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REVIEW ARTICLE

Advanced glycation end products and diabetic retinopathy

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Abstract Retinopathy is a serious microvascular complication of diabetes and a major cause of blindness in young adults, worldwide. Early diabetic retinopathy is characterized by a loss of pericytes from retinal capillaries, the appearance of acellular capillaries and microaneurysms, and a breakdown of the blood–retinal barrier. In later stages, this can evolve into the proliferative phase in which there is neovascularization of the retina, which greatly increases the probability of vision loss. Advanced glycation end products (AGEs) which accumulate under hyperglycemic conditions are thought to play an important role in the pathogenesis of diabetic retinopathy. AGEs arise primarily by the modification of amine groups of proteins by reactive dicarbonyls such as methylglyoxal. Intracellular proteins including anti-oxidant enzymes, transcription factors and mitochondrial proteins are targets of dicarbonyl modification and this can modify their functional properties and thus compromise cellular physiology. Likewise, modification of extracellular proteins by dicarbonyls can impair cell adhesion and can generate ligands that can potentially bind to cell surface AGE receptors that activate pro-inflammatory signaling pathways. AGE inhibitors have been shown to provide protection in animal models of diabetic retinopathy and currently are being evaluated in clinical trials.

Keywords Diabetic complications · Advanced glycation endproducts · Dicarbonyls · Methylglyoxal · Endothelial cells · Pericytes

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Introduction

Retinopathy, a serious microvascular complication of diabetes, is the leading cause of new cases of blindness in individuals between the ages of 30 and 70 years (Aiello et al. 1998; Frank 2004) and, based on current incidence of diabetes and demographics, it has been projected that the number of Americans with diabetic retinopathy will triple to 16 million by 2050 (Saaddine et al. 2008). The non-proliferative form of diabetic retinopathy (DR) is characterized by a loss of pericytes from retinal capillaries, microaneurysm formation, increased retinal capillary permeability, thickening of the capillary basement membrane, and impaired perfusion. Progression of the disease to the proliferative phase, characterized by neovascularization of the retina, greatly increases the probability of vision loss and this can be compounded by macular oedema, a result of the breakdown of the blood–retinal barrier which occurs in both non-proliferative and proliferative DR (Frank 2004). Large prospective studies of both type 1 and type 2

diabetics have shown that a tight control of glycemia reduces the occurrence of DR and other microvascular complications compared to diabetics on a conventional therapy (The Diabetes Control and Complications Trial Research Group 1993; United Kingdom Prospective Diabetes Study (UKPDS) Group 1998). Hyperglycemia therefore appears to be directly or indirectly implicated in the pathogenesis of diabetic complications. A consequence of elevated glucose levels in blood and tissues is increased modification of proteins, nucleic acids, and lipids with advanced glycation end products (AGEs) which can have profound effects on cellular function. Here, we will review the current views on the role of AGEs in the initiation and evolution of DR.

Pathophysiology of diabetic retinopathy

Pericyte dropout

One of the earliest morphological changes in DR is a disappearance of pericytes from retinal capillaries (Bergers and Song 2005; Ejaz et al. 2008) and this is thought to be the initiating factor for many of the clinical features of DR including microaneurysms (Robison et al. 1990; Lindahl et al. 1997; Hellström et al. 2001), the appearance of acellular capillaries (Hammes et al. 2004a, b), breakdown of the blood–retinal barrier (Hellström et al. 2001) and neovascularization (Enge et al. 2002; Hammes et al. 2004a). Pericytes are perivascular cells of mesenchymal origin located within the basal lamina of the microvessel (Allt and Lawrenson 2001; Armulik et al. 2005; Bergers and Song 2005) that are believed to have a contractile role similar to that of smooth muscle cells in larger vessels (Schönfelder et al. 1998; Allt and Lawrenson 2001). Compared to capillaries in other tissues, pericyte coverage of capillaries in the retina is very high (Frank et al. 1987, 1990). A complex system of paracrine communication between endothelial cells and pericytes exists during both angiogenesis and in the mature vessel that is mediated by signaling molecules that include platelet-derived growth factor β (Hirschi et al. 1998; Benjamin et al. 1998; Hellström et al. 1999), activated transforming growth factor β (Antonelli-Orlidge et al. 1989) (Sato and Rifkin 1989), vascular endothelial growth factor (VEGF) (Darland et al. 2004), angiopoietin 1 (Ang1) (Sundberg et al. 2004), and its antagonist, angiopoietin 2 (Ang2) (Hanahan 1997; Joussen 2001).

