

Diabetes and Vascular Disease Research

<http://dvr.sagepub.com>

Protein kinase C ² inhibition: a novel therapeutic strategy for diabetic microangiopathy

Iskandar Idris and Richard Donnelly

Diab Vasc Dis Res 2006; 3; 172

DOI: 10.3132/dvdr.2006.026

The online version of this article can be found at:
<http://dvr.sagepub.com/cgi/content/abstract/3/3/172>

Published by:



<http://www.sagepublications.com>

On behalf of:

International Society of Diabetes Vascular Disease

Additional services and information for *Diabetes and Vascular Disease Research* can be found at:

Email Alerts: <http://dvr.sagepub.com/cgi/alerts>

Subscriptions: <http://dvr.sagepub.com/subscriptions>

Reprints: <http://www.sagepub.com/journalsReprints.nav>

Permissions: <http://www.sagepub.co.uk/journalsPermissions.nav>

Citations <http://dvr.sagepub.com/cgi/content/refs/3/3/172>

Protein kinase C β inhibition: a novel therapeutic strategy for diabetic microangiopathy

ISKANDAR IDRIS, RICHARD DONNELLY

Abstract

Biochemical mechanisms involved in hyperglycaemia-induced vascular damage include alterations in cellular signalling by activation of protein kinase C (PKC). Twelve isoforms of PKC have been characterised according to their structure and co-factor requirements. Activation of PKC is mediated primarily through increased release of diacylglycerol (DAG). Adverse effects of PKC and DAG on vascular function include increased permeability, endothelial cell activation, altered blood flow, leukocyte adhesion and abnormal growth factor signalling.

A highly selective and orally active PKC- β isoform-selective inhibitor, ruboxistaurin, has been developed. Initial studies suggest that this agent decreased the development of sight-threatening macular oedema and the occurrence of visual loss. It did not, however, prevent the progression of diabetic retinopathy.

Diabetes Vasc Dis Res 2006;3:172–8

Key words: diabetes, vascular function, retinopathy, protein kinase C, ruboxistaurin.

Introduction

Chronic hyperglycaemia is the main aetiological factor for the development of microvascular complications in diabetes (e.g. retinopathy, neuropathy and nephropathy). Two landmark studies, the Diabetes Control and Complications Trial (DCCT)¹ and the United Kingdom Prospective Diabetes Study (UKPDS),² have established that intensive glycaemic control can retard the occurrence and progression of these vascular complications. However, follow-up data of the DCCT cohort, the DCCT/EDIC (Epidemiology of Diabetes Interventions and Complications),³ showed that in the group originally allocated to 'conventional control' the risk of vascular complications persisted despite conversion to

intensive glycaemic management. This phenomenon of 'metabolic memory' highlights the importance of attaining tight glycaemic control from the outset of diagnosis – a task that may not be possible in many patients with diabetes, not least because of the risks of hypoglycaemia and problems associated with treatment compliance.

An increased understanding of the pathophysiology of diabetic microangiopathy and the mechanisms of glycaemic vascular damage might facilitate the development of new therapeutic agents that ameliorate microvascular complications, even or especially when tight glycaemic control is unattainable.

This review will outline the pathophysiology of diabetic microvascular complications, describe mechanisms that have been proposed to explain the molecular pathogenesis of diabetic vasculopathy, focus on the clinical and therapeutic significance of glucose-induced activation of protein kinase C (PKC) and review clinical evidence on the efficacy and tolerability of the PKC- β inhibitor ruboxistaurin for the treatment of sight-threatening diabetic retinopathy.

Diabetic microangiopathy

Several biochemical mechanisms have been identified in the pathogenesis of hyperglycaemia-induced vascular damage. They include formation of glucotoxins by: 1) increased flux via the aldose reductase pathway;⁴ 2) accelerated formation of advanced glycation end products (AGEs);⁵ 3) increased oxidative stress;⁶ and 4) glucotoxin-induced alterations in cellular signalling by activation of PKC (figure 1).⁷

Following exposure of vascular tissues to glucotoxins, the ensuing changes in cellular structure and function lead to endothelial hyperpermeability, activation of inflammatory cells and abnormal expression of vascular and neurotrophic factors. In addition, because of cross-talk between different signalling pathways, hyperglycaemia induces increases in capillary pressure, hypoxia⁸ and/or increased activity of the local renin-angiotensin-system.⁹ These changes lead to tissue-specific vascular damage. In the retinal vasculature, for example, one of the earliest and most specific changes is the death of pericytes (retinal contractile cells), leading to endothelial cell proliferation and microaneurysm formation. Changes in vascular haemodynamics and autoregulation result initially in increased retinal blood flow, venous dilatation and beading as well as intraretinal microvascular abnormalities.

