Can protein kinase C inhibition and vitamin E prevent the development of diabetic vascular complications?

Sven-Erik Bursell a, George L. King b,*

a Beetham Eye Institute Eye Research, Harvard Medical School, Boston, MA, USA
b Research Division of Joslin Diabetes Center, Harvard Medical School, One Joslin Place, Boston, MA 02215, USA

Abstract

Hyperglycemia causes vascular complications of diabetes possible by the activation of protein kinase C (PKC). We have provided substantial evidence that activation of PKC can lead to a whole host of vascular dysfunction in diabetes. The activation of PKC induced by hyperglycemia appears to be due to an increase in diacylglycerol (DAG) levels, a physiological activator of PKC. Studies involving cultural cells, animal models of diabetes and patients have shown that inhibition of PKC by specific PKC-\(\alpha\) inhibitor was able to reverse many of the vascular dysfunctions in the retina, kidney and cardiovascular systems induced by either hyperglycemia or diabetes. In addition high doses of vitamin E were shown to decrease the level of DAG and PKC induced by diabetes or hyperglycemia. Thus animal and clinical studies have shown that high doses of vitamin E treatment can apparently reverse some of the changes in the retinal and renal vessels. © 1999 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Protein kinase C; Vitamin E; Diabetic vascular complications

1. Introduction

The variety of changes induced by hyperglycemia and diabetes are not surprising since the flux of glucose and its metabolites are known to affect many cellular pathways. Multiple theories have been proposed to explain the pathogenesis of the various complications of diabetes related to hyperglycemia involving retina, glomeruli, peripheral nerves, cardiovascular tissues, wound healing and pregnancy.

Glucose can react non-enzymatically with the primary amines of proteins forming glycated compounds or oxidants [1]. These products can secondarily act on inflammatory cells or vascular cells directly via receptor or non-receptor mediated processes to cause vascular dysfunction [2,3]. Excessive glucose can also be transported intracellularly, mainly by the glucose transporter GLUT-1, and the resulting metabolism can cause changes in the redox potential, increase sorbitol production via aldose reductase, or alter signal transduction pathways such as the activation of diacylglycerol (DAG) and protein kinase C (PKC) pathway [4–10]. It is probable that all hyperglycemia induced intra- and extracellular changes and their
adverse effects are being mediated through the alteration of some signal transduction pathways.

The effect of hyperglycemia on the activation of the DAG-PKC pathway has been extensively studied. This pathway has been reported to regulate permeability, contractility, extracellular matrix, cell growth, angiogenesis, cytokine actions and leucocyte adhesions, all of which are abnormal in diabetes [11,12].

1.1. PKC

The family of PKCs include at least 11 isoforms (α, β1, β2, γ, δ, ε, ζ, η, θ, λ, μ), representing major downstream targets for lipid second messengers or phorbol esters [11–13]. The classical PKC isoforms (α, β1, β2, γ) are Ca\(^{2+}\) dependent containing two cysteine-rich, zinc finger like motifs (C1 region), which are the binding sites of DAG or phorbol ester, and a Ca\(^{2+}\)/phospholipid (C2 region). New PKCs (δ, ε, η, θ, μ), are DAG sensitive but Ca\(^{2+}\)-independent due to the absence of the C2 region. Atypical PKCs (ζ, λ) which are insensitive to DAG and lack one of cysteine-rich motif in the C1 region, but they can be activated by phosphatidylerine.

The source of DAG causing PKC activation can be derived from the hydrolysis of phosphatidylinositides (PI) or from the metabolism of phosphatidylcholine (PC). Recent data, however, have shown that each isoform can be regulated by more than one lipid second messengers [9] such as the activation of PKC ζ by PIP3 [12–14]. Multiple isoforms can be expressed in different cell types, but despite extensive study, the attribution of a specific function to a specific isoform cannot be consistently established, suggesting that several isoforms can possibly mediate a similar range of functions and their actions are cell specific [12,15].