The loss of pericytes from retinal vessels in DR appears to be, at least in part, due to apoptosis. Increased numbers of apoptotic pericytes (Podestà et al. 2000; Mizutani et al. 2004) that can express Bax (Podestà et al. 2000), a pro-apoptotic member of the Bcl family of regulators of apoptosis, are present in the retinal vessels of diabetic patients. A number of mechanisms have been proposed for the induction of pericyte apoptosis in the diabetic retina including AGEs (discussed in detail below), increased glucose metabolism by the polyol pathway, and oxidative stress (Frank 2004; De La Cruz et al. 2004). Nuclear factor-κB has been shown to be activated in retinal pericytes of diabetic humans and rats and in cultured bovine retinal pericytes exposed to hyperglycemic conditions and this leads to up-regulation of the pro-apoptotic proteins Bax and tumor necrosis factor-α (TNFα) (Romeo et al. 2002). The loss of retinal pericytes has also been attributed to an increased retinal expression of Ang2 in diabetes (Hammes et al. 2004b). Intravitreal injection of mice with Ang2 (Hammes et al. 2004b) or retina-specific over-expression of Ang2 (Feng et al. 2007) reduces the numbers of pericytes in retinal vessels whereas, mice, in which one Ang2 allele had been inactivated by gene targeting, do not show retinal pericyte dropout when they are rendered diabetic by administration of streptozotocin (STZ) (Hammes et al. 2004b). Ang1 protects cultured bovine pericytes from hyperglycemia- and TNFα-induced apoptosis whereas apoptosis is exacerbated by Ang2 (Cai et al. 2008). Increased Ang2 levels in diabetes may also promote pericyte migration from retinal capillaries further contributing to pericyte loss (Pfister et al. 2008).

Acellular capillaries and impaired retinal perfusion

The mechanisms that have been proposed to be responsible for the loss of pericytes from retinal vessels have also been invoked for the subsequent loss of endothelial cells. In addition, increased leukocyte adhesion to retinal endothelial cells in experimental diabetes can lead to endothelial cell apoptosis (Li et al. 2002) through a Fas–Fas ligand pathway (Joussen et al. 2003). Diabetes-induced leukocyte adherence to retinal microvessels is reduced in intercellular adhesion molecule-1- (ICAM-1) and in CD18-deficient mice and both lines are partially protected from retinal endothelial cell death and formation of acellular capillaries in long-term diabetes (Joussen et al. 2004). Likewise, administration of a neutralizing anti-Fas ligand antibody to diabetic rats reduces retinal endothelial cell death without altering the adhesion of leukocytes to retinal vessels (Joussen et al. 2003). ICAM-1 (McLeod et al. 1995), Fas and the Fas ligand (Joussen et al. 2003) are all up-regulated in diabetes. IL-1β levels are also increased in the diabetic retina and blockade of IL-1β signalling can alleviate capillary degeneration in DR (Vincent and Mohr 2007). The loss of endothelial cells from retinal capillaries is aggravated by the inability of endothelial progenitor cells (EPCs) from diabetic individuals to participate in the vascular repair of

acellular retinal capillaries (Caballero et al. 2007). Impaired recruitment of EPCs to ischemic tissue in diabetes likely reflects both defective EPCs (Liu et al. 2005; Caballero et al. 2007) and inadequate production of EPC-mobilizing cytokines (Liu et al. 2005). Circulating EPCs are reduced in patients with moderate/severe non-proliferative DR but are increased in patients with severe proliferative retinopathy (Brunner et al. 2009). The reduced retinal perfusion that results from capillary loss leads to ischemia, which is thought to be a stimulus for the breakdown of the blood–retinal barrier and the neovascularization of the retina.

