Review

Involvement of Advanced Glycation End Products in the Pathogenesis of Diabetic Retinopathy

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Key Words

Advanced glycation end products • Diabetic retinopathy • Endothelial dysfunction • Inflammation • Angiogenesis

Abstract

Diabetic retinopathy (DR) is a common and devastating microvascular complication of diabetes and a major cause of acquired blindness in young adults. Advanced glycation end products (AGEs) accumulated under hyperglycemic conditions are thought to play an important role in the pathogenesis of DR. AGEs can exert their deleterious effects by acting directly to induce aberrant crosslinking of extracellular matrix proteins, to increase vascular stiffness, altering vascular structure and function. Moreover, AGEs binding to the receptor for AGEs (RAGE) evokes intensive intracellular signaling cascades that leading to endothelial dysfunction, elaboration of key proinflammatory cytokines and proangiogenic factors, mediating pericyte apoptosis, vascular inflammation and angiogenesis, as well as breakdown of the inner blood-retinal barrier (BRB), the end result of all these events is damage to the neural and vascular components of the retina. Elucidation of AGE-induced mechanisms will help in the understanding of the complex cellular and molecular pathogenesis associated with DR. Novel anti-AGEs agents or AGE crosslink “breakers” are being investigated, it is hoped that in next few years, some of these promising therapies will be successfully applied in clinical context, aiming to reduce the major economical and medical burden caused by DR.

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Introduction

Diabetic retinopathy, the most common microvascular complication of diabetes mellitus (DM), is one of the leading causes of new onset blindness among adults. It was initially assumed that nearly all patients of type 1 diabetes and more than 60% of patients with type 2 diabetes result in some degree of DR 15–20 years after diagnosis [1]. However, recently a Q. Liu and J. Wu contributed equally to this work.

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large amount of work implied that, the pathological changes that end up to microvascular complications begin within days to weeks of onset of diabetes. As shown in the UK Prospective Diabetes Study (UKPDS) and the Hoorn Study, around 20% of the DM patients had microvascular diabetic complications including retinopathy and neuropathy at the time of diagnosis [2]. Likewise, the Gutenberg health study (GHS) even shows a prevalence of 8.2% for DR among the pre-diabetic population in Mid-Western Germany (7.5% mild non-proliferative diabetic retinopathy (NPDR); 0.5% moderate and 0.3% with severe NPDR) [3].

The early stage of DR (non-proliferative phase, NPDR) is characterized by a loss of pericytes from retinal capillaries, the formation of acellular capillaries and microaneurysms, increased vascular permeability and thickening of the capillary basement membrane [1]. Changes in the permeability characteristics of retinal endothelial cells therefore exert irreversible damages to the BRB [4], which is a key event in the early pathology of DR. Left untreated, the disease can evolve into the proliferative phase (PDR), characterized by neovascularization of the retina, bleeding and leakage from the vulnerable new vessels results in fibrovascular epiretinal membranes, vitreous hemorrhage, and tractional retinal detachment, greatly increasing the probability of vision loss [1].

The morbidity of DR in pre-diabetic population highlights the necessity for more researches going deep into molecular levels, facilitating detection of DR in its early stage thus allowing for timely intervention. Despite that hyperglycemia has been proved directly or indirectly implicated in the pathogenesis of DR [5, 6], mechanisms involved are still failed to be fully elucidated. Elevated glucose levels in blood and tissues subsequently upregulate the modification of proteins, lipids, and nucleic acids after contact with aldose sugars, turned into AGEs [7], cross-talk between AGEs and other interconnected elements or pathways can evoke profound effects on cellular functions, which eventually give rise to the clinical picture of DR, this is what we review and will be discussed in detail in this article.