1.2. Mechanisms of hyperglycemia-induced PKC activation

The increases in total DAG content, induced by hyperglycemia has been demonstrated in a variety of tissues including retina [16], aorta, heart [17], and renal glomeruli [18,19] in both diabetic animal models and in patients (Table 1). This has also been observed in classically termed ‘insulin sensitive’ tissues such as the liver and skeletal muscle [20,21]. In all vascular cells studied, increasing glucose levels from 5 to 22 mM in the media elevated the cellular DAG contents [17]. Increased DAG levels in response to elevated glucose may not occur immediately and can take as long as 3–5 days after elevating glucose levels [18,22]. Xia et al. [22] have also shown that increased DAG content was chronically maintained in the aorta of diabetic dogs even after 5 years of disease. These results clearly indicate that the activation of the DAG-PKC pathway can be chronically sustained.

Agonist-stimulated hydrolysis of phosphoinositol (PI) or phosphatidylcholine (PC) can also increase Cellular DAG content by phospholipase (PL) C or D [11–13]. Since inositol phosphate products are not increased by hyperglycemia in

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Summary of DAG levels and PKC activities in cultured cells exposed to high glucose levels (15.6–25 mM) and tissues isolated from diabetic animals</th>
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<tbody>
<tr>
<td>Cultured cells</td>
<td>Diacylglycerol</td>
</tr>
<tr>
<td>Retinal endothelial cells</td>
<td>↑</td>
</tr>
<tr>
<td>Aortic endothelial cells</td>
<td>↑</td>
</tr>
<tr>
<td>Aortic smooth muscle cells</td>
<td>↑</td>
</tr>
<tr>
<td>Renal mesangial cells</td>
<td>↑</td>
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<tr>
<td>Pericytes</td>
<td>→</td>
</tr>
<tr>
<td>Tissues</td>
<td></td>
</tr>
<tr>
<td>Retina (diabetic rats and dogs)</td>
<td>↑</td>
</tr>
<tr>
<td>Heart (diabetic rats, mice, dog)</td>
<td>↑</td>
</tr>
<tr>
<td>Aorta (diabetic rats and dogs)</td>
<td>↑</td>
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<tr>
<td>Renal glomeruli (diabetic rats)</td>
<td>↑</td>
</tr>
<tr>
<td>Brain (diabetic rats)</td>
<td>ND(^a)</td>
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<tr>
<td>Peripheral nerve (diabetic rats)</td>
<td>→↑</td>
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<tr>
<td>Liver</td>
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\(^a\) ND, not determined.
aortic cells and glomerular mesangial cells, increases in PI hydrolysis are most likely not involved in diabetes [22,23]. Increases in DAG content could also arise from PC metabolism since Yasanare et al. [24] have reported that PLD activity was increased by elevated glucose levels in aortic smooth muscle cells. Most studies, however, have shown that the source of glucose-induced DAG increases was from the de novo synthesis pathway, in aortic endothelial cells [25], aortic smooth muscle cells [22] and glomeruli [18]. These studies clearly established that the increased DAG content was partially derived from glycolytic intermediates [26–28]. Palmitic acid and oleic acid are the predominate fatty acids incorporated into DAG through the de novo pathway and from the metabolism of PC, which is consistent with the findings in vascular tissues from diabetic animals [25,29].

The activation of PKC by hyperglycemia may be tissue specific since it was noted in the retina [16], aorta, heart [17], and glomeruli [8,18], but not clearly demonstrated in the brain [16] and peripheral nerves [30] (Table 1). Similar increases in DAG levels and PKC activation have also been shown in multiple types of cultured vascular cells in response to increased glucose levels [8,16,22,31] (Table 1). Thus, it is likely that DAG-PKC pathway is activated by the hyperglycemic-diabetic state in all vascular cells.

Amongst the various PKC isoforms in vascular cells, PKC β and δ isoforms appear to be preferentially activated as shown by immunoblotting studies in aorta and heart of diabetic rats [17] and in cultured aortic smooth muscle cells [32] exposed to high levels of glucose. However, increases in other isoforms such as PKC α, β2, and ε in the retina [18], and PKC α, β1, and δ in the glomerular cells from diabetic rats have also been noted [33,34]. These results demonstrated that diabetes and hyperglycemia will activate the DAG-PKC pathways in many tissue types including vascular tissues, and thus glucose and its metabolises can cause many cellular abnormalities. However, for a hyperglycemia induced change to be credible as a causal factor of diabetic complications, it has to be shown to be chronically altered, difficult to reverse, to cause similar vascular changes when activated without diabetes, and to be able to prevent complications when it is inhibited. Based on the evidence presented, DAG-PKC pathway activation appears to fulfil the first two criteria. In the following sections, data will be presented to support a fulfilment of the final two criteria with respect to the DAG-PKC pathway.