Breakdown of the blood–retinal barrier

A breakdown of the blood–retinal barrier, which can eventually lead to macular edema, occurs early in human diabetic subjects and in animal models of diabetes (Cunha-Vaz et al. 1975; Antonetti et al. 1998). The increased vascular permeability has been attributed to a local increase in VEGF concentration (Podestà et al. 2000). Within the retina, VEGF is produced by retinal pigment epithelial cells, pericytes, astrocytes, Müller glia and endothelial cells (Caldwell et al. 2003). Local hypoxia and the increased levels of inflammatory cytokines, AGEs (see below) and reactive oxygen species that occur in diabetes can induce VEGF gene expression (Caldwell et al. 2003). VEGF can provoke breakdown of the blood–retinal barrier directly or indirectly. VEGF directly alters endothelial tight junctions by suppressing expression of occludin (Antonetti et al. 1998). Exogenous VEGF also up-regulates ICAM-1 expression in retinal vessels (Miyamoto et al. 2000) and in cultured retinal endothelial cells (Mamputu and Renier 2004) and ICAM-1-mediated leukostasis can increase retinal vascular permeability (Del Maschio et al. 1996; Miyamoto et al. 2000) by triggering the disorganization of adherens (Del Maschio et al. 1996) and tight junctions (Bolton et al. 1998) and by promoting endothelial cell apoptosis (see above). Local or systemic administration of Ang1 to diabetic rats normalizes retinal levels of VEGF and ICAM-1 and leukocyte adherence in retinal vessels and prevents blood–retinal barrier breakdown (Joussen et al. 2002; Sima et al. 2004). Ang1 may act at several levels as VEGF-induced retinal vascular leakage is also suppressed in Ang1 transgenic mice (Nambu et al. 2004). Although retinal Ang1 expression is not altered in experimental diabetes, expression of the Ang1-antagonist, Ang2, is up-regulated (Ohashi et al. 2004). Whereas Ang2 acts synergistically with VEGF to increase capillary permeability (Peters et al. 2007), pigment epithelium-derived factor (Liu et al. 2004b), whose levels are decreased in diabetes, (Boehm et al. 2003) and glial cell-derived neurotrophic factor (Nishikiori et al. 2007), like Ang1, counteract the effects of VEGF on retinal vascular permeability.

Proliferative retinopathy

Progression to proliferative DR involves neovascularization of the superficial retina that can lead to blood leakage from immature vessels into the vitreous. Extension of vessels into the vitreous cavity can also provoke retinal detachment (Frank 2004). As was the case for blood–retinal barrier breakdown, increased local concentrations of VEGF are central to the etiology of proliferative DR (Caldwell et al. 2003). An excellent review of the molecular and cellular events involved in the evolution of proliferative retinopathy has recently been published (Hammes et al. 2011).

Advanced glycation end products and diabetic retinopathy

Elevated levels of advanced glycation end products (AGEs) are believed to play a causative role in DR (Stitt 2003). AGE levels are increased in hyperglycema and immunoreactive AGEs are detected in the retinas of diabetic patients (Stitt et al. 1997; Hammes et al. 1999) and experimentally diabetic rats (Thornalley et al. 2003) and, in type 1 diabetic patients, AGE-modified collagen levels predict long-term progression of DR even after adjustment for HbA_{1c} levels (Genuth et al. 2005). AGEs can arise from modification of protein amine groups by reducing sugars and by reactive dicarbonyls, such as glyoxal, methylglyoxal (MG) and 3-deoxyglucosone (Singh et al. 2001; Ulrich and Cerami 2001). MG concentrations are elevated 5–6-fold in type 1, and 2–3-fold in type 2 diabetic patients. The high reactivity of MG with proteins and its elevated concentration in the plasma suggest that it is a major glycating agent in diabetic patients (Thornalley 1998). Increased serum MG-derived hydroimidazolone (MG-hydroimidazolone) AGE adducts are associated with DR in type 2 diabetes independently of HbA_{1c} levels and are higher in patients with proliferative retinopathy than in patients with non-proliferative retinopathy (Fosmark et al. 2006). When quantified by liquid chromatography / tandem mass spectroscopy, MG-imidazolone is the predominant AGE adduct in glomeruli, retina, sciatic nerve and plasma proteins of diabetic rats (Thornalley et al. 2003) and in lenses of humans (Ahmed et al. 2003). Immunohistochemical analyses show that there is a marked increase in immunoreactive N^{ε}(carboxymethyl)lysine (CML) and MG-hydroimidazolone AGEs in the retinas of diabetic humans and rats with MG-hydroimidazolone immunoreactivity, in