AGEs and AGE-RAGE Interaction

AGEs accumulating under hyperglycemic conditions are acknowledged to play a causative role in both the microvascular and macrovascular complications of diabetes including retinopathy, neuropathy, nephropathy, also atherosclerosis [8, 9]. Under hyperglycemia, AGEs are formed through the non- enzymatic glycation reaction (called the “Maillard reaction”), which begins with the transformation of reversible, unstable Schiff base adducts to relatively stable, covalently bound Amadori rearrangement products [10]. Additional oxidative and dehydrated reactions of Amadori products contribute to the formation of irreversible forms of protein-bound AGEs [11]. Moreover, apart from endogenous formation of AGEs, they can also be generated from exogenous sources including tobacco, smoke, and diet [12]. Food-processing methods, such as prolonged heat processing and microwave cooking, can also accelerate the generation of glyco-oxidation and lipo-oxidation adducts, thus increasing the proportion of AGEs ingestion along with food [12, 13].

Prolonged deposition of AGEs has been proved to be detrimental to cells and tissues via several mechanisms [11]. Aberrant crosslinks between AGEs and key molecules of extracellular matrix result in a decrease in the elasticity and an increase the stiffness of vessels, consequently increase the thickness and rigidity of the vessel wall [14, 15]. In addition, binding of AGEs to different cellular receptors can trigger multiple cell signaling pathways. RAGE is the first discovered and so far the most studied receptor for AGEs. It is an immunoglobulin superfamily protein of 35-kDa, functions primarily as a multiligand transmembrane binder for several molecules [8]. RAGE localizes in various cell types such as macrophages, monocytes, endothelial cells (ECs), smooth muscle cells and hepatocytes [16], in eyeball, it is constitutively expressed on vascular endothelial cells, pericytes, microglia, Müller glia and retinal pigmented epithelium (RPE) cells, with expression notably increased under diabetic conditions [17].

AGEs binding to RAGE induces activation of various downstream pathways such as p21ras and mitogen-activated protein kinases (MAPKs) pathways, with subsequent translocation of nuclear factor- κ B (NF- κ B) and elevated synthesis of growth factors, pro-inflammatory cytokines, as well as adhesion molecules, end up in a disorder of cell functions [11, 18]. Additionally, RhoA/ROCK has been associated to AGE-RAGE axis, AGEs bond to RAGE mediates endothelial activation, proliferation, migration, and tubulogenesis by inducing moesin phosphorylation via RhoA/ROCK pathway [19]. Increased oxidative stress has also been reported upon AGE-RAGE ligation through activation of NADPH [18]. Although multiple pathways have been reported, the proximal signaling proteins that initiate transduction upon AGE-RAGE interaction are still under study.

However, the involvement of AGEs and AGE-RAGE interaction in the development of retinopathy remain suspected for years. Emerging clinical evidence demonstrated that high vitreous levels of AGEs might instigate the proliferative changes in retinal capillaries of NPDR subjects to more severe DR [20, 21]. AGEs acting through RAGE increase the retinal endothelial cell permeability, resulting in vascular leakage and breakdown of BRB [22]. Two distinct mechanisms were proposed in a review published recently, AGE-RAGE interaction facilitates angiogenesis by relieving the restriction on ECs growth due to the apoptotic cell death of pericytes and by autocrine and paracrine induction of vascular endothelial growth factor (VEGF) proteins by vascular wall cells [23].

AGE-mediated pathogenesis in DR

Pericyte Dropout

Pericytes are perivascular cells of mesenchymal origin, embedded in the basal lamina of the microvessels, believed to have a contractile property similar to smooth muscle cells in larger vessels, and is essential for the maintenance of microvascular integrity and homeostasis [18]. Notably, retinal vessels are covered with pericytes at the highest density in the body, indicating their crucial status in ocular diseases. Loss of pericytes is considered as one of the earliest morphological hallmark of DR, subsequently predispose the vessels to EC injury, thrombogenesis, the appearance of acellular capillaries and neovascularization [24].

