

PKC, Oxidative Stress and Retinal Endothelial Cell Dysfunction

The inhibitory effect of gliclazide on AGE-induced retinal endothelial cell dysfunction is outlined.

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Hyperglycemia is a major risk factor for the development and progression of diabetic microvascular complications such as neuropathy, nephropathy and retinopathy. Signaling pathways linking hyperglycemia with diabetic retinopathy include increased flux through the polyol pathway, protein kinase C (PKC) activation, oxidative stress and advanced glycation end products (AGEs) formation.¹⁻⁶ Activation of PKC, specifically the beta isoform, is widely recognized as a key biochemical event involved in both the early- and late-stage manifestations of diabetic retinopathy.^{4,7,8} Hyperglycemia is one main metabolic element that induces activation of the PKC pathway in retinal cells.⁹⁻¹¹

Overwhelming evidence indicates that hyperglycemia results in the generation of reactive oxygen species (ROS), leading to increased oxidative stress. Oxidative stress is a common element that links major signaling pathways implicated in hyperglycemia-induced vascular damage.^{1,12-14} Generation of oxidative stress and PKC activation are inter-related events in vascular cells exposed to high glucose. ROS act as upstream stimuli for PKC activation,¹³ and PKC enhances their production through activation of the ROS-producing enzyme, NAD(P)H oxidase.¹⁵ Evidence that vitamin E normalizes the level of PKC activation in the retina of diabetic animals identified oxidative stress as a key determinant of increased retinal PKC activity.¹⁶

LINK WITH DR

The accelerated formation and accumulation of AGEs in the retina is one major mechanism linking chronic hyperglycemia with diabetic retinopathy.¹⁷ Support for a causal role of AGEs in the development of diabetic retinopathy has been drawn from animal studies.¹⁸⁻²⁰ The toxic effect of AGEs in diabetic retinopathy involves cell-mediated effects via receptor for advanced glycation end products (RAGE), leading to oxidative stress.²¹⁻²² PKC activation, upregulation of vascular endothelial growth factor (VEGF) and increased

leukocyte adhesion to retinal endothelial cells represent potential key mediators of the deleterious effect of AGEs in diabetic retinopathy.²³⁻²⁶

Recently, we reported that AGEs induced bovine retinal endothelial cell (BREC) proliferation through induction of VEGF expression in these cells. We also documented the involvement of this growth factor in the stimulatory effect of AGEs on monocyte adhesion to retinal endothelial cells. Finally, we characterized the signaling pathways involved in the effects of AGEs on retinal endothelial cell proliferation and leukostasis and identified PKC and oxidative stress as key determinants of these effects.

This review summarizes the results of these studies and outlines the molecular mechanisms involved in the inhibitory effect of gliclazide, a sulfonylurea with antioxidant activities, on AGEs-induced retinal endothelial cell dysfunction.

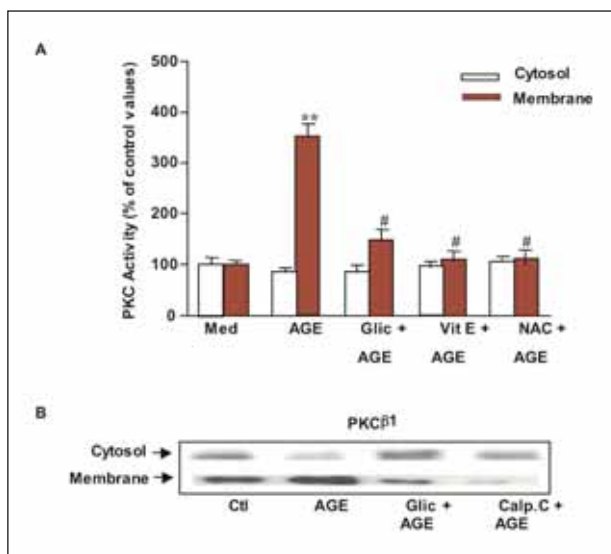


Figure 1. PKC activity increased as BRECs were treated with AGEs (A). PKC-beta 1 translocation was induced (B).

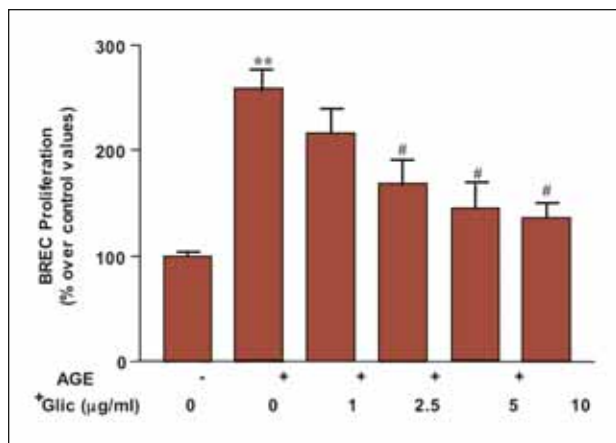


Figure 2. Cells treated with either PKC inhibitors or gliclazide.