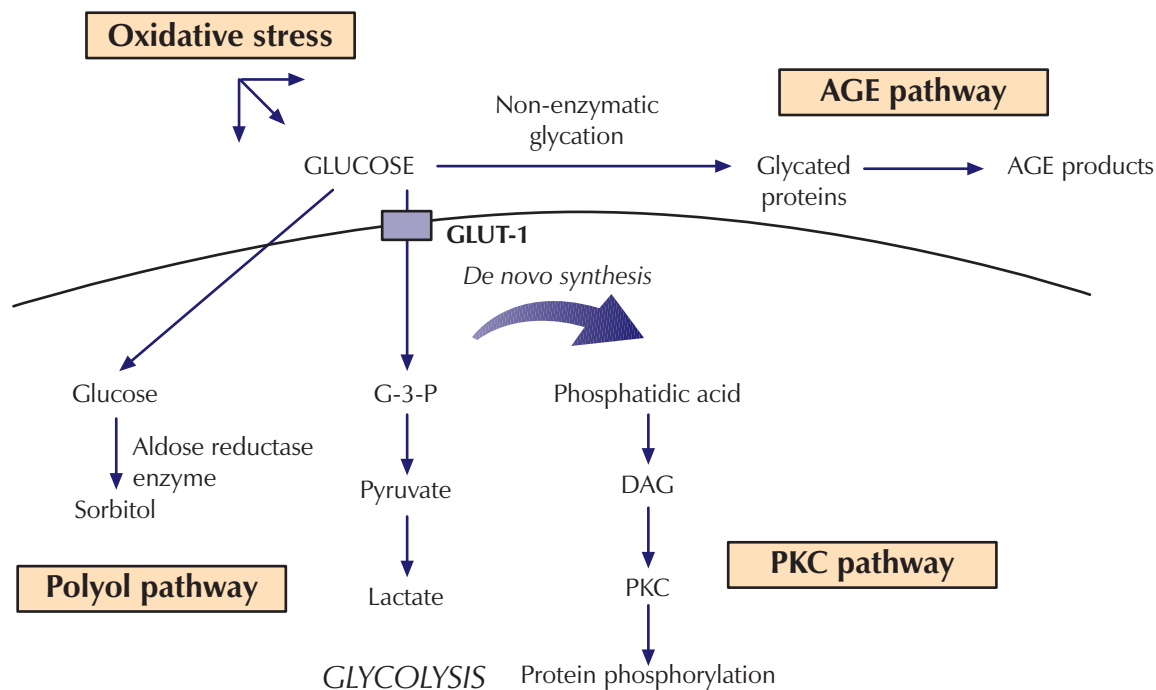
The loss of endothelial barrier function is also an early pathophysiological feature of diabetic microangiopathy. Marked increases in albumin permeation have been reported in the eye, sciatic nerve, aorta and kidney obtained from

John Peace Diabetes Centre, Kings Mill Hospital, Sherwood Forest Hospitals NHS Trust, Mansfield Road, Nottinghamshire, UK.
Iskandar Idris, Consultant in Diabetes and Endocrinology
 University of Nottingham, Derby Hospitals NHS Foundation Trust, UK.
Richard Donnelly, Professor and Associate Dean (Graduate Entry Medicine)

Correspondence to: Dr Iskandar Idris

John Peace Diabetes Centre, Kings Mill Hospital, Sherwood Hospitals NHS Trust, Mansfield Road, Sutton in Ashfield, Nottinghamshire, NG17 4JL, UK.
 Tel: + 44 (0)162 362 2515; Fax: + 44 (0)162 367 2332
 E-mail: iidris@aol.com

Figure 1. Glucose enters vascular cells and undergoes glycolysis, with a variable amount (depending upon tissue activity of aldose reductase) entering the polyol pathway. Under conditions of hyperglycaemia and increased glycolysis, glyceraldehyde-3-phosphate (G-3-P) is converted to phosphatidic acid and *de novo* synthesis of DAG occurs. Newly-synthesised DAG is rich in palmitate, and DAG activates PKC, especially the β_{II} isoform in vascular cells



Key: DAG - diacylglycerol; PKC = protein kinase C; AGE = advanced glycation end products

three-week-old diabetic rats.¹⁰ The mechanism of endothelial barrier dysfunction is not fully understood, but alterations in intracellular contractile events seem to be associated with characteristic morphological changes of the endothelial cells:¹¹ increased endothelial permeability to macromolecules; a change in the shape ('rounding up') of endothelial cells; and activation of second messenger systems.

Hyperglycaemia-induced increases in vascular permeability involve phosphorylation and relaxation of specific cytoskeletal and adhesion proteins,¹² decreased cell-to-cell contact and an increase in albumin permeability mediated in part via an effect on nitric oxide synthase.¹³ In addition, hyperpermeability changes in response to bradykinin, angiotensin II and platelet activating factor (PAF) are also mediated by hyperglycaemia.¹⁴

Progressive capillary narrowing and/or microthrombosis lead to impairment of retinal blood flow. When a large segment of the retina is affected, retinal ischaemia occurs and stimulates growth factor production. Whilst various growth factors have been postulated to play an important role in the development of diabetic retinopathy, vascular endothelial growth factor (VEGF) is the most extensively studied. VEGF (also known as vascular permeability factor - VPF) is a homodimeric glycoprotein produced by the vascular smooth muscle.¹⁵ VEGF expression is induced by hypoxia¹⁶ and by various metabolic stimuli such as platelet-derived growth factor, angiotensin II¹⁷ and high extracellular glucose.¹⁸ By virtue of its powerful angiogenic effect and

potent permeability properties, VEGF is strongly implicated in the development of neovascularisation and retinal leakage (macular oedema).

One study also showed that, regardless of contributions made by other growth factors, VEGF signalling appears to play a critical role in the pathogenesis of retinal new vessel formation: inhibition of VEGF receptor kinase activity alone is adequate to block retinal neovascularisation completely.¹⁹ Increasing evidence has also emerged to suggest the important role of renal VEGF in producing the glomerular protein leakage seen in diabetic nephropathy.²⁰ This is characterised initially by glomerular hyperfiltration, followed by expansion of extracellular matrix within the renal mesangium (predominantly mediated by increased type IV collagen and fibronectin, induced by transforming growth factor- β , TGF- β) as well as morphological changes in podocyte cell structure.