early diabetes, being restricted to the microvasculature (Hammes et al. 1999)

MG metabolism and its contribution to AGE formation

While MG can be generated by a number of metabolic events (Fu et al. 1996; Thornalley 1998; Januszewski et al. 2003; Deng and Yu 2004), in diabetes, it is thought that the major source of MG is triose phosphate intermediates of glycolysis (Thornalley 1998). When cultured bovine aortic endothelial cells are exposed to hyperglycemic conditions, intracellular MG and MG-derived AGEs accumulate within 1 week (Shinohara et al. 1998; Du et al. 2003). Hyperglycemia induces an overproduction of superoxides by the mitochondrial electron transport chain (Nishikawa et al. 2000; Du et al. 2003; Hammes et al. 2003; Brownlee 2005) which activates the enzyme poly (ADP-ribose) polymerase (PARP) that, in turn, modifies and inhibits the key glycolytic enzyme, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Du et al. 2003). An important consequence of GAPDH inactivation is an accumulation of triose phosphates (glyceraldehyde-3-phosphate and dihydroxyacetone phosphate) that can undergo spontaneous dephosphorylation to MG. While retinal GAPDH activity has been shown to be reduced in STZ-treated rats (Kanwar and Kowluru 2009), at the cellular level the situation in the retina may be more complex. It has been reported that, unlike retinal pigment epithelium and Müller cells that increase glucose consumption under hyperglycemic conditions, human retinal endothelial cells do increase glucose uptake (Busik et al. 2002) but exhibit neither increased glucose consumption, increased generation of reactive oxygen species nor activation of pro-inflammatory and pro-apoptotic signaling pathways (Busik et al. 2008). In contrast to this lack of response to hyperglycemia, increased glucose consumption and production of reactive oxygen species and activation of pro-inflammatory and pro-apoptotic signaling pathways are apparent upon exposure of retinal endothelial cells to the cytokine IL-1β, even under normoglycemic conditions (Busik et al. 2008). It was therefore proposed that hyperglycemia-induced changes in retinal endothelial cell metabolism might be a paracrine phenomenon mediated by cytokines from adjacent cells (Busik et al. 2008). As a source of cytokines, Müller cells appear to be excellent candidates given their proximity to retinal capillaries (Bringmann et al. 2006) and their production of IL-1β in response to hyperglycemia (Busik et al. 2008). Moreover, it has been shown that MG modification of transcription factors in hyperglycemia can alter gene expression in Müller cells (Yao et al. 2007) and, under hypoxic conditions, Müller cells increase retinal capillary endothelial permeability (Tretoacj et al. 2008).