DATA, RESULTS

To obtain the data, we incubated BRECs (passages three through six) for different periods of time with AGEs or VEGF. The cells were either previously treated with PKC inhibitors, antioxidants, sulfonylureas or left untreated. BRECs proliferation and surface expression of intercellular adhesion molecule-1 (ICAM-1) were determined. Levels of VEGF mRNA and protein in AGEs-treated BRECs were assessed. Monocyte adhesion to BRECs was quantitated by measuring monocyte myeloperoxidase activity. Finally, PKC activity and PKC-beta 1 translocation were determined.

Three major results were uncovered. We found that AGEs increased VEGF expression in BRECs through generation of oxidative stress and downstream activation of the PKC pathway. When BRECs were treated with AGEs, membrane PKC activity increased (Figure 1a) and PKC-beta1 translocation from the cytosol to the membrane was induced (Figure 1b). The AGEs-induced increase in PKC activation was inhibited by calphostin C (a PKC inhibitor), gliclazide, N-acetyl cysteine and vitamin E (Figure 1a). These agents also abolished the stimulatory effect of AGEs on VEGF expression.

We also found that VEGF, PKC and oxidative stress were involved in AGEs-induced BRECs proliferation. Incubation with AGEs or VEGF significantly increased cell proliferation. Preincubation of BRECs with a VEGF antibody abolished AGEs-induced BRECs proliferation (BRECs proliferation [percent over control values]: medium: 100 ± 6; AGEs: 174 ± 14, $P < .01$, AGEs + anti-VEGF: 75 ± 8, VEGF: 214 ± 18, $P < .01$). A similar inhibitory effect was observed when cells were treated with the PKC inhibitors (calphostin C and chelerythine) or with gliclazide (Figure 2).

AGEs increased monocyte adhesion to BRECs and ICAM-1 expression in these cells. According to our data, adhesion was mediated through VEGF-induced ICAM-1 expression—an oxidative stress sensitive effect involving PKC. Gliclazide effectively inhibited these AGEs effects (Figure 3).

DISCUSSION

Increased activation of PKC has been identified in diabetic vascular tissues and in vascular cells exposed to high glucose levels and oxidative stress.^{14,28-31} Vascular abnormalities associated with hyperglycemia-induced PKC activation include altered vascular blood flow,³² increased endothelial cell permeability,³³⁻³⁴ leukostasis⁸ and neovascularization.³⁵⁻³⁶ Evidence indicates that PKC activation is implicated in the pathogenesis of diabetic retinopathy.⁴ First, activation of PKC, especially beta isoform, is enhanced in the diabetic retina and mediates retinal blood flow abnormalities in early diabetes.^{8,37-39} Second, treatment with a PKC-beta inhibitor significantly reduced PKC activity in the retina of diabetic animals and concomitantly decreased diabetes-induced increase in retinal mean circulation time.³⁷ Third, transgenic animals overexpressing PKC-beta in vascular tissues developed retinal hemodynamic abnormalities similar to those observed in human diabetic retinopathy.⁴⁰

Evidence points to a causal role for AGEs in the pathogenesis of diabetic retinopathy. Possible pathogenic mechanisms linking AGEs to diabetic retinopathy include PKC activation and oxidative stress. There is evidence supporting AGEs involvement in PKC activation in the diabetic retina.

We and others have demonstrated that AGEs induced the translocation of PKC-beta1 in cultured BRECs and that inhibition of AGEs formation by aminoguanidine partially inhibited diabetes-induced increase in retinal PKC activity.²³⁻²⁴

Oxidative stress is a well known inducer of PKC activation in endothelial cells³¹ and increased oxidative stress is documented in retinal cells exposed to AGEs.⁴¹⁻⁴² Thus, PKC activation that we reported in AGEs-treated BRECs may involve oxidative stress. Consistent with this hypothesis, our data demonstrated that antioxidants inhibit AGEs-induced PKC activation in BRECs.

We found that gliclazide, a second-generation sulfonylurea with free radical scavenging effects,⁴³ also blocked AGEs-induced PKC activation in BRECs. Gliclazide mimicked the inhibitory effect of antioxidants on retinal PKC activation whereas glyburide, a sulfonylurea without antioxidant activity, had no effect on this parameter. This indicated that the antioxidant effects of gliclazide account for its suppressive effect on PKC activation in BRECs.