Protein kinase C and glycaemic vascular dysfunction

Adding and removing phosphate groups is an important physiological mechanism which regulates the activity of various cellular proteins. Key metabolic enzymes, for example, are switched on and off by kinases (enzymes that add phosphate groups) and phosphatases (enzymes that remove phosphate groups), which are themselves regulated by other biochemical signals such as hormones and growth factors. Kinases are broadly divided into those that phosphorylate proteins at tyrosine residues (tyrosine kinases)

Table 1. Different isoenzymes of PKC

Group A Conventional (cPKCs)	Group B Novel (nPKCs)	Group C Atypical (aPKCs)
Ca ²⁺ - and PL-dependent	Ca ²⁺ -independent and PL-dependent	Ca ²⁺ - and PL-independent
α	δ	ζ
β _I	ε	ι/λ
β _{II}	η	
γ	θ	
	μ	

Key: PL = phospholipid; PKC = protein kinase C

PKC-λ and PKC-ι may be species homologues

and those that phosphorylate them at serine and threonine sites (serine/threonine kinases). Two major serine/threonine kinases are widely distributed in all tissues: cyclic-AMP-dependent protein kinase (also known as protein kinase A) and PKC.

First described 40 years ago, PKC is not a single enzyme but rather a family of structurally and functionally related proteins, derived from multiple genes and from alternative splicing of single mRNA transcripts. Twelve isoforms of PKC have been cloned and characterised according to structure and co-factor requirements (table 1): group A (classical) PKC isoforms, e.g. PKC-α, -β_I and -β_{II}, require the presence of both calcium and phospholipid for enzyme activation; group B (novel) isoforms are calcium-independent; and group C (atypical) PKC isoforms are both calcium- and phospholipid-independent.²¹ Individual isoforms have different patterns of tissue distribution, substrate specificity and co-factor requirements.

Activation of PKC is primarily mediated by increased release of intracellular diacylglycerol (DAG).²² DAG is generated from several different sources: receptor-mediated hydrolysis of inositol phospholipids; *de novo* synthesis from phosphatidic acid; breakdown of phosphatidylcholine; and via the liberation of free fatty acids from precursor lipids by the action of phospholipase A₂.²³ In diabetes, excess glucose that is transported into vascular cells (by GLUT-1 transporter) is metabolised mostly by glycolysis. The increase in glycolysis results in increased *de novo* synthesis of DAG, which is the main endogenous activator of PKC. Increases in total DAG and PKC activation have been demonstrated in various tissues of animals and human subjects associated with diabetic vascular complications, including retina,²² aorta, heart²⁴ and renal glomeruli.²⁵

Investigators at the Joslin Diabetes Centre in Boston were first to observe that different PKC isoforms respond differently to hyperglycaemia.²² This may suggest that different species of DAG (varying in fatty acid composition)

Table 2. Hyperglycaemia induces increases in DAG and PKC activation. In particular, activation of the PKC-β isoform leads to various unwanted pathological effects, which can be broadly grouped under three headings: altered membrane transport, increased gene expression and changes in endothelial and vascular smooth muscle (VSM) function

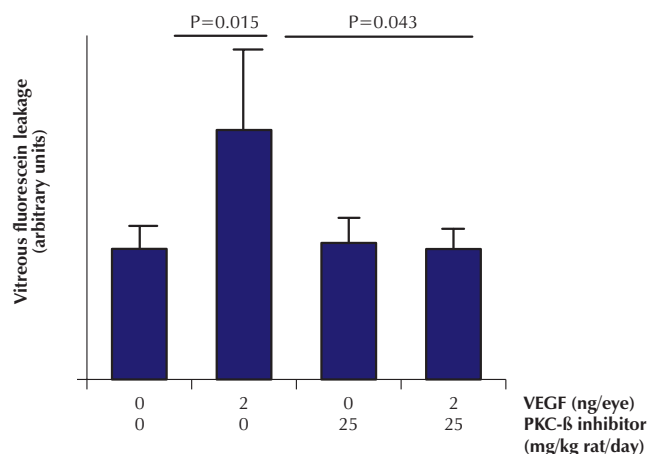
Membrane transport
Increased PLA ₂ activity Increased arachidonic acid activity Increased PGE ₂ activity Reduced Na ⁺ -K ⁺ -ATP-ase
Gene expression
VEGF permeability and angiogenesis TGF-β matrix production Growth factors, e.g. β fibroblast growth factor Vascular smooth muscle hyperplasia Intracellular adhesion molecules Endothelin-1
Endothelial and vascular smooth muscle function
Increased reactive oxygen species Impaired nitric oxide synthase regulation Angiotensin II and endothelin-1 responsiveness Impaired platelet-mediated vasodilatation

Key: DAG = diacylglycerol; PKC = protein kinase C; PLA₂ = phospholipase A₂; PGE₂ = prostaglandin E₂; VEGF = vascular endothelial growth factor

selectively activate one or more PKC isoforms in different vascular tissues. The same investigators showed that hyperglycaemia-induced DAG (rich in palmitate) preferentially activates the PKC-β_I and -β_{II} isoforms relative to other isoforms in the retina and renal tissues.²⁶ In addition, glucose appears to regulate proliferative responses in retinal endothelial cells via the PKC-β_I isoform.²⁷ In humans, PKC activity in circulating monocytes correlates with plasma glucose concentrations in subjects both with and without diabetes.