The glyoxalase system composed of glyoxalase 1 (GLO-1) and glyoxalase 2 (GLO-2) is largely responsible for intracellular detoxification of α-oxoaldehydes. GLO-1 is a cytosolic enzyme (Thornalley 1998), whereas cytosolic and mitochondrial forms of GLO-2 exist (Cordell et al. 2004). MG is the primary cellular substrate for GLO-1 and it accumulates in the presence of GLO-1 inhibitors (Thornalley 1998). An accumulation of intracellular MG can also occur under conditions of oxidative stress due to a depletion of the GLO-1 cofactor, GSH (Abordo et al. 1999). Human GLO-1 is a zinc-containing homo-dimer comprised of two 184-amino acid subunits (Ranganathan et al. 1993; Kim et al. 1993) whose crystal structure has been solved (Cameron et al. 1997, 1999). Human GLO-1 shows genetic polymorphism but this appears to influence neither catalytic activity (Thornalley 1993) nor susceptibility to complications in diabetic patients (McLellan et al. 1993). The human GLO-1 promoter has been partially characterized and functional insulin-responsive and metal-responsive elements were identified (Ranganathan et al. 1999). In diabetic patients, GLO-1 activity is increased in blood cells (McLellan et al. 1994; Ratliff et al. 1996) while GLO-1 activity is decreased in the liver but increased in skeletal muscle of rats with STZ-induced diabetes (Phillips et al. 1993). An end stage renal disease patient has been described who has very low levels of immunoreactive GLO-1 and GLO-1 enzyme activity, high levels of circulating AGEs and recurrent macrovascular disease in the absence of the usual risk factors (Miyata et al. 2001). It was proposed that a defective GLO-mediated detoxification of reactive α-oxoaldehydes was responsible for the elevated AGE levels and the vascular disease. Over-expression of GLO-1 in cultured endothelial cells prevents hyperglycemia-induced AGE formation and increased macromolecular endocytosis (Shinohara et al. 1998) whereas hyperglycemia-provoked apoptosis of retinal pericytes is exacerbated by GLO-1 inhibition (Miller et al. 2006). GLO-1 over-expression also protects cultured leukemia cells from anti-tumor agent-induced apoptosis (Sakamoto et al. 2000) and prevents hyperglycemia-induced inactivation of hypoxia-inducible factor 1α (Liu et al. 2005). It has been recently reported that the beneficial effects of inhibitors of the rennin–angiotensin system on diabetic retinal vascular pathology may in part result from their ability to prevent angiotensin II-mediated down-regulation of GLO-1 expression (Miller et al. 2010).

Mechanisms of α-oxoaldehyde- and AGE-mediated pathogenesis in DR

AGEs and α-oxoaldehydes can interfere with normal cell function by several distinct mechanisms. It has been estimated that between 0.1 and 0.2% of lysine and arginine residues of cellular and extracellular proteins are glycated

(Thornalley et al. 2003) and it is likely that these AGE modifications alter protein function (Bidasee et al. 2003, 2004; Ahmed and Thornalley 2007). Proteins modified by MG can be targeted for proteasomal degradation (Du et al. 2007; Rabbani and Thornalley 2008). Exposure of cultured retinal Müller cells to high glucose results in MG-modification of the transcriptional co-repressor mSin3A that, in turn, leads to increased expression of Ang2 (Yao et al. 2007). This can be prevented by over-expression of GLO-1. The role of Ang2 in the pathogenesis of DR is discussed above. Likewise, ischemia-induced vasculogenesis in diabetic mice is impaired due to MG-modification of hypoxia inducible factor 1 (HIF-1) (Liu et al. 2005) and its co-activator, p300 (Thangarajah et al. 2009). It has been recently reported that cultured aortic endothelial cells exposed transiently (16 h) to hyperglycemia show prolonged (6 days) Set7 histone methyltransferase-mediated methylation of histone 3, recruitment of methylated histone 3 to the promoter of the gene encoding the p65 NF-κB subunit and up-regulation of the transcription of both p65 and of downstream NF-κB-regulated genes. This could be prevented by overexpression of uncoupling protein 1 (UCP-1), manganese superoxide dismutase (SOD2), or GLO-1 which suggests a role for both oxidative free radicals and MG in this cellular response to hyperglycemia (El-Osta et al. 2008). The same epigenetic and transcriptional changes were seen in aortic endothelial cells isolated from non-diabetic mice, six days after the mice were challenged with 6 h of hyperglycemia and in aortic endothelial cells isolated from normoglycemic *UCP-2*$^{+/-}$ and GLO-1 knockdown mice. It was proposed that protracted MG-induced epigenetic changes provoked by transient hyperglycemic episodes could contribute to diabetic macrovascular and microvascular complications and to "metabolic memory" (El-Osta et al. 2008).