AGEs accumulate in retinal pericytes during diabetes, mediating pericyte survival and function that consequently leading to pericyte loss [24]. However, the loss of pericytes from retinal vessels with DR appears to be, at least in part, attributed to apoptosis [25]. Studies have established that the AGEs stimulating ROS production in cultured retinal pericytes is able to elicit apoptotic cell death of pericytes. In addition, ROS is thought to be produced via the p47phox- and Rac1-dependent nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activation [25]. The generated ROS induce pericyte apoptosis possibly by stimulating intracellular ceramide formation via activation of phosphatidyl-choline phospholipase C coupled to acidic sphingomyelinase [26]. Furthermore, AGEs evoke the translocation of NF- κ B to the nucleus under hyperglycemia, particularly in pericytes but not in endothelial cells, which determines the downstream consequences of AGE-stimulated ROS evaluation in pericytes [25, 27].

NF- κ B is recognized as a crucial redox-sensitive transcription factor, in its inactive condition, it is stabilized in a latent form in the cytoplasm, functioning to be pro-survival whereas masking the nuclear localization signal. Once activated, it will be dissociated from I κ B α and translocates into the nucleus to modulate the transcription of its target genes, eventually leads to the upregulation of proapoptotic proteins Bax, inducible nitric oxide synthase (iNOS) and tumor necrosis factor- α (TNF- α) [27-29]. Decreased ratio of Bcl-2/Bax, subsequently increases the activity of caspase-3, a key enzyme in the execution of apoptosis of pericytes [27]. The induction of iNOS produces large quantities of NO, which is also a well-known mediator of apoptosis [29]. Moreover, as a secreted cytokine, TNF- α was speculated could reach neighboring ECs and transfer their phenotype to proinflammatory and procoagulant, resulting in capillary occlusion and EC apoptosis [27].

Additionally, induction of endoplasmic reticulum (ER) stress and the unfolded protein response (UPR) were assessed in diabetic mice and human retinal pericytes exposed to AGEs recently, to elucidate their role in DR [30]. According to Chung and associates' research, under the application of AGEs, multiple ER stress markers, specifically phosphorylated eukaryotic translation initiation factor-2 α (p $\text{eIF-2}\alpha$) and CCAAT-enhancer-binding protein homologous protein (CHOP) levels were significantly upregulated, whereas this can be attenuated by ursodeoxycholic acid (UDCA), a known ameliorator of ER stress. Consequently, vascular integrity was protected and pericyte loss was reduced in the retina of STZ-induced diabetic mice [30]. UPR is supposed as a cellular mechanism activated to overcome ER stress and restore cellular homeostasis, however it may induce apoptotic death in cases of prolonged and severe ER stress [30, 31]. Apart from UPR, autophagy is also regarded as a cytoprotective mechanism utilized by pericytes, until a critical stress threshold is exceeded [32]. The interplay between autophagy and apoptosis is critical for pericyte survival, once the apoptotic mechanisms are activated, both UPR and autophagy shifts from a pro-survival to a lethal role [32].

Endothelial Dysfunction

Vascular endothelial cells, lining the inner surface of the vessels, constitute a size-selective and semi-permeable barrier. However, in diabetes, certain inflammatory stimulates target ECs, resulting in hyperpermeability [15]. Therefore, the ensuing impairment of BRB further induces macular edema and vascular hemorrhage, compromising retina integrity [33]. Previous researches *in vitro* have verified that the accumulation of AGEs in the blood greatly contributes to the disruption and exacerbation of the retinal vascular permeability in DM. A complex system of paracrine interaction between ECs and pericytes exists during both angiogenesis and in the mature vessel, that is mediated by signaling molecules including platelet-derived growth factor β (PDGF- β), activated transforming growth factor β (TGF- β), VEGF, angiopoietin 1 (Ang1), and its antagonist, angiopoietin 2 (Ang2) [34]. Lost pericytes leave behind a cavity in the capillary wall known as a pericyte ghost, whereas lost endothelial cells lead to become acellular capillaries that finally affect visual function [35]. Those mechanisms that have been proposed to be responsible for the pericytes loss have also been partially invoked for the subsequent loss of ECs and the increase of endothelial permeability. Thus, instead of ECs death, some more specific mechanisms mediating cell to cell junctions will be discussed here.