2. Functional alterations in vascular cells induced by DAG-PKC activation

Multiple cellular and functional abnormalities in the diabetic vascular tissues have been attributed to the activation of DAG-PKC pathways as described in the following paragraphs and Fig. 1.

2.1. Vascular blood flow

Abnormalities in vascular blood flow and contractility have been found in many organs of diabetic animals or patients including the kidney, retina, peripheral arteries, and microvessels of peripheral nerves. In the retina of diabetic patients without clinical retinopathy [35–38] and animals [39–43] with short durations of disease, retinal blood flows have been shown to be decreased. However, retinal blood flow may be nor-
mal or increased with longer duration of retinopathy [44, 37, 38]. Multiple lines of evidence have supported that the decreases in retinal blood flow are due to PKC activation. For example, introduction of PKC agonist such as phorbol esters into the retina will decrease retinal blood flow [16]. Decreases in retinal blood flow in diabetic rats have been reported to be normalized by PKC inhibitors [16, 19]. In non-diabetic animals, the intravitreal injection of a DAG kinase inhibitor resulted in increased retinal DAG levels, activation of PKC and a concomitant reduction in retinal blood flow (131). DAG kinase metabolizes DAG to phosphatidic acid and its inhibition will result in an increase in the available DAG pool. The results from this study showed that increased retinal DAG levels resulted in retinal blood flow decreases comparable to those measured in the diabetic rats. In addition to the retina, decreases in blood flow have also been reported in the peripheral nerves of diabetic animals, which can be normalized by PKC inhibition [45, 46], although some reports have noted increases in neuronal blood flow in diabetic rats [6]. One of the potential mechanisms by which PKC activation could be causing vasoconstriction in the retina is by increasing the expression of endothelin-1 (ET-1) [47, 48]. The decrease in blood flow to the retina could lead to local hypoxia which is a potent inducer of vascular endothelial growth factor (VEGF) causing increases in permeability and microaneurysms [49, 50].

Elevated renal glomerular filtration rate (GFR) and modest increases in renal blood flow are characteristic findings in insulin-dependent diabetes mellitus (IDDM) patients [51, 52] and experimental diabetic animals [51–53]. Diabetic glomerular hyperfiltration is likely to be the results of hyperglycemia-induced decreases in arteriolar resistance, especially at the level of afferent arterioles [54, 55], resulting in an elevation of increases of glomerular filtration pressure. Multiple mechanisms have been proposed to explain the increases in GFR and glomerular filtration pressure including an enhanced activity of angiotensin [56] and culturation in prostanoïd productions [57–59]. It is possible that the activation of DAG-PKC may also play a role in the enhancement of angiotensin actions since angiotensin mediates some of its activity by the activation of DAG-PKC pathway [57]. In addition, increases in vasodilatory prostanoïds such as prostaglandin E2 (PGE2) and PGI2 could also be involved in causing glomerular hyperfiltration in diabetes [58, 59]. The enhanced production of PGE2 induced by diabetes and hyperglycemia could be the result of sequential activation of PKC and cytosolic phospholipase A2 (cPLA2), a key regulator of arachidonic acid synthesis [60–63].

In the microvessels, increases in nitric oxide (NO) activities, a potent vasodilator, may also enhance glomerular filtration [64]. Urinary excrections of NO2/NO3, stable metabolites of NO, have been reported to be increased in diabetes of short duration [64–66], possibly due to enhanced expression of inducible NO synthase (iNOS) gene and increased production of NO in mesangial cells [67]. In addition, both increases in iNOS gene expression and NO production can be mimicked by PKC agonist and inhibited by PKC inhibitors when induced by hyperglycemia [67], suggesting that NO production might be increased in diabetes through PKC-induced iNOS overexpression. In addition, Graier et al. have suggested that NO production was enhanced by the elevation of glucose levels, possibly by the increased flux of Ca2+ and its activation of eNOS [68]. However, Craven et al. have reported that the production of glomerular NO and its second messenger, cyclic guanosine monophosphate, in diabetic rats in response to cholinergic agents were decreased and that PKC inhibitors restored the glomerular cGMP production [69]. Several authors have also reported that elevated levels of glucose decreased inducible NOS expression in vascular smooth muscle cells and that these effects of glucose were reversed by PKC-β inhibitors [70, 71]. Thus, PKC can regulate renal hemodynamics by increasing or decreasing NO production dependent on the cell type and tissue location.