MG formed intracellularly is thought to be the major source of extracellular AGEs (Brownlee 2001). AGE cross-linking of proteins in the vessel wall increases vascular stiffness (Wolffenbuttel et al. 1998; Vasan et al. 2003) and AGE modification of extracellular matrix (ECM) proteins can decrease pericyte (Beltramo et al. 2002) and EPC (Bhatwadekar et al. 2008b) adherence. AGE-modified extracellular proteins can also cause injury via binding to cell surface AGE receptors, of which, the best characterized is RAGE, the Receptor for AGEs. RAGE, a type 1 integral membrane protein, is a member of the immunoglobulin superfamily and binds a number of ligands in addition to AGEs including β-amyloid peptide and other β–sheet fibrils (Yan et al. 1996), S100/calgranulins (Hofmann et al. 1999), amphoterin [also called high-mobility group box 1 (HMBG1)] (Hori et al. 1995) and β2-integrins (Chavakis et al. 2003). Although RAGE has been shown to be an endothelial cell adhesive receptor that is important in leukocyte recruitment to sites of inflammation (Chavakis et al. 2003) and to mediate transport of β-amyloid peptide across the blood–brain barrier (Deane et al. 2003), it functions primarily as a signaling receptor. Binding of ligands to RAGE activates a variety of signaling pathways that can lead to increased oxidative stress and the synthesis of local growth factors, cytokines and adhesion molecules (Bucciarelli et al. 2002a, b; Hudson et al. 2003; Yan et al. 2003). The role of RAGE and its ligands in retinal disease is the subject of recent reviews (Barile and Schmidt 2007; Takeuchi et al. 2010). AGEs acting through RAGE increase retinal vascular permeability (Warboys et al. 2009) and early diabetic retinopathy is exacerbated in STZ-injected mice that over-express RAGE in endothelial cells (Kaji et al. 2007) whereas blockade of RAGE signaling by injection of a soluble form of RAGE reduces retinopathy in mouse models of type 1 (Kaji et al. 2007) and type 2 (Barile et al. 2005) diabetes. While there is now strong evidence for RAGE contributing to diabetic complications, including DR, there is a growing consensus that S100/calgranulins and HMGB1, but not AGE-modified proteins, represent the true patho-physiological ligands for RAGE in diabetes (Ahmed and Thornalley 2007; Ramasamy et al. 2007; Heizmann 2007). When cultured endothelial cells are exposed to hyperglycemic conditions, expression of both RAGE and RAGE ligands is increased and this can be prevented by overexpression of UCP-1, SOD2 or GLO-1 (Yao and Brownlee 2009).

As described above, AGEs accumulate within the retinal vasculature of diabetic patients. This is likely the result of both local synthesis (Chibber et al. 1999) and deposition of circulating AGEs (Stitt 2003). AGE-modified soluble proteins (Yamagishi et al. 2004; Chibber et al. 2004) and AGE-modified extracellular matrix (AGE-ECM) proteins (Liu et al. 2004a; Stitt et al. 2004b), including α-oxoaldehyde-modified fibronectin (Liu et al. 2004a), can elicit apoptosis in cultured pericytes. This AGE-induced pericyte death has been attributed to signaling through RAGE (Yamagishi et al. 2004), induction of oxidative stress (Liu et al. 2004a), inhibition of integrin-mediated protein kinase B/Akt phosphorylation (Liu et al. 2004a), and reduced survival signaling by PDGF (Stitt et al. 2004b). AGEs also up-regulate ICAM-1 expression in cultured bovine retinal endothelial cells and could, therefore, contribute to diabetic retinal microvascular leukostasis (Moore et al. 2003; Mamputu et al. 2004) and the appearance of acellular capillaries (see above). Intravenous administration of AGE-modified albumin (AGE-albumin) to non-diabetic rats (Lu et al. 1998; Stitt et al. 2004a) and mice (Treins et al. 2001) increases retinal VEGF mRNA. Increased VEGF message is seen in the ganglion, inner nuclear, and retinal pigment epithelial cell layers of the retina after AGE-albumin intravenous injection into rats and