Worthy of note, the integrity of BRB is maintained by the existence of junctional complexes between adjacent ECs and adhesive interactions between ECs and neighboring pericytes. Phosphorylation and reorganization of the junction proteins is deemed as characteristic events in the development of barrier dysfunction [36]. Occludin, claudin-5 and zonula occludens (ZO) family are reported to be the principal tight junction (TJ) proteins found in retinal endothelial cells (RECs) [37, 38]. Particularly, ZO-1 is a cytoplasmic protein that links occludin to the other intracellular junction structures [36]. Adherens junctions (AJ) are mediated by VE-cadherin which promotes Ca^{2+} -dependent homophilic cell to cell contacts, and interact intracellularly with actin cytoskeleton through catenins [36], while AGEs stimulation resulted in cleavage of VE-cadherin from the cell surface [39]. Treatments regarding to restore the expression of junction proteins are able to protect RECs from hyperglycemic insult to maintain their barrier properties [38, 40]. Signaling events downstream of the AGE-RAGE pathway that dysregulate the junctional molecules were demonstrated in the last few years but still remained to be determined. One known mechanism was proposed with regard to the activation of protein kinase C (PKC) and its isoforms. PKC δ activation, related to its subcellular translocation and subsequent decrease of tight junction proteins ZO-1 and ZO-2, is involved in increased vascular permeability in AGE-treated RECs [41]. Further research proved that suppression of PKC ζ can also attenuates AGE-induced tight junction protein loss, therefore to prevent vascular leakage in diabetic retinopathy [38].

As previously mentioned, actin-binding proteins (ABPs) like catenin act as accessory molecules bridge that linking VE-cadherin to the actin cytoskeleton, thereby control

cytoskeleton function and thus junctional integrity [42]. In endothelium, the actin filament (F-actin) is a structural unit of highly organized, double-stranded protein assemblies that include actin bundles, actin networks, cortical actin filaments and membrane cytoskeleton [43]. All of these structures together with microtubules and intermediate filaments constitute the cytoskeleton. Previous studies have demonstrated that AGEs can significantly increase endothelial monolayer permeability via disorganized endothelial F-actin cytoskeleton and VE-cadherin distribution [44]. Here, under AGEs stimulation, the cortical actin rim along the cell periphery is rearranged into stress fibres which span throughout the cell. In parallel to disorganization of junctional complexes, stress fibres are believed to increase centripetal tension which counteracts tension derived from the cortical actin rim and therefore may mediate cell-cell border retraction and give rise to paracellular gap formation [42]. A recent study shed light on the activation of Ezrin-radixin-moesin (ERM) protein moesin, which is known to work as a linker to regulate actin-membrane interactions in a signal-dependent manner. The inhibition of moesin expression could attenuate the formation of F-actin stress fiber and the hyper-permeability response in AGE-stimulated RECs [44]. Moreover, further results revealed that the phosphorylation of moesin is triggered via Rho kinase (ROCK) and MAPKs activation, indicating a complex signaling system elicited by AGEs that includes the activation of ROCK and p38 α MAPK and the phosphorylation of ERM proteins, especially moesin [45].

Vascular Inflammation

As an organism in response to noxious stimuli, chronic low-grade subclinical inflammation has been implicated in many of the signature vascular lesions of DR and may represent the inciting and final common pathway leading to the pathology. This conception was supported by the clinical results from Hoorn Study, a population-based cohort study, which recruited 625 patients and suggested that the prevalence of retinopathy was positively associated with tertiles of C reactive protein (CRP) and soluble intercellular cell adhesion molecule 1 (sICAM-1) [46]. Furthermore, it has been recognized that hyperglycemia deteriorates vasculature via inducing endothelial activation and proinflammatory phenotype of endothelial cells, which is marked by upregulation of cell surface adhesion molecules such as ICAM-1 and vascular cell adhesion molecule-1 (VCAM-1) [40].