In the macrovessels, increases in contractility observed in diabetes are due to a delay in the relaxation response after contraction induced by cholinergic agents [72–75]. These abnormal responses can also be prevented by PKC inhibitor
suggesting that PKC activation play a general role in causing abnormal peripheral hemodynamics in diabetes.

2.2. Vascular permeability and neovascularization

Increased vascular permeability is another characteristic systemic vascular abnormality in diabetic animals where increased permeability to albumin can occur as early as 4–6 weeks of diabetes [69], suggesting endothelial cell dysfunctions. PKC activation can directly increase the permeability of albumin and other macromolecules through barriers formed by endothelial cells [70,71] and skin chamber granulation tissues [72] probably by phosphorylating cytoskeletal proteins forming the intracellular junctions [73,74]. Interestingly, phorbol ester-induced increases in endothelial permeability may be regulated by PKC β1 activation [75], which is consistent with the preferential activation of PKC β isoforms in diabetes.

PKC activation could also regulate vascular permeability and neovascularization via the expression of growth factors, such as the vascular endothelial growth factor/vascular permeability factor (VEGF/VPF) which is increased in ocular fluids from diabetic patients and has been implicated in the neovascularization process of proliferative retinopathy [49]. We have reported that both the mitogenic and permeability-inducing actions of VEGF/VPF are partly due to the activation of PKC β isoform via the tyrosine phosphorylation of phospholipase C [76]. In vivo studies in the rat have also shown that VEGF increase in retinal vascular permeability are mediated via the PKC pathway [77]. Inhibition by PKC β selective inhibitor LY333531 can decrease endothelial cell proliferation, angiogenesis [76], and permeability induced by VEGF [77]. In addition, Williams et al. [50], have shown that the expression of VEGF was increased in aortic smooth muscle cells by elevating glucose concentration and was inhibited by PKC inhibitors.

In the kidney, the expression of transforming growth factor-β (TGF-β) has been shown to be increased in the glomeruli of diabetic patients and experimental animals. Similar increases of TGF β have also been reported in cultured mesangial cells exposed to high glucose levels [9]. Since TGF β can directly cause the overexpression of extracellular matrix, PKC inhibitors have shown both to inhibit TGF β expression by hyperglycemia and may prevent the mesangial expansion observed in diabetic nephropathy [7,9,11].

2.3. Na⁺-K⁺ ATPase

Na⁺-K⁺ ATPase, an integral component of the sodium pump, is involved in the maintenance of cellular integrity and functions such as contractility, growth, and differentiation [5]. It is well established that Na⁺-K⁺ ATPase activity is generally decreased in the vascular and neuronal tissues of diabetic patients and experimental animals [5,78–80]. Recently, we have shown that elevated glucose level (20 mM) will increase PKC and cPLA₂ activities leading to increases of arachidonic acid release and PGE₂ production resulting in decreases in Na⁺ K⁺ ATPase activity. Inhibitors of PKC or PLA₂ prevented glucose-induced reduction in Na⁺ K⁺ ATPase activities in aortic smooth muscle cells and mesangial cells [61].

2.4. Extracellular matrix components

Thickening of capillary basement membrane is one of the early structural abnormalities observed in almost all the tissues including the vascular system in diabetes [81,82]. Histologically, increases in type IV and VI collagen, fibronectin, and laminin and decreases in proteoglycans are observed in mesangium of diabetic patients [83,84]. These effects can be replicated in mesangial cells incubated in increasing glucose levels (5–20 mM) which were prevented by general PKC inhibitors [85–90]. As described above, the increased expressions of TGF-β have been implicated in the development of mesangial expansion and basement membrane thickening in diabetes [91–96]. Ziyadeh et al. have reported that neutralizing TGF-β antibodies significantly reduced collagen synthesis and gene expression of type (IV) collagen and fibronectin in renal cortex of diabetic rats and cultured mesangial cells exposed to high
glucose level [91–98]. Since PKC activation can increase the production of ECM and TGF β expression, it is not surprising that several reports have shown that PKC inhibitors can also prevent hyperglycemia- or diabetes-induced increases in ECM and TGF β in mesangial cells or renal glomeruli [32].