Clinical and experimental studies have since documented a number of undesirable effects of PKC activation in vascular tissues which contribute to both the structural and functional changes associated with diabetic complications. Glucose-induced activation of PKC regulates gene expression, indirectly via stimulation of mitogen-activated protein (MAP) kinase and directly by encoding for proteins involved in vascular contractility such as fibronectin, type IV collagen,²⁸ caldesmon²⁹ and nitric oxide synthase.³⁰ Growth factors and proteins involved in vascular permeability and scar tissue formation, especially VEGF and TGF-β respectively, are also regulated by PKC at the level of gene transcription.¹⁹ Increased adhesion of leukocytes, in part secondary to upregulation of ICAM-1, neutrophil integrins and other pro-inflammatory molecules, leads to retinal capillary leakage, occlusion and microthrombosis.^{31,32} In addition to the high

Figure 2. Oral administration of the PKC- β inhibitor (LY333531) to normal rats prevents the increase in vitreous fluorescein leakage following intravitreal injection of VEGF³⁹



expression of PKC isoforms in circulating white cells and platelets and the close correlation between PKC enzyme activity in circulating monocytes³³ and plasma glucose concentrations, leukocyte adhesion to retinal endothelial cells has been shown to involve several PKC-dependent pathways.³⁴

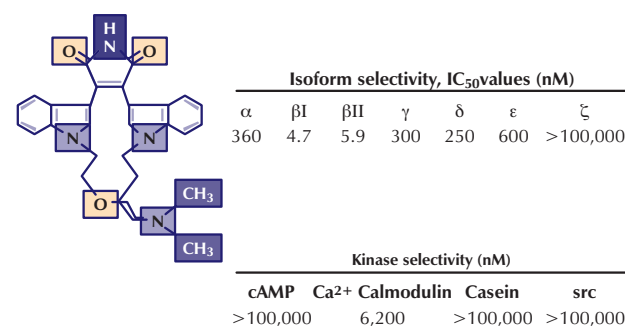
Thus, DAG and PKC appear to be critical intracellular signalling molecules which can adversely affect vascular function through increased permeability, endothelial cell activation, alterations in blood flow, leukocyte adhesion and abnormal growth factor signalling (table 2).

Relationship between PKC and VEGF in microangiopathy

VEGF is a cytokine with powerful angiogenic and mitogenic actions which has a major role in retinal and renal leakage (macular oedema and albuminuria, respectively) and neo-vascularisation. *In vitro* studies have shown that hyperglycaemia induces a dose-dependent increase in VEGF peptide production in human vascular smooth muscle cells. Ocular levels of VEGF correlate with new vessel formation in patients with diabetes,³⁵ whilst intra-vitreous administration of neutralising chimeric proteins attenuates the angiogenic response to VEGF in experimental models.³⁶ VEGF binds to the vascular endothelium via two major receptors; fms-like tyrosine kinase (Flt) and fetal liver kinase 1 (Flk-1), also known as VEGF-R1 and VEGF-R2, respectively. VEGF-R1 is expressed on both endothelial and non-endothelial cell types, whereas VEGF-R2 is expressed only on endothelial cells, especially of the retinal microcirculation.³⁷ The binding of VEGF to either receptor triggers the release of DAG, which in turn leads to activation of PKC α , β and δ .³⁸

The pathological response to VEGF appears to be dependent upon selective activation of specific PKC isoforms, but PKC- β seems to be especially important in VEGF signalling. In an *in vivo* study, intravitreal injection of VEGF was shown to activate PKC rapidly in the retina; the effect of VEGF on retinal vascular permeability appears to be mediated predominantly by the β isoform of PKC, with > 95% inhibition

Figure 3. Structure of LY333531, a macrocyclic bis-indolylmaleimide, which is an orally active PKC- β inhibitor. IC₅₀ values (i.e. concentration in nM required to achieve 50% inhibition of enzyme activity) for LY333531 with respect to each PKC isoform and related intracellular kinases⁴⁶



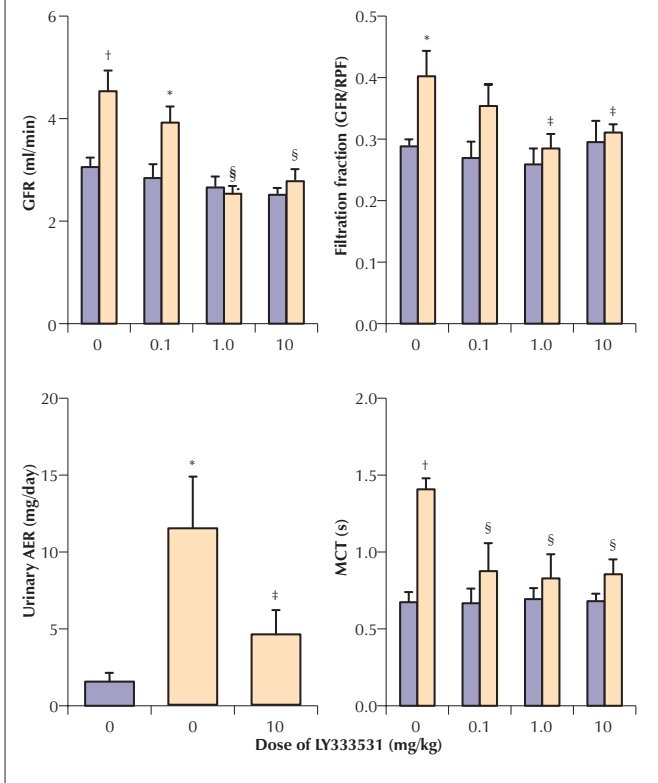
of VEGF-induced permeability by intravitreal or oral administration of a PKC β -isoform-selective inhibitor (figure 2).³⁹ Further support for the role of the PKC- β isoform in mediating advanced diabetic retinopathy was seen in studies using genetically modified animals. The angiogenic response to retinal ischaemia is increased in mice overexpressing the PKC- β II isoform and the mitogenic action of VEGF was increased two-fold in retinal endothelial cells overexpressing the PKC- β I and β II isoforms.⁴⁰ Importantly, VEGF gene transcription in response to hypoxia and/or hyperglycaemia is also partly dependent upon PKC activation,^{18,41} evidence perhaps of a feedback loop regulating VEGF and PKC activities.