VEGF is a primary initiator of proliferative diabetic retinopathy and a potential mediator of nonproliferative retinopathy.⁴⁴⁻⁴⁶ Induction of VEGF expression is documented in isolated retinal cells in response to AGEs,^{25,47,48} thus suggesting a role for VEGF as mediator of AGEs-induced retinal vascular alterations. Our results supported this possibility and demonstrated that AGEs exert a direct stimulatory effect on VEGF expression in retinal

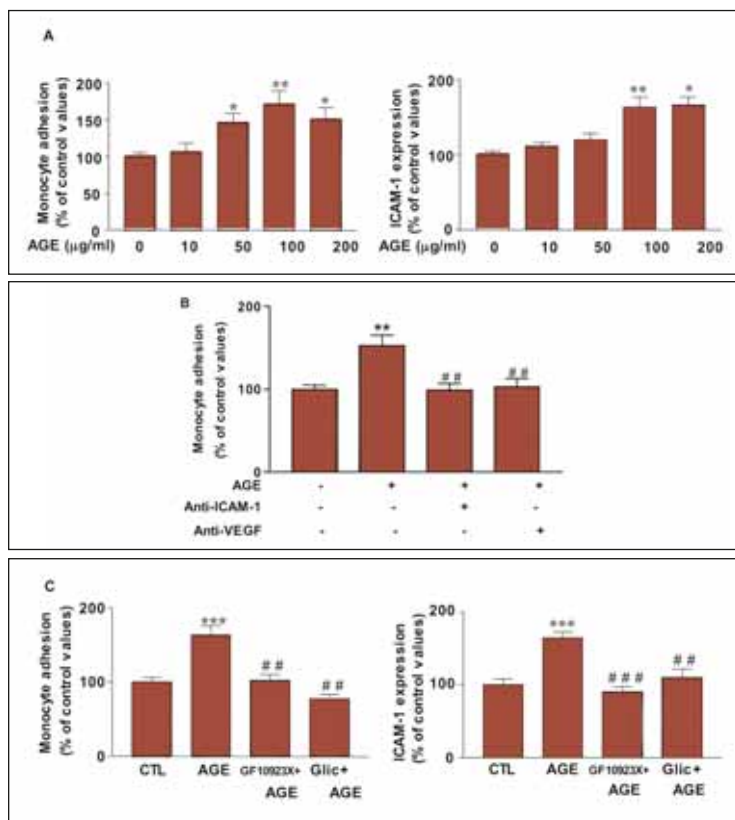


Figure 3. Gliclazide inhibits the effects of AGEs.

endothelial cells and that this growth factor is involved in the stimulatory effect of AGEs on BREC proliferation.

Our data showing an inhibitory effect of gliclazide in AGEs-induced VEGF expression and BREC proliferation indicate a potential benefit of gliclazide in diabetic retinopathy. Because PKC-beta mediates VEGF-induced growth in BRECs,⁴⁹ it is tempting to postulate that this drug may inhibit BREC proliferation through inhibition of AGEs-induced PKC activation. Alternatively because mitogen-activated protein kinase (MAPK) is involved in AGEs-induced BREC proliferation,⁵⁰ and that VEGF activates MAPK in BRECs,⁵¹ gliclazide may inhibit this biological event through inhibition of MAPK activation.

VEGF is a potent mitogen for retinal cells and acts as a proinflammatory cytokine enhancing retinal vascular ICAM-1 expression and leukostasis in vivo.⁵²⁻⁵³ We found that VEGF upregulated ICAM-1 expression in cultured BRECs and enhanced human monocyte adhesion to these cells.⁵⁴ Our findings that immunoneutralization of VEGF inhibited ICAM-1 expression and monocyte adhesion in AGEs-treated BRECs clearly identify this growth factor as the key determinant of the in vitro effect of AGEs on ICAM-1 induction and monocyte adhesion to BRECs.

We used isolated retinal endothelial cells. It seems reason-

able to postulate that VEGF released by AGEs-treated BRECs acts as an autocrine-activating stimulus for retinal endothelial cells leading to increased ICAM-1 expression and monocyte adhesion. Consistent with the role of oxidative stress in AGEs-induced leukostasis, we found that antioxidants as well as gliclazide suppressed AGEs-induced retinal endothelial cell ICAM-1 expression and monocyte adhesion.⁵⁴

These and previous results correspond, showing that gliclazide and alpha-lipoic acid reduce retinal leukostasis in diabetic rats.⁵⁵⁻⁵⁶ It has been shown that retinal leukostasis in diabetes is associated with increased retinal ICAM-1 expression and that blockade of ICAM-1 prevents diabetic retinal leukostasis.⁵⁷ Furthermore, it has been demonstrated that ROS are critical in VEGF signalling⁵⁸ and that VEGF-induced ICAM-1 expression involves the activation of oxidative stress sensitive pathways including PKC.⁵⁹ Our data also demonstrated that antioxidant agents and PKC inhibitors inhibit VEGF-induced ICAM-1 expression and monocyte adhesion to retinal endothelial cells.

In summary, these data show that AGEs increase VEGF expression in cultured retinal endothelial cells and identify this growth factor as a key mediator of the in vitro effect of AGEs on retinal endothelial cell proliferation and leukostasis. They further demonstrate the involvement of PKC and oxidative stress in these AGEs effects. Finally, data suggest the potential utility of gliclazide, a sulfonylurea with antioxidant and PKC inhibitory properties, as a therapeutic strategy for preventing AGEs-induced retinal endothelial cell alterations in diabetes. ■

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