2.5. Selective PKC β isoform inhibition

Numerous studies have used PKC inhibitors such as staurosporine, H-7, and GF109203X to examine the role of PKC activation in diabetic vascular complications, but long-term studies involving PKC inhibitors have not been possible due to their toxicity’s associated with their non-specificity with respect to other kineses [19,99]. Since analysis of retina, kidney and cardiovascular tissues of diabetic rats showed that the PKC β isoforms were preferentially activated [17,19,32], a specific inhibitor for PKC isoforms could potentially be more effective and less toxic than the general isoform non-specific PKC inhibitor.

Recently, we have reported that increases in albuminuria and abnormal retinal and renal hemodynamics in diabetic rats can be ameliorated by an orally available PKC β isoform selective inhibitor LY333531. These physiological changes are concomitant with the inhibition of diabetes-induced PKC activation in retina and renal glomeruli [19]. LY333531 prevented the overexpression of TGF-β, α1(IV) collagen, and fibronectin in renal glomeruli of diabetic rat’s [33] (Fig. 1). These results suggested that activation of PKC β isoforms are involved in the development of some of the early abnormalities of diabetic vascular complications.

2.6. Vitamin E and PKC inhibition

Oxidative stress has been postulated as an underlying cause of diabetic vascular complications [100–104]. Antioxidants such as vitamin E have received considerable interest with respect to their potential ability to ameliorate diabetic complications. There has also been considerable interest in the use of vitamin E as an antioxidant agent for potential beneficial effects in coronary disease and cancers. Results from large multicenter clinical trials are now becoming available. A study on coronary heart disease in women [105] and in men [106] showed that increased vitamin E intake was associated with a significant risk reduction for coronary heart disease. Additionally a recently published study involving male smokers in Finland [107], showed a 32% decrease in the incidence of prostate cancer in subjects taking 50 mg of vitamin E per day. Interestingly, in this study there was a 23% increase in prostate cancer in those subjects randomized to taking beta-carotene. Clinical studies aimed at characterizing the effect of vitamin E in the eye have focused primarily on the potential benefit of vitamin E in age related macular degeneration [108,109], retinitis pigmentosa [110] and retinopathy of prematurity [111]. The results from these studies are suggestive that vitamin E specifically and other antioxidants in general may be beneficial in treating macular degeneration and retinopathy of prematurity. There have, however, been no clinical studies aimed at investigating the effect of vitamin E in diabetes.

In rat retina, vitamin E levels were five-fold higher than in other tissues such as the aorta [112]. Vitamin E supplementation further increased these retinal vitamin E levels. Other investigators have shown that vitamin E is present in primate and human retinas [113–115], that the distribution of vitamin E suggests an antioxidant protective effect for age related macula degeneration and that the level of vitamin E in the retina correlates with serum vitamin E levels [113].

Vitamin E, in parallel with its antioxidant potential, has the additional interesting property of inhibiting the activation of the DAG-PKC pathway in vascular tissues and cultured vascular cells exposed to high glucose levels [32,112]. When retinal vascular endothelial cells exposed to high glucose were treated with vitamin E (d-α-tocopherol), DAG decreased and PKC activation was normalized [32,112]. We have reported that vitamin E can inhibit PKC activation, probably, by decreasing DAG levels [32,112], since the direct addition of vitamin E to purified PKC α or β isoforms in vitro did not have any inhibitory effect [112]. These results are consistent with other
studies demonstrating that d-α-tocopherol will inhibit PKC activation [116–118]. In 1991 Boscoboinik et al. [116] first demonstrated that PKC activation was inhibited by d-α-tocopherol in a manner unrelated to d-α-tocopherol’s antioxidant action [116–119]. They also showed that the magnitude of the inhibition was related to the level of PKC activation [120] with little effect of d-α-tocopherol if cellular PKC was not activated.