Increased numbers of leukocytes in retinal vasculature of diabetic mice in animal study has been shown to begin as early as 1 week following experimental diabetes onset [47]. Intercellular adhesion molecules including ICAM-1 and VCAM-1 facilitate the adhesion and transmigration of leukocyte to endothelium, which is known as leukostasis, contributing to early DR [48]. Once activated, these polymorphonuclear leukocytes (PMNs) create an oxidative burst that damages ECs [49]. With repeated aggressions, the ECs dropout and a platelet/fibrin thrombus is formed. Eventually, so does pericytes also disappear, resulting in capillary degeneration and occlusion [18, 40, 49]. The vascular inflammation also promotes generation of VEGF which therefore increases vascular permeability and later progresses angiogenesis [49]. Previous study observed that leukostasis was markedly increased in nondiabetic RAGE-transgenic mice and this change was exacerbated in diabetes, indicating that the overexpression of RAGE and the increased interaction of AGEs and RAGE are sufficient to induce leukostasis in these animal models [50]. Reportedly, ICAM-1 antibody completely suppressed the AGE-elicited T cell adhesion to ECs [51]. Moreover, endothelial ICAM-1 gene transcription and expression are demonstrated to be regulated through an oxidative stress-sensitive mechanism, since that administration of pigment epithelium-derived factor (PEDF) or pyridoxal phosphate, an AGE inhibitor, attenuated retinal levels of 8-hydroxydeoxyguanosine, an oxidative stress marker, and subsequently suppressed ICAM-1 gene expression and retinal leukostasis in diabetic rats [51]. Meanwhile, PEDF was once reported to inhibit the AGE-elicited ICAM-1, VEGF and monocyte chemoattractant protein-1 (MCP-1) up-regulation by blocking the NADPH oxidase-mediated ROS generation [26].

Herein, AGEs would be one of the key proinflammatory factors in the progression of diabetic retinopathy. AGE-RAGE interaction induces the activation of Ras-mitogen activated

protein kinase pathway with NADPH oxidase-mediated ROS generation and subsequent translocation of NF- κ B, consequently leading to the transcription of target genes including growth factors (VEGF), adhesion molecules (ICAM-1, VCAM-1), as well as proinflammatory cytokines and chemokines (IL-6, IL-1 β , TNF- α , MCP-1) [11, 26]. Likewise, AGEs also increased RAGE mRNA levels in ECs via the intracellular ROS generation [52], which formed a positive feedback loop that transduced the AGE signals again, ended up in a vicious cycle of RAGE activation and inflammatory factor production. In addition, attenuation of the AGE-RAGE axis with soluble RAGE (sRAGE), a broad-based inhibitor of the various AGEs receptors, ameliorated blood-retinal barrier breakdown and leukostasis, as well as the expression of ICAM-1 [50].

AGE-stimulated inflammatory cytokines mediate the synthesis of acute phase proteins which can initiate and support inflammatory process in the vascular wall. Serum level of TNF- α displayed a significant correlation with the severity of diabetic retinopathy in type 2 diabetic patients [53], genetically deficient of TNF- α had less diabetes-induced increase in vascular permeability and leukostasis [54]. One proposed mechanism of TNF- α regulating vascular permeability is through PKC ζ /NF- κ B signaling to downregulate junctional protein like claudin-5 and ZO-1 expression [55]. However, orchestrated interactions were involved between AGEs and the induction of pro-inflammatory cytokines and chemokines. Thus, the role of high mobility group box 1 (HMGB1) and silent mating type information regulation 2 homolog 1 (Sirt 1) was once studied recently, HMGB1 knockdown by shRNA targeting HMGB1 blunted the promotion of above-mentioned cytokines and chemokines in AGE-treated RPE, suggesting that HMGB1 mediated in this AGE-induced process, moreover, Sirt 1 was down-regulated by AGEs and was confirmed to regulate the cytokines and chemokines promotion via inhibiting the nuclear-to-cytoplasmic translocation and release of HMGB1 [56].

