats through a decreased activation of critical NAD(P)H subunits [41]. It can also normalize eNOS activity in glomerular endothelial cells that is decreased in response to glucose-induced PKC activation [42]. eNOS plays an important role in renal protection by maintaining normal glomerular function through the inhibition of thrombosis, leukocyte adhesion/activation, apoptosis, and oxidative stress.

Tubulointerstitial macrophage accumulation is also associated with declining renal function in diabetic nephropathy.
These inflammatory cells are rich in the profibrotic growth factor, TGF-β, such that their presence in areas of injury is frequently associated with tissue fibrosis. The migration of macrophages occurs in response to the site-specific production of chemokines, with osteopontin closely associated with macrophage trafficking into the tubulointerstitium of the kidney. Diabetic rats exhibit increases in osteopontin expression in cortex tubular epithelial cells in association with macrophage infiltration, interstitial fibrosis, and TGF-β activity. RBX treatment significantly attenuates these parameters, suggesting that inhibition of osteopontin expression may be an additional mechanism by which inhibition of the PKC-β isofrom confers a protective effect on the kidney [43].

In addition to hyperglycaemia, hypertension and the renin-angiotensin system have been consistently implicated in the pathogenesis of diabetic nephropathy. Each of these pathogenetic factors may induce changes in cellular function by the activation of PKC-β. In a severely hypertensive diabetic animal model, the (mRen-2)27 rat (a transgenic animal that has the entire mouse renin gene, Ren-2, inserted into the Sprague-Dawley genome) exposed to streptozotocin, subsequent RBX treatment reduces albuminuria, glomerulosclerosis, and tubulointerstitial fibrosis despite persistent hypertension and hyperglycaemia [44]. These findings suggest the potential role of this therapeutic strategy in the treatment and prevention of diabetic nephropathy.

**Diabetic neuropathy**

Nakamura et al. compared the effects of PKC-β inhibition by RBX on diabetic nerve dysfunction with effects of an aldose reductase inhibitor, NZ-314 (NZ) [45]. Streptozotocin-induced diabetic rats were treated with or without RBX and/or NZ for 4 weeks, and motor nerve conduction velocities (MNCV), coefficients of variation of the electrocardiogram R-R interval (CVR-R), sciatic nerve blood flow (SNBF), peak latencies of oscillatory potentials on electrotinograms, PKC activities in both membrane-associated and cytosolic fractions of sciatic nerves, and polyol content in the tail nerves were measured. Untreated diabetic rats demonstrated delayed MNCV, decreased CVR-R, reduced SNBF, and prolonged peak latencies of oscillatory potentials. Treatment with RBX as well as NZ prevented all these deficits in diabetic rats. There were no significant differences in the PKC activities in membrane-associated or cytosolic fractions of sciatic nerves between normal and diabetic rats. Treatment with neither RBX nor NZ altered these PKC activities. Nerve myo-inositol depletion in diabetic rats was ameliorated not only by NZ, but also by RBX. Hence, even though in contrast to diabetic retinopathy and nephropathy, no PKC-β activation has been demonstrated in diabetic neuropathy, these observations suggest that inhibition of PKC-β by RBX may have a beneficial effect in preventing the development of diabetic nerve dysfunction. As mentioned earlier in the present review, it has been suggested that endoneurial ischaemia may be a major contributing factor in the development of diabetic neuropathy. Thus, increased PKC-β activity in the endoneurium of diabetic rats resulting in vasoconstriction could be at least partly responsible for the observed nerve dysfunction and inhibition of PKC-β activity by RBX would also increase the endoneurial blood flow, leading to an amelioration of nerve functions. Hence, both aldose reductase inhibitors and PKC inhibitors involve different pathways, suggesting that they may have different clinical efficacies.

Cotter et al. [46] showed that in streptozotocin-induced diabetic rats, an 8-week period of diabetes caused deficits in both sciatic motor and saphenous nerve sensory conduction velocities, which were reversed by RBX. Diabetic rats had mechanical and thermal hyperalgesia. RBX treatment did not affect mechanical thresholds, but corrected thermal hyperalgesia. Sciatic nerve and superior cervical ganglion blood flow were both reduced by 50% in diabetic rats; this deficit was almost completely corrected by 2 weeks of RBX treatment. Thus, PKC-β contributes to the neural and vascular complications of experimental diabetes [46]. In addition, a 2-week period of RBX treatment was reported to correct the reductions in both sciatic motor and saphenous sensory observed conduction velocities after 8 weeks of diabetes in streptozotocin-induced diabetic rats. A dose of 10 mg/kg/day resulted in non-diabetic nerve conduction velocity values and also completely corrected the 50% diabetic reduction in sciatic endoneurial blood flow [47]. Hyperalgesia and C-fiber hyperexcitability to mechanical stimuli observed in diabetic rats were reduced by intradermal injections of agents that inhibit PKC [48]; RBX was effective for treating diabetic hyperalgesia in STZ-induced diabetic rats through amelioration of the decrease in the neuronal NOS-cGMP system [49]. Thus, experimental data with RBX provides a basis for clinical trials in neuropathy.

**Macrovascular complications**

Different studies have shown that some of the hyperglycaemia-induced PKC effects, such as hyperglycaemia-induced endothelial dysfunction in healthy humans [50], glucose induction of several NAD(P)H oxidase subunits in cultured endothelial cells [51], downregulation of eNOS expression in cultured endothelial cells [52], and increased vascular muscle cell proliferation [53], are prevented and/or reversed by RBX. However, further experimental studies are needed before a decision concerning whether specific PKC inhibition should be tested in clinical studies of atherosclerosis is made.