Therapeutic potential of isoform-selective PKC inhibition

Early PKC inhibitors developed for experimental use, such as staurosporine, the isoquinolinesulphonamides and H7, blocked all the PKC isoforms and were not specific for PKC. Increased understanding of the biochemistry and functional roles of different PKC isoforms has led to renewed interest in developing isoform-selective blockade of PKC activation for therapeutic use – for example, using antisense oligonucleotides or macrocyclic-bis-indolyl-maleimide compounds. The chemical characterisation and *in vivo* pharmaceutical profile of a highly selective and orally active PKC- β isoform-selective inhibitor, ruboxistaurin mesylate (LY333531), was reported in *Science* by researchers in Boston⁴² (figure 3). This compound showed considerable selectivity for inhibiting PKC- β I and β II, with IC₅₀ values (drug concentration required to inhibit isoenzyme activity by 50%) of around 5 nM (i.e. more than 100 times lower than the IC₅₀ values for other PKC isoforms and kinases).

Reduction of retinal blood flow occurs early in diabetic retinopathy due to increased endothelin-1 (ET-1) production/responsiveness mediated by increased PKC activity.^{43,44} In the retina of diabetic animals, ruboxistaurin has been shown to decrease retinal PKC activity, restore Na⁺-K⁺-ATPase activity,⁴⁵ reduce VEGF-induced angiogenesis and permeability,³⁹ and normalise retinal blood flow.⁴⁶ Systemic administration of ruboxistaurin in a pig model of retinal vein occlusion also

Figure 4. Effect of oral LY333531 on renal and retinal vascular function in non-diabetic and STZ-diabetic rats. Untreated diabetic animals show increases in glomerular filtration rate (GFR), renal filtration fraction (GFR) corrected for renal plasma flow (RPF), urinary albumin excretion rate (AER) and retinal mean circulation time (MCT). Oral treatment with LY333531 0.1 -10 mg/kg/day ameliorated these renal and retinal haemodynamic abnormalities⁴²



attenuated new vessel formation in the retina and optic nerve.⁴⁷

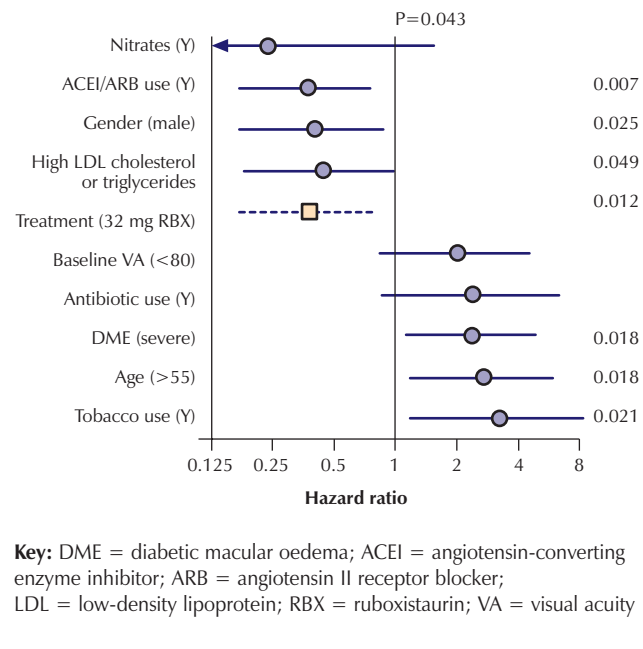
In addition to the favourable effects of PKC-β inhibition in experimental models of retinopathy, ruboxistaurin has been shown to prevent or reverse vascular dysfunction in diabetic nephropathy (figure 4).⁴² PKC inhibitors have also been shown to decrease cardiac and endothelial cell dysfunction and systemic oxidative stress in animal models of diabetes or insulin resistance.^{38,48}

Clinical evaluation of ruboxistaurin

A phase I tolerability and pharmacokinetic study in healthy volunteers^{49,50} was followed by phase II efficacy studies in patients with diabetes.⁵¹ In those with type 1 and type 2 diabetes and minimal or no evidence of diabetic retinopathy, ruboxistaurin produced a dose-dependent increase in retinal blood flow, maximal after 32 mg daily for one month.

Large international randomised controlled trials were subsequently initiated to evaluate the safety and efficacy of ruboxistaurin in larger groups of patients during longer-term administration and at different doses. A combined phase II/III trial studied 252 patients in the diabetic retinopathy arm (PKC-diabetic retinopathy study [DRS]) and 426 patients in the diabetic macular oedema arm (PKC-diabetic macular

Figure 5. Cox proportional hazard model for moderate visual loss (MVL). Severe DME is defined as retinal thickening (or adjacent hard exudates) located at or within 100 μm of the centre of the macula. Horizontal bars indicate 95% CIs, and P values are indicated to the right⁵²



edema [DME]).⁵² In the PKC-DRS trial, the aim was to evaluate whether the agent slowed the progression of non-proliferative diabetic retinopathy or prevented laser treatment. In this study, diabetic retinopathy severity was 47b to 53e (moderate-to-severe non-proliferative diabetic retinopathy [NPDR]) with no prior panretinal photocoagulation but patients could have had prior focal treatment or diabetic macular oedema at baseline. The primary end point was the progression of diabetic retinopathy (defined as 3+ step change in the Early Treatment Diabetic Retinopathy Study (ETDRS) retinopathy person severity scale or 2+ change in the ETDRS retinopathy eye severity scale) or panretinal photocoagulation.