Recently, the activation of DAG kinase has been suggested to be one potential site of action for vitamin E to inhibit PKC. Results indicate an indirect effect through activation of DAG kinase; increased metabolic breakdown of DAG to phosphatidic acid which resulted in decreased DAG levels and decreased PKC activation [121]. Koya et al. [122] confirmed these results in the kidney and showed that glomerular dysfunction in diabetic rats could be prevented by d-α-tocopherol treatment through PKC inhibition most probably mediated by increased DAG kinase activity.

In vivo studies in the diabetic rat have shown that the decreased retinal blood flow is related to elevation of retinal DAG levels, inhibition of DAG kinase [123] and the activation of PKC [16,32], particularly the β isoform of PKC [19,123]. The results from these studies showed that the effects of increased DAG levels and PKC activation on retinal hemodynamics in non-diabetic rats can mimic that the hemodynamic response measured in untreated diabetic rats.

In diabetic rats, intraperitoneal injection of vitamin E prevented the increases in both DAG and active PKC levels in the retina, aorta, heart, and renal glomeruli of diabetic rats [120,123]. Functionally, vitamin E treatment prevented the abnormal hemodynamics in retina and kidney of diabetic rats in parallel with the inhibition of DAG-PKC activation [120,123]. In addition, increased albuminuria was prevented by vitamin E treatment in diabetic rats [123]. Normalization of the physiological parameters studied in these diabetic rats was achieved despite chronically maintained elevated blood glucose levels. Thus, it is possible that some of the PKC activation induced by diabetes could also be the result of excessive oxidants, which are known to activate PKC and can be produced by hyperglycemia leading to the development for the vascular dysfunctions in early stage of diabetes [124].

In diabetic patients with no or minimal diabetic retinopathy retinal blood flow was reduced to an extent comparable to that measured in diabetic rats [35,37]. Studies have shown that the reduction in retinal blood flow in these patients is associated with level of glycemic control [35].

The results from a recently completed clinical study [127] showed that short duration (4 months), high-dose vitamin E treatment in 36 IDDM patients (average duration of diabetes 4.3 ± 2.7 years) with no or minimal diabetic retinopathy signigicantly normalized retinal blood flow and normalized the elevated renal creatinine clearance levels measured in these patients. This physiological normalization in the early stages of diabetes could potentially result in an amelioration of the risk for development of retinal or renal complications. Retinal blood flow changes were used to assess hemodynamic function and served as the endpoint for demonstrating the effectiveness of vitamin E treatment. The average retinal blood flow, at entry to the study, of the IDDM patients in was 17.3% lower than in the non-diabetic subjects and was in agreement with results from prior studies [35,37]. After 4 months of 1800 IU (1350 mg) vitamin E taken daily in these diabetic patients, there was a significant increase in retinal blood flow and an 88% normalization compared to non-diabetic patients. The normalization in retinal blood flow was attained despite the fact that the level of glycemic control remained unchanged during the course of the study. Interestingly, retinal blood flow normalization by vitamin E treatment was most marked in the diabetic patients with retinal blood flows comparable to flows in non-diabetic subjects showed no significant changes. Additionally there were no significant changes in the level of serum vitamin E comparing patients with low retinal blood flows and those with retinal blood flow in the normal range.

We have also recently completed a clinical study aimed at investigating in 27 diabetic patients the safety and vascular pharmacodynamic effects of a PKC-β selective inhibitor [128]. The results from this study demonstrated that the
PKC-β inhibitor provided a statistically significant dose-responsive effect on retinal MCT and retinal blood flow with normalized MCT and retinal blood flow being achieved at the highest dose used in the study. This study was performed as a double-masked, randomized, parallel, multiple-dose study of 1 month duration. As with vitamin E, the oral PKC inhibitor had no significant effect of glycohemoglobin or fasting glucose levels, suggesting that improved glycemic control was not the likely mechanism of the PKC inhibitor action.

Both clinical studies described above used retinal blood flow as an endpoint to assess retinal physiological function. The results from both these clinical studies and from our prior studies using the rat animal model, indicate that both the reduction in retinal blood flow seen in early diabetes and the normalization of the retinal blood flow following either vitamin E treatment with the PKC inhibitor can be achieved independent of any potential benefits realized through an improvement in level of glycemic control.