Angiogenesis

Vessel wall thickening and coagulation, leading to vascular occlusion and ischemia. Vessels ischemia is verified as one of the strongest triggers for the secretion of multiple growth factors such as VEGF, eventually contributing to angiogenesis [23]. Angiogenesis is actually an extremely regulated process conducted by pro-angiogenic (like VEGF) and anti-angiogenic (like PEDF) endogenous factors [49]. VEGF, a potent mitogen to ECs, is considered as a crux factor in the pathogenesis of DR. Endothelial cells, pericytes, Müller cells, microglia, astrocytes, retinal pigment epithelium and neurons have all been known to produce VEGF at some point in retinal development [57]. Besides its pro-angiogenic role in promoting the growth of new vessels, its role of proinflammatory which therefore causes the breakdown of BRB, ECs growth and neovascularization, vascular hyperpermeability in the ischemic retina had also aroused a lot of interest [58, 59].

AGEs act on pericytes via interaction with RAGE to stimulate VEGF expression. Whereas PEDF could prevent the progression of DR by attenuating the deleterious effects of AGEs on pericytes [60]. As stated, pericytes can control ECs proliferation, their removal from the vascular wall may lead to peripheral endothelial sprouting/pruning and formation of aberrant capillaries [61]. Besides the well accepted mechanism mentioned above that AGE-RAGE interaction might promote VEGF gene transcription in microvascular ECs by NADPH oxidase-mediated ROS generation and subsequent NF- κ B activation via Ras-MAPK pathway [62], there are also many novel pathways that lead to the AGE-stimulated VEGF over-expression were revealed these years, which in turn provides exciting new possibilities for therapeutic targets.

Worthy of note, angiogenesis and inflammation share a group of common mediators and signaling pathways that act synergistically in this pathogenesis. Pro-inflammatory cytokines may directly induce tube formation via engagement of target ECs or, indirectly, by inducing leukocytes and/or endothelial cells to produce proangiogenic mediators [49]. Lai et al. reported that the elevation of N^ε-(carboxymethyl) lysine (CML, a major advanced glycation end products) levels causing the activation of the TPL2/ATF4/SDF1 α pathway is a key promoter for endothelial inflammation and subsequent neovascularization [35]. As

a member of the MAP3K serine/threonine protein kinase family, tumor progression locus 2 (TPL2/Cot/MAP3K8) is induced by pro-inflammatory cytokines and supposed to be a novel functional signaling that integrates the intricate inflammatory cascade implicated in the development and progression of hyperglycemia-associated complications [63]. Nevertheless, TPL2 inhibitors can thwart TPL2-mediated VEGF by inactivating the transcription factors (like the activating transcription factor-4, ATF4) involved in angiogenic factor triggered angiogenesis [63].

PLA₂/COX-2/PG pathway is another widely researched pathway that was also shared by inflammation and angiogenesis. Reportedly, activation of cytosolic phospholipase A₂ (cPLA₂) requires phosphorylation on serine residues, mediated by extracellular signal-regulated protein kinase-1 and -2 (ERK1-2) that are activated downstream of AGE-RAGE [64]. Moreover, AGEs-evoked NF-κB activation contribute to the expression of cyclooxygenase-2 (COX-2), which possess a modulator role of angiogenesis via interaction with VEGF [65], and in the context of proliferative retinopathies, COX-2 mainly generates prostaglandin E₂ (PGE₂). Hence the PLA₂/COX-2/PG pathway largely depends on the AGE-RAGE interaction. In Giurdanella and colleagues' study, they proposed the existence of an autocrine/paracrine feed-forward mechanism that AGE-RAGE activates cPLA₂/COX-2/PG axis which can induce VEGF-A expression, and VEGF-A, in turn, can further increase the activation of cPLA₂/COX-2/PG axis. The occurrence of such an autocrine loop eventually lead to the pericyte damage and tube formation [66]. Thus, the anti-VEGFs afford protection of pericytes from detrimental effects of AGEs by inhibiting this loop.