In the DME arm, the primary outcome was the slowing or reversal of the progression of macular oedema or the prevention of laser treatment. The diabetic retinopathy severity was 20 to < 47 (mild-to-moderate NPDR). Patients had no prior photocoagulation (panretinal or focal) but could have macular oedema at baseline. The Cox proportional models for both studies are as shown (figure 5). Whilst the overall result for these two studies was largely equivocal, subanalysis of the two studies showed that ruboxistaurin decreased the development of sight-threatening macular oedema and the occurrence of visual loss, most efficacious at the 32 mg dose and where HbA_{1C} was less than 10% at baseline. Ruboxistaurin did not prevent the progression of diabetic retinopathy nor the combined outcome of DME progression or the application of laser photocoagulation. Ruboxistaurin did, however, appear to be well tolerated and pooled data from both PKC-DRS and PKC-DME (n=937) showed that there was no consistent pattern of adverse events to suggest a

causal relationship between ruboxistaurin and any spontaneously reported adverse event.

Failure to reach the primary outcomes in these studies may be due to a variety of reasons. One likely possibility was that the study was underpowered (i.e. power calculation was based on a previously documented retinopathy progression rate when glycaemic and blood pressure control were likely to be less intensive). Combining phase II with phase III trials in order to fast-track the clinical development of ruboxistaurin has resulted in inadequate knowledge about the most appropriate dose range that should be utilised within the context of a large RCT. Furthermore, it is our view that, given the experimental data favouring use of ruboxistaurin to ameliorate leakage and ischaemia in macular oedema, further clinical trials should not exclude patients receiving laser treatment but rather should evaluate ruboxistaurin as add-on therapy to 'usual care'.⁵³

Nevertheless, given the knowledge obtained from these two studies, two large high-powered single dose trials (PKC-DRS2 and PKC-DMES2), evaluating the impact of ruboxistaurin 32 mg on these end points, have recently been completed. A combined meta-analysis from the PKC-DRS and PKC-DRS2 studies showed that, compared to placebo, ruboxistaurin significantly reduced the occurrence of sustained moderate visual loss by 41% and reduced the encroachment of clinically significant macular oedema to the centre of the macula. Ruboxistaurin also increased the chance of visual acuity improvement in patients with moderately severe to very severe non-proliferative diabetic retinopathy.⁵⁴

The therapeutic potential of ruboxistaurin has also been observed in diabetic neuropathy and nephropathy. In a multinational phase II RCT to assess sensory symptoms and nerve function in patients with diabetic neuropathy, ruboxistaurin improved sensory symptoms and nerve fibre functions in a subgroup of patients with less severe diabetic neuropathy but did not prevent the progression of diabetic neuropathy as measured by nerve conduction velocity.⁵⁵ A more recent multicentre pilot study investigated the effects of ruboxistaurin on one-year diabetic nephropathy outcomes among 123 patients with type 2 diabetes and persistent albuminuria (mean albumin-creatinine ratio [ACR] 764 mg/g). The investigators reported that ruboxistaurin (32 mg/day) produced a 24% reduction of ACR and a slower decline in renal function compared with baseline.⁵⁶ The beneficial effect of ruboxistaurin is additive to intensive glycaemic control plus the inhibition of the renin-angiotensin-system, suggesting perhaps distinct vascular pathways from those by which angiotensin II and PKC- β activation mediate their pathogenic effects in patients with diabetic nephropathy. It is important to note, however, that whilst ruboxistaurin showed a reduction of ACR when compared with baseline levels, the changes were not significant when compared with those achieved by placebo when subjected to the conventional inter-group comparison. Information obtained from this 'proof of concept' study will no doubt form the basis for larger, adequately powered studies to evaluate the therapeutic effects of ruboxistaurin on diabetic nephropathy.

Conclusion

Hyperglycaemia-induced *de novo* synthesis of DAG in vascular cells leads to preferential activation of the PKC- β isoform, which is strongly implicated in the pathogenic processes involved in diabetic microangiopathy such as ischaemia, leakage, neovascularisation and abnormal vasodilator function. Ruboxistaurin is an orally active β -specific PKC inhibitor and appears to be well tolerated in large phase II and phase III clinical trials of intermediate duration (1–4 years). Preliminary evidence from these clinical studies has led to some degree of optimism with regards to its efficacy in a subgroup of patients with diabetic microangiopathy, although more large studies are needed to establish its efficacy for the treatment of diabetic microangiopathy when used in combination with established therapies.

Conflict of interest

It was supported in part through an unrestricted educational grant from Eli Lilly & Co to the University of Nottingham. RD has received consultancy fees from Eli Lilly & Co for attending scientific advisory board meetings for ruboxistaurin.

References

1. 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* 1998;**352**:837-53.
2. 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* 1993;**329**:977-86.
3. Epidemiology of Diabetes Interventions and Complications (EDIC) study group. Sustained effect of intensive treatment of type 1 diabetes mellitus on development and progression of diabetic nephropathy. *JAMA* 2003;**290**:2159-67.
4. Brownlee M. Biochemistry and molecular cell biology of diabetic complications. *Nature* 2001;**414**:813-20.
5. Singh R, Barden A, Mori T, Beilin L. Advanced glycation end products: A review. *Diabetologia* 2001;**44**:129-46.
6. Scott JA, King GL. Oxidative stress and antioxidant treatment in diabetes. *Ann NY Acad Sci* 2004;**1031**:204-13.
7. Way KJ, Katai N, King GL. Protein kinase C and the development of diabetic vascular complications. *Diabetic Med* 2001;**18**:945-59.
8. Catrina SB, Okamoto K, Pereira T, Brismar K, Poellinger L. Hyperglycemia regulates hypoxia-inducible factor-1 α protein stability and function. *Diabetes* 2004;**53**:3226-32.
9. Cooper ME. Pathogenesis, prevention and treatment of diabetic nephropathy. *Lancet* 1998;**352**:213-19.
10. Williamson JR, Chang K, Tilton RG et al. Increased vascular permeability in spontaneously diabetic BB/W rats and in rats with mild versus severe streptozocin-induced diabetes. Prevention by aldose reductase inhibitors and castration. *Diabetes* 1987;**36**:813-21.
11. Galdal KS, Evensen SA, Nilsen E. Thrombin induced shape changes of cultured endothelial cells: metabolic and functional observations. *Thrombin Res* 1983;**32**:57-66.
12. Stasek JE Jr, Patterson CE, Garcia JG. Protein kinase C phosphorylates caldesmon77 and vimentin and enhances albumin permeability across cultured bovine pulmonary artery endothelial cell monolayers. *J Cell Physiol* 1992;**153**:62-75.
13. Ramirez MM, Kim DD, Duran WN. Protein kinase C modulates microvascular permeability through nitric oxide synthase. *Am J Physiol* 1996;**271**(4 Pt 2):H1702-H1705.
14. Adamson RH, Zeng M, Adamson GN, Lenz JF, Curry FE. PAF- and bradykinin-induced hyperpermeability of rat venules is independent of actin-myosin contraction. *Am J Physiol Heart Circ Physiol* 2003;**285**:H406-H417.
15. Ferrara N. Vascular endothelial growth factor. The trigger for neovascularization in the eye. *Laboratory Invest* 1995;**72**:615-18.
16. Shweiki D, Itin A, Soffer D, Keshet E. Vascular endothelial growth factor induced by hypoxia may mediate hypoxia-initiated angiogenesis.