**Ruboxistaurin and diabetic complications: clinical evidence**

Experimental evidence and animal data suggest that PKC-βII inhibitors, and more specifically RBX, may offer an important new approach for the treatment of diabetic
microvascular complications. More specifically, much evidence demonstrates a relationship between PKC-β activation and diabetic retinal complications. Diabetic retinopathy has thus been the primary focus of initial clinical trials. Results of both phase-I and -II trials have also been promising and the first results of a phase-III trial, the PKC-DRS study, were published in Diabetes, in July 2005 [54]. The purpose of that study was to evaluate the safety and efficacy of orally administered RBX in subjects with moderately severe to very severe nonproliferative diabetic retinopathy. In this multicenter, double-blind, randomized, placebo-controlled study, 252 subjects received placebo or RBX (8, 16, or 32 mg/day) for 36 to 46 months. Compared with placebo, 32 mg/day RBX was associated with a delayed occurrence of moderate visual loss and of sustained moderate visual loss. Post-hoc analysis showed that sustained moderate visual loss tended to occur more frequently in eyes with more severe retinopathy and in those with definite diabetic macular oedema at baseline. Even though RBX reduced the risk of visual loss, it did not prevent progression of diabetic retinopathy. RBX was well tolerated and none of the patients experienced any significant adverse effects. In another study, Strom et al. evaluated the effects of oral RBX treatment on the permeability of the blood-retinal barrier in 41 patients with diabetic macular edema who were assigned to an 18-month randomized, placebo-controlled, double-blind trial (4, 16, or 32 mg/day RBX or placebo) [55]. Retinal vascular leakage was assessed using vitreous fluorometry at baseline and after 3, 12, and 18 months, demonstrating a significant interaction between RBX treatment at any dosage and baseline permeability. RBX treatment was associated with a reduction of retinal vascular leakage in patients that had diabetic macular edema and markedly elevated leakage at baseline [55]. These data suggest that clinical benefit from RBX treatment may be most prominent in patients with severe macular edema at baseline. The efficacy of RBX in diabetic eye disease is currently under evaluation in two ongoing trials to test its efficacy in slowing the evolution of macular edema.

The impact of RBX on diabetic neuropathy has also been tested in humans. In a phase-II trial, 205 patients with diabetes mellitus and diabetic peripheral neuropathy were randomized to 32 mg RBX/day (66 patients), 64 mg/day RBX (71 patients), and to placebo (68 patients) [56]. No changes in vibration detection threshold, the primary predefined endpoint, were observed between different treatment groups. Among the 83 patients with significant symptoms at baseline, there was a reduction from baseline at 12 months in the neuropathy total symptom score-6 (NTSS-6) [57] in the RBX 64 mg/day group compared with placebo. Hence, RBX treatment appeared to be of benefit only for a subgroup of patients with less severe symptomatic diabetic peripheral neuropathy by relieving sensory symptoms and improving nerve fiber function. RBX was well tolerated. One long-term, phase-III trial is currently evaluating RBX for the treatment of diabetic peripheral neuropathy. This study has now been completed [58]. The primary objective was to determine the potential of RBX to reduce the frequency and intensity of 6 painful and non-painful sensory symptoms of diabetic peripheral neuropathy as measured by a change in the NTSS-6 total score; secondary objectives included evaluation of changes seen during neurological examination.

Lastly, the effects of ruboxistaurin on diabetic nephropathy have also been evaluated in a clinical study. In a recent issue of the journal Diabetes Care, Tuttle et al. [59] have reported encouraging results of a pilot study regarding the effects of ruboxistaurin on nephropathy in type 2 diabetic patients with intensive glycaemic control and blood pressure regulation by ACE inhibitor or angiotensin receptor blocker. In this multicenter, double-blind, placebo-controlled study, 123 type 2 diabetic patients with proteinuria (mean albumin-to-creatinine ratio 764 mg/g) and near-normal serum creatinine received either 32 mg/day ruboxistaurin or placebo for up to 1 year. Patients were taking stable doses of angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers, or both, for 6 months prior to and throughout the study and RBX significantly reduced albuminuria by 24%, compared with an insignificant 9% reduction in patients taking placebo. Blood glucose levels and blood pressure control were similar at the beginning of and throughout the study. Reductions in albuminuria with RBX were seen after one month of treatment and remained consistent throughout the study. In addition, patients taking placebo experienced a significant loss of kidney function after 1 year, whereas kidney function was stable in patients treated with RBX. Thus, there was a slower decline of renal function in patients treated with RBX when compared with the baseline. These results suggest that when added to angiotensin-converting enzyme inhibitors and/or angiotensin II receptor blockers, RBX may be helpful in further slowing the progression of kidney disease.

Conclusions

Many different pathways are activated by hyperglycaemia and may be involved in the pathophysiology of diabetic complications. Thus, blocking one of these pathways might not be sufficient to prevent or treat diabetic complications. However, experimental evidence shows that PKC, and more specifically, its β isofrom, plays a key role in most pathophysiological processes associated with diabetic vascular complications. Therefore, specific inhibition of the
PKC-β isoform does appear to be a promising approach for the treatment of these complications. The first clinical trials have shown that it may be effective in slowing the evolution of microvascular complications. Thus RBX appears to be promising even though long-term studies are needed to evaluate its efficacy and side effects.

References