AGE-albumin raises VEGF mRNA and protein levels in cultured retinal endothelial cells (Mamputu and Renier 2002), pericytes (Yamagishi et al. 2002), and pigmented epithelial cells (Lu et al. 1998; Treins et al. 2001). The augmented retinal VEGF mRNA following AGE-albumin administration to rats is accompanied by a breakdown of the blood-retinal barrier and an increase in vesicular–vacuolar organelles in endothelial cells (Stitt et al. 2004a) that would be consistent with increased transendothelial transport. In cultured endothelial cells (Otero et al. 2001; Navaratna et al. 2007) and in vivo (Navaratna et al. 2007), AGEs can also trigger the disruption of adherens junctions (Otero et al. 2001). In addition to increasing retinal vascular permeability, AGE-induced upregulation of VEGF could stimulate angiogenesis during the proliferative phase of DR (Lu et al. 1998). It should be noted that, in a number of the studies cited above, conditions were used for in vitro glycation of proteins that result in levels of AGE modification that greatly exceed those which occur in vivo under patho-physiological conditions. This should be considered in evaluating the conclusions that were drawn from these studies. The AGE inhibitor, pyridoxamine, protects against the appearance of acellular capillaries and the increased expression of genes encoding protein components of the retinal capillary basement membrane (Stitt et al. 2002) and lessens Müller cell dysfunction (Curtis et al. 2011) in the retinas of STZ-induced diabetic rats. Likewise, LR90, another AGE inhibitor, reduces retinal pericyte loss and acellular capillaries in STZ-treated rats (Bhatwadekar et al. 2008a). Thus, AGEs could participate at multiple steps in the pathogenesis of DR. A simplified model of how dicarbonyl stress and AGEs might contribute to the early stages of diabetic retinopathy is presented in Fig. 1.

Conclusions

Reduction of AGEs and AGE precursors such as MG appears to be a promising strategy to reduce diabetic complications. As was demonstrated in the in the DCCT and UKPDS trials discussed above (The Diabetes Control and Complications Trial Research Group 1993; United Kingdom Prospective Diabetes Study (UKPDS) Group 1998), maintaining a tight control of blood glucose levels can prevent onset and progression of DR and this may in part be through a reduction of AGEs (Monnier et al. 1999; Genuth et al. 2005). Recent trials have, however, underlined the limitations in the use of intensive glycemic control to minimize diabetic complications (The Advance Collaborative Group 2008; The Action to control Cardiovascular Risk in Diabetes Study Group 2008). The promising results using inhibitors of AGE formation and AGE cross-link breakers in alleviating complications in diabetic animal models give hope that they will prove to be useful