- Nature* 1992;**359**:843-5.
17. Williams B. Angiotensin II, VEGF and diabetic retinopathy. *Lancet* 1998;**351**:837-8.
 18. Williams B, Gallacher B, Patel H, Orme C. Glucose-induced protein kinase C activation regulates vascular permeability factor mRNA expression and peptide production by human vascular smooth muscle cells in vitro. *Diabetes* 1997;**46**:1497-503.
 19. Ozaki H, Seo MS, Ozaki K et al. Blockade of vascular endothelial cell growth factor receptor signalling is sufficient to completely prevent retinal neovascularization. *Am J Pathol* 2000;**156**:697-707.
 20. Lenz T, Haak T, Malek J, Grone HJ, Geiger H, Gossmann J. Vascular endothelial growth factor in diabetic nephropathy. *Kidney Blood Press Res* 2003;**26**:338-43.
 21. Idris I, Gray S, Donnelly R. Protein kinase C activation: isozyme-specific effects on metabolism and cardiovascular complications in diabetes. *Diabetologia* 2001;**44**:659-73.
 22. Shiba T, Inoguchi T, Sportsman JR, Heath W, Bursell S, King GL. Correlation of diacylglycerol and protein kinase C activity in rat retinal to retinal circulation. *Am J Physiol* 1993;**265**:E783-E793.
 23. Koya D, King GL. Protein kinase C activation and the development of diabetic complications. *Diabetes* 1998;**47**:859-66.
 24. Inoguchi T, Battan R, Handler E, Sportsman JR, Heath W, King GL. Preferential elevation of protein kinase C isoform ϵ II and diacylglycerol levels in the aorta and heart of diabetic rats: differential reversibility to glycaemic control by islet cell transplantation. *Proc Natl Acad Sci USA* 1992;**89**:11059-63.
 25. Craven PA, Davidson CM, DeRubertis FR. Increase in diacylglycerol mass in isolated glomeruli by glucose from de novo synthesis of glycerolipids. *Diabetes* 1996;**39**:667-74.
 26. Inoguchi T, Pu X, Kunisaki M, Higashi S, Feener EP, King GL. Insulin's effect on protein kinase C and diacylglycerol induced by diabetes and glucose in vascular tissues. *Am J Physiol* 1994;**267**:E369-E379.
 27. Knott RM, Keenan FE, Muckersie E et al. Glucose dependent decrease DNA synthesis in bovine retinal endothelial cells is mediated by protein kinase C- α . *Biochem Soc Trans* 1998;**26**:S260.
 28. Wakasaki H, Koya D, Schoen FJ et al. Targetted overexpression of protein kinase C- β_{II} isoform in myocardium causes cardiomyopathy. *Proc Natl Acad Sci USA* 1997;**94**:9320-5.
 29. Bogatcheva NV, Birukova A, Borbiev T et al. Caldesmon is a cytoskeletal target for PKC in endothelium. *J Cell Biochem* 2006 [Epub ahead of print].
 30. Sharma K, Danoff T, DePierro A, Ziyadeh FN. Enhanced expression of inducible nitric oxide synthase in murine macrophages and glomerular mesangial cells by elevated glucose levels: possible mediation via protein kinase C. *Biochem Biophys Res Commun* 1995;**207**:80-8.
 31. Schroder S, Palinski W, Schmidt-Schonbein GW. Activated monocytes and granulocytes, capillary non-perfusion and neovascularization in diabetes retinopathy. *Am J Pathol* 1991;**139**:81-100.
 32. Miyamoto K, Khosrof S, Bursell SE et al. Vascular endothelial growth factor (VEGF)-induced retinal vascular permeability is mediated by intracellular adhesion molecule-1 (ICAM-1). *Am J Pathol* 2000;**56**:1733-9.
 33. Ceolotto G, Gallo A, Miola M et al. Protein kinase C activity is acutely regulated by plasma glucose concentration in human monocytes in vivo. *Diabetes* 1999;**48**:1316-22.
 34. Park CW, Kim JH, Lee JH et al. High glucose-induced intercellular adhesion molecule-1 (ICAM-1) expression through an osmotic effect in rat mesangial cells is PKC-NF-kappa B dependent. *Diabetologia* 2000;**43**:1544-53.
 35. Aiello LP, Avery RL, Arigg PG et al. Vascular endothelial growth factor in ocular fluid of patients with diabetic retinopathy and other retinal disorders. *N Engl J Med* 1994;**331**:1480-7.
 36. Aiello LP, Pierce EA, Foley ED et al. Suppression of retinal neovascularization in vivo by inhibition of vascular endothelial growth factor (VEGF) using soluble VEGF-receptor chimeric proteins. *Proc Natl Acad Sci USA* 1995;**92**:10457-61.
 37. Jakeman LB, Winer J, Bennett GL, Altar CA, Ferrara N. Binding sites for vascular endothelial growth factor are localised on endothelial cells in adult rat tissues. *J Clin Invest* 1992;**89**:244-53.