However, in a further study conducted by the same group, a conflict result was concluded. Giving the verified evidence that RAGE activation strongly induces VEGF expression in ECs through NF-κB, therefore VEGF blockade is supposed to neutralize the VEGF-mediated consequences of RAGE activation [67]. Nevertheless, ERK1-2/cPLA₂/COX-2 can be activated as a downstream pathway of VEGF, but they can also be activated in another pathway directly downstream of AGE-RAGE interaction. Thus, with blockade of RAGE, we may expect an entire reversion of HG-induced cPLA₂ phosphorylation and PGE₂ release, while with VEGF blockade we may expect only a partial reversion [33]. This may be one of the reasons for the failure of current anti-VEGF medication in eye diseases that needs however further confirmation.

Though it is well established that medications by intravitreal injection (IVI) of neutralizing antibody for VEGF alleviate the pathologic processes of vascular leakage and angiogenesis in DR, resistance to VEGF blockade is still the most challenging curative issue. Since it has been widely accepted that the proangiogenic effect of AGEs in retinal neovascularization has been mainly associated with their ability to upregulate the expression and secretion of VEGF-A. Recently, there is data suggesting that, besides VEGF-A, VEGF-C, another member of the VEGF family produced by RPE cells, also plays a role in AGEs-induced retinal angiogenesis [68]. VEGF-C sustains retinal neovascularization, by potentiating the angiogenic effect of VEGF-A and by preventing retinal endothelial cell apoptosis [68]. Interestingly, upon AGEs presence, upregulation of VEGF-C gene expression in RPE and endothelial cells occurs even after VEGF-A blocking, or in another word, VEGF-C may compensate for treatments that reduce VEGF-A [69]. And this may be another explanation that clinically, more than half of patients do not improve after treatment with monoclonal antibodies against VEGF-A.

Therapeutic viewpoints

The mainstays of current therapy for DR are surgery for vitreous hemorrhage and retinal detachment, and treatment with anti-angiogenic drugs, like anti-VEGF monoclonal antibodies, VEGF receptor blockers or corticosteroids, for macular oedema and angiogenesis. However, these therapies are often started in late stages of DR, and are designed to prevent further deterioration rather than restore impaired vision [70]. On the other hand, intensive treatment of diabetes may prevent onset and progression of DR as shown by the Diabetes

Control and Complications Trial (DCCT) and UKPDS studies [71]. But it is difficult to provide intensive monitoring to all diabetics given the high prevalence of DM in practical terms, moreover, recent trials have underlined the limitations in the use of intensive glycemic control to minimize diabetic complications [72].

Our current literature on the molecular basis of diabetic retinopathy stresses the important pathogenic role of advanced glycation end products and the irreversible crosslinked signaling cascades downstream the AGE-RAGE interaction. Based on these results, three potential therapeutic targets for diabetic retinopathy are discussed: (1) inhibition of AGEs formation; (2) blockade of the AGEs/RAGE interaction; and (3) suppression of the AGEs/RAGE-mediated downstream signaling cascades.