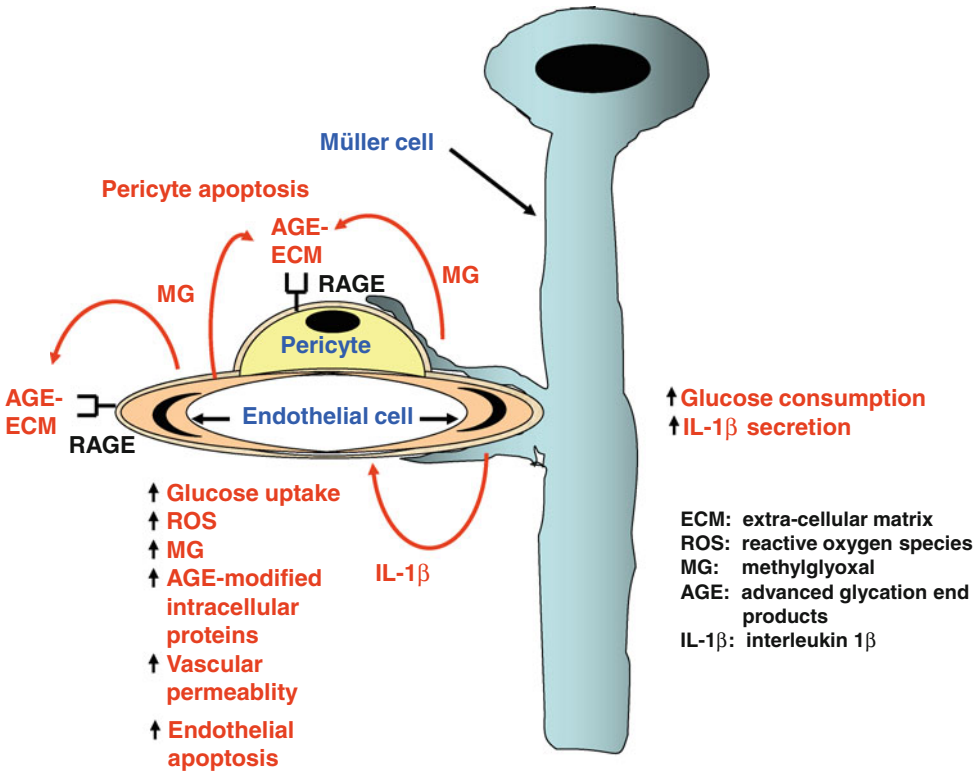

Fig. 1 A model showing putative cellular interactions that lead to dicarbonyl-mediated endothelial cell and pericyte death under hyperglycemic conditions. Hyperglycemia results in the production and secretion of both MG and cytokines by Müller cells. Müller cell-derived cytokines would increase glucose consumption in adjacent retinal endothelial cells that, in turn, would lead to generation of reactive oxygen species and MG. Pericyte and endothelial cell apoptosis could be mediated in part by AGE-modified extracellular matrix proteins or other RAGE ligands whose secretion is stimulated by hyperglycemia and that bind to cell surface RAGE

therapeutic agents. The current status of these agents in terms of clinical trials has been documented in recent reviews (Goh and Cooper 2008; Mohamed and Wong 2008). Potential agents directed at targets upstream of AGE formation that include catalytic anti-oxidants (Brownlee 2005), PARP inhibitors (Zheng et al. 2004) and transketolase activators (Benfotiamine) (Babaei-Jadidi et al. 2003; Hammes et al. 2003; Du et al. 2008; Alkhalaf et al. 2010) are being tested in animal models and in humans. The advantage of catalytic anti-oxidants, PARP inhibitors and benfotiamine is that they can potentially block other pathways (hexosamine pathway, polyol pathway, protein kinase C activation) as well as AGE formation that have been implicated in the pathogenesis of diabetic complications (Brownlee 2005). In addition to endogenously formed AGEs, AGEs present in the diet can be absorbed by the intestine and can contribute to AGE-mediated toxicity (Vlassara et al. 2002; Huebschmann et al. 2006; Sebekova and Somoza 2007; Yamagishi et al. 2007) which would indicate that dietary AGE restriction could be a non-pharmaceutical approach to protect against DR and other diabetic complications. Other potential therapeutic targets exist downstream of AGE formation. Based on abundant data from animal studies, down-regulation of RAGE expression, blockade of binding of ligands to RAGE or interference with RAGE signaling pathways would be expected to provide protection against both microvascular and macrovascular diabetic complications. A 6-month safety/efficacy trial of TTP488, an orally bioavailable, small molecule RAGE antagonist in type 2 diabetic patients has recently been completed but the results have not yet been published (http://clinicaltrials.gov/ct2/show/study/NCT00287183). VEGF, whose expression is up-regulated by RAGE-activated signaling pathways, has an important role in the development of proliferative DR and in the increased retinal vascular permeability that leads to diabetic macular oedema. Intraocular administered oligonucleotide aptamers, antibody-based, RNA antisense, and small interfering RNA, anti-VEGF agents are presently in clinical trials for DR (Mohamed and Wong 2008).

It is hoped that, in the near future, some of these therapies that target AGEs and AGE-mediated signaling pathways will prove successful in reducing DR and other diabetic complications. Moreover, further definition of the molecular mechanisms that are involved in the development and the normal physiology of the retina as well as in the pathogenesis of diabetic complications should reveal additional therapeutic targets for controlling DR. This should be facilitated through the use of animal models in which dicarbonyl stress is genetically manipulated in specific cell types (endothelial cells, pericytes, Müller cells, etc.) by over-expressing or knocking down the glyoxalase enzymes involved in the detoxification of dicarbonyls. Likewise, identification of the protein targets of AGE modification through proteomics will help to establish the molecular basis of the pathogenesis of DR.

Conflict of interest None.

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