 38. Xia P, Aiello LP, Ishii H et al. Characterization of vascular endothelial growth factor's effect on the activation of protein kinase C, its isoforms and endothelial cell growth. *J Clin Invest* 1996;**98**:2018-26.
 39. Aiello LP, Bursell SE, Clermont A et al. Vascular endothelial growth factor induced retinal permeability is mediated by protein kinase C in vivo and suppressed by an orally effective beta-isoform selective inhibitor. *Diabetes* 1997;**46**:1473-80.
 40. Suzuma K, Takahara N, Suzuma I et al. Characterization of protein kinase C beta isoform's action on retinoblastoma protein phosphorylation, vascular endothelial growth factor-induced endothelial cell proliferation, and retinal neovascularization. *Proc Natl Acad Sci USA* 2002;**99**:721-6.
 41. Aiello LP, Northrup JM, Keyt BA, Takagi H, Iwamoto MA. Hypoxic regulation of vascular endothelial growth factor in retinal cells. *Arch Ophthalmol* 1995;**113**:1538-44.
 42. Ishii H, Jirousek MR, Koya D et al. Amelioration of vascular dysfunctions in diabetic rats by an oral PKC-beta inhibitor. *Science* 1996;**272**:728-31.
 43. Takagi C, Bursell SE, Lin YW. Regulation of retinal haemodynamic in diabetic rats by increased expression and action of endothelin-1. *Invest Ophthalmol Vis Sci* 1996;**37**:2504-18.
 44. Chakravarty U, Hayes RG, Stitt AW, McAuley E, Archer DB. Constitutive nitric oxide synthase expression in retinal vascular endothelial cells is suppressed by high glucose and advanced glycation end products. *Diabetologia* 1998;**41**:945-52.
 45. Kowluru RA, Jirousek MR, Stramm L, Farid N, Engerman RL, Kern TS. Abnormalities of retinal metabolism in diabetes or experimental galactosemia: V. Relationship between protein kinase C and ATPases. *Diabetes* 1998;**47**:464-9.
 46. Jirousek MR, Gillig JR, Gonzalez CM et al. (S)-13-[(dimethylamino)methyl]-10,11,14,15-tetrahydro-4,9:16,21-dimetheno-1H,13H-dibenzof[e,k]pyrrolo[3,4] [1,4,13] oxadiazacyclohexadecene-1,3(2H)-dione (LY333531) and related analogues: isozyme selective inhibitors of protein kinase C- β . *J Med Chem* 1996;**39**:2664-71.
 47. Danis RP, Bingaman DP, Jirousek M, Yang Y. Inhibition of intraocular neovascularization caused by retinal ischaemia in pigs by PKCbeta inhibition with LY333531. *Invest Ophthalmol Vis Sci* 1998;**39**:171-9.
 48. Abiko T, Abiko A, Clermont AC et al. Characterization of retinal leukostasis and hemodynamics in insulin resistance and diabetes: role of oxidants and protein kinase-C activation. *Diabetes* 2003;**52**:829-37.
 49. Demolle D, de Suray JM, Onkelinx C. Pharmacokinetics and safety of multiple oral doses of LY333531, a PKC beta inhibitor, in healthy subjects. *Clin Pharm Ther* 1999;**65**:189.
 50. Demolle D, de Suray JM, Vandenhende F et al. LY333531 single escalating oral dose study in healthy volunteers. *Diabetologia* 1998;**41**(suppl 1):A354.
 51. Aiello LP, Clermont A, Arora V, Davis MD, Sheetz MJ, Bursell SE. Inhibition of PKC beta by oral administration of ruboxistaurin is well tolerated and ameliorates diabetes-induced retinal hemodynamic abnormalities in patients. *Invest Ophthalmol Vis Sci* 2006;**47**:86-92.
 52. The PKC-DRS Study Group. The effect of ruboxistaurin on visual loss in patients with moderately severe to very severe non-proliferative diabetic retinopathy: initial results of the Protein Kinase C beta inhibitor Diabetic Retinopathy Study (PKC-DRS) multicentre randomized clinical trial. *Diabetes* 2005;**54**:2188-97.
 53. Donnelly R, Idris I, Forrester JV. Protein kinase C inhibition and diabetic retinopathy: a shot in the dark at translational research. *Br J Ophthalmol* 2004;**88**:145-51.
 54. Aiello LP, Vignati L, Sheetz MJ et al. Effect of ruboxistaurin (RBX) on Diabetic Macular Edema (DME) and visual loss: Meta-analysis of the PKC-DRS and PKC-DRS-2. *Diabetes* 2006;**55**(S1):A54.
 55. Vinik AI, Bril V, Kempler P et al; the MBBQ Study Group. Treatment of symptomatic diabetic peripheral neuropathy with the protein kinase C beta inhibitor ruboxistaurin mesylate during a 1-year randomized, placebo-controlled, double-blind clinical trial. *Clin Ther* 2005;**27**:1164-80.
 56. Tuttle KR, Bakris GL, Toto RD, McGill JB, Hu K, Anderson PW. The effect of ruboxistaurin on nephropathy in type 2 diabetes. *Diabetes Care* 2005;**28**:2686-90.