These clinical results combined with prior animal studies provide the support for performing further clinical studies aimed at evaluating whether vitamin E treatment is effective in reducing the risks for the development and/or progression of retinal or renal complications in diabetic patients. This will require the initiation of multicenter clinical trials aimed at answering the question of whether high doses of vitamin E can prevent the development of microvascular complications in diabetes.

3. Summary

The results presented above are consistent with the activation of the DAG-PKC signal transduction pathway may play a role in the pathogenesis of diabetes. The initiating factors are chiefly metabolic with hyperglycemia as the main triggering element. The finding that the secondary metabolic products of glucose such as glycation products and oxidants can also increase DAG-PKC suggest that the activation of DAG-PKC could be a common downstream mechanism by which multiple byproducts of glucose are exerting their adverse effects. It is not surprising that changes in the DAG-PKC pathway can play a role in diabetic microvascular complications as this signal transduction pathway is known to regulate many vascular actions and functions as described above [11,12]. The correlation between the activation of DAG-PKC and diabetic vascular and neurological complications are substantial in rodent models of diabetes [7,8,17–19,31]; however, there is limited data available to indicate that DAG-PKC levels are increased in the vasculature of diabetic patients. This is primarily due to the difficulty of obtaining fresh human vascular or neurological tissues for the measurement of DAG-PKC levels. Thus, further studies are needed to confirm whether DAG-PKC activation plays a role in the development of diabetic complications. First, the activation of the DAG-PKC pathway needs to be chronically inhibited in a long-term animal model of diabetes in order to demonstrate which of the various retinal and renal pathologies can be prevented. Long-term experiments to chronically inhibit PKC can be accomplished through the use of specific PKC isoform inhibitors or by characterizing the pathologic changes in transgenic mice strains lacking a specific PKC isoform. These experimental approaches are now possible since a specific and relatively nontoxic oral inhibitor of PKC β isoforms is now available and can be used to test which of the vascular dysfunctions are due to PKC β isoform activation [19].

Second, most of the reported findings to date have been performed in animal tissues and not in human vascular tissues. Thus, there may be differences between human and animal vascular tissue responses in relation to glucose metabolism and PKC isoform expression. A PKC-β isoform inhibitor will only be useful in diabetic patients if the same profile of PKC isoforms are activated or expressed in diabetic patients as in the diabetic rodent models. In addition, the secondary markers of PKC activation need to be identified since they can be used to monitor the effectiveness of PKC inhibition when treated with intensive glycemic control or with PKC inhibitors. Progress has been made to identify some of these potential
secondary parameters of vascular pathologies, such as the levels of VEGF, changes in retinal hemodynamics, and endothelial cell function [40,49,50,61].

The most important requirement for determining the role of activation of the DAG-PKC pathway in the vascular complication of diabetic patients has to be clinical trials using specific PKC isoform inhibitors. These trials are now in progress; specifically with the orally available PKC β inhibitor. The need for clinical trials is vital as multiple agents have been shown to be capable of reversing vascular abnormalities induced by hyperglycemia in rodent models of diabetes. None of these agents, however, has been shown to be effective in clinical trials [125,126], clearly indicating the difficulties in extrapolating results to humans from those obtained using rodent models for diabetic complications. An additional potential problem with any therapeutic PKC inhibitor used clinically is the issue of toxicity since PKC activation is involved in so many vital functions of the cell. This is especially true in the clinical arena as these agents can be used by patients over long periods of time.

Thus, a large body of evidence has suggested that the activation of the DAG-PKC pathway by hyperglycemia and diabetes plays a role in the development of some of the vascular dysfunctions and neurovascular changes noted in diabetes. However, definitive studies as described above are on-going and should determine clearly the role of DAG-PKC in the development of the various complications of diabetic patients. It is also likely that hyperglycemia and diabetes may affect other signal transduction pathways besides the DAG-PKC pathway since a number of these other pathways can also regulate vascular functions. Definitive studies using inhibitors of PKC β isoform or antioxidants will be needed to determine their effective role in preventing diabetic complications.

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