Maintaining an intensive control of blood glucose levels may in part be through the reduction of AGEs formation. Over the last decades, available knowledge of the mechanisms involved in AGEs formation has led to attempts to inhibit the formation of AGEs. Different sources including several endogenous glycolytic and oxidative pathways, as well as exogenous diet-derived sources were reported [73, 74]. A number of compounds such as benfotiamine, aminoguanidine, pyridoxamine, carnosine and phenyl thiazolium bromide have been investigated on the property of anti-AGEs activity [73]. As a well-known AGEs inhibitor, pyridoxamine ameliorated DR in experimental diabetic rats and as yet it is the only compounds that passed the phase III clinical trials [75]. In a recently published review, they proposed that, as for preventing lifestyle-related diseases like diabetic complications, daily intake of AGEs inhibitors in natural products are preferred to prescribed drugs [76]. Therefore, this article summarized several antioxidants such as citric acid which can effectively inhibit CML formation, giving hope that daily intake of citric acid from fruits such as lemons, limes and oranges and possibly through supplementation is able to play a beneficial and therapeutic role in DR [76].

Other potential therapeutic targets exist downstream of AGEs formation. A new class of molecules, the soluble RAGEs (sRAGEs), has recently added its contribution to this complex scenario. Three major variants of RAGEs are existed, including full-length type, NH₂-truncated type and COOH-truncated type, the last type is secreted extracellularly thus detected in serum as endogenous secretory RAGEs (esRAGEs) [77]. Total circulating sRAGEs is a sum of esRAGE and RAGE that are proteolytically cleaved from cellular surface by action of matrix metalloproteinases (MMPs) [78]. Unlike cell-surface RAGE, sRAGEs contributes to the removal/detoxification of AGEs by acting as a decoy [79]. Blocking the interaction of AGEs and RAGE with sRAGEs is demonstrated to ameliorate diabetic leukostasis and BRB breakdown in experimental models [50]. Moreover, in a clinical research, they found that the sRAGE levels significantly declined as the retinal complication advanced from simple and then to proliferative retinopathy compared to those in the no-retinopathy group, which continuously remained a high level of sRAGE [80]. These results indicate that sRAGE can effectively confer protection on vessels against AGEs and hopefully to become a useful biomarker to indicate individual variations in susceptibility to DR.

PEDF is a once reported glycoprotein that belongs to the superfamily of serine protease inhibitors with complex neurotrophic, neuroprotective, antiangiogenic, anti-oxidative, and anti-inflammatory properties, which are accomplished by the core capability of inhibiting the ROS generation and this is the focus of the research work by Yamagishi and co-workers [26]. Intravenous administration of AGEs to normal rats not only induced retinal vascular hyperpermeability by stimulating VEGF expression, but also decreased retinal PEDF levels [81]. Conversely, intravitreal injection of PEDF gene-modified human umbilical cord mesenchymal stem cells (PEDF-MSCs) could down-regulate the expression of VEGF in diabetic rats [82].

Fig. 1. Simplified overview of the AGE-induced molecular pathways to the development of DR. AGEs crosslinking of proteins or binding to receptors located on the cellular surface elicit the activation of different downstream molecular pathways, leading to pericyte dropout, endothelial dysfunction and vascular inflammation, moreover, increasing levels of proangiogenic factors result in the formation of neovascularization. Key: TJ proteins: tight junction proteins; ROS: reactive oxygen species; BRB: blood-retinal barrier.

Conclusion

At present, AGEs measurement has still not taken a precise role in clinical practice, but its relevance in the pathogenesis of Diabetic Retinopathy has been widely shown. AGE-RAGE signaling involved in the determination of DR is highly regulated in a context-specific manner (Fig.1), including the activation of several interrelated pathways which all tie in to several key mechanisms namely initiated pericyte apoptosis, increased pro-inflammatory mediators, dysfunction of retinal microvascular ECs and enhanced VEGF secretion, they all occurs against a background of the various metabolic derangements that are inherent to DM.

The degree of cross talks between AGEs and downstream interconnected elements leading to the full-blown clinical expression of DR, making it difficult to truly separate any one event from another. It is hoped that, those discovered molecules that target AGEs and AGE-mediated signaling pathways will prove successful in cell therapy of DR vascular damage. Nevertheless, further investigation 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.

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Disclosure Statement

The authors declare to have no competing interests.

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