



Review

Advanced glycation and retinal pathology during diabetes

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Abstract:

Of all microvascular complications of diabetes mellitus, retinopathy remains the most common. This disease presents major therapeutic problems for the ophthalmologist and despite many decades of intense research it still constitutes a major cause of blindness in the Western world. This review outlines the pathological characteristics of diabetic retinopathy and proposes a link between disease progression with the formation and accumulation of advanced glycation endproducts (AGEs). AGEs form *in vivo* from the reaction of glucose and/or α -oxoaldehydes leading to chemical modifications on the amino groups of proteins, lipids and DNA. These heterogenous adducts can modify the structure and function of proteins and lead to intra-molecular and intermolecular cross-link formation. As reported in a range of clinical investigations and determined by mechanistic *in vitro* and *in vivo* studies, AGEs accumulate in the diabetic retina where they may be effectors of retinal vascular and neural cell dysfunction. Evidence now points towards a pathogenic role for advanced glycation in the initiation and progression of diabetic retinopathy and this review will examine the current state of knowledge of AGE-related pathology in the retina at a cellular and molecular level. It will also describe how ongoing pharmaceutical strategies to inhibit AGE formation and thereby attenuate their pathogenic influence during chronic hyperglycemia may play a significant role in the treatment of diabetic retinopathy.

Key words:

advanced glycation endproducts, diabetes, age, retinopathy, eye

Abbreviations: 3-DG – 3-deoxyglucosone, AGEs – advanced glycation endproducts, BM – basement membrane, CMA – carboxymethyl-arginine, CML – N -(carboxy-methyl)lysine, DAG – diacylglycerol, Et-1 – endothelin-1, GO – glyoxal, iBRB – inner blood retinal barrier, ICAM-1 – intracellular adhesion molecule-1, MGO – methylglyoxal, RAGE – receptor for AGEs, RAS – renin-angiotensin system, sRAGE – soluble RAGE, VEGF – vascular endothelial growth factor

Introduction to diabetic retinopathy

Retinopathy is one of the commonest microvascular complications of diabetes and still a major cause of

registerable blindness in the working population of many developed countries [20]. With type 1 diabetes of 10 years duration, the prevalence of diabetic retinopathy is around 80% and this increases to ~95% by 20 years [40, 41]. Overall, diabetic retinopathy is slightly less common in type 2 patients but is still the most frequent microvascular complication suffered by this group [40]. There are few preventative measures and sight-threatening diabetic retinopathy can, at present, only be treated or contained to some extent by focal or pan-retinal laser photocoagulation or vitreoretinal surgery but often at the expense of functional retina and visual performance [62].

Diabetic retinopathy is quintessentially a disease of the intra-retinal vasculature, although there is a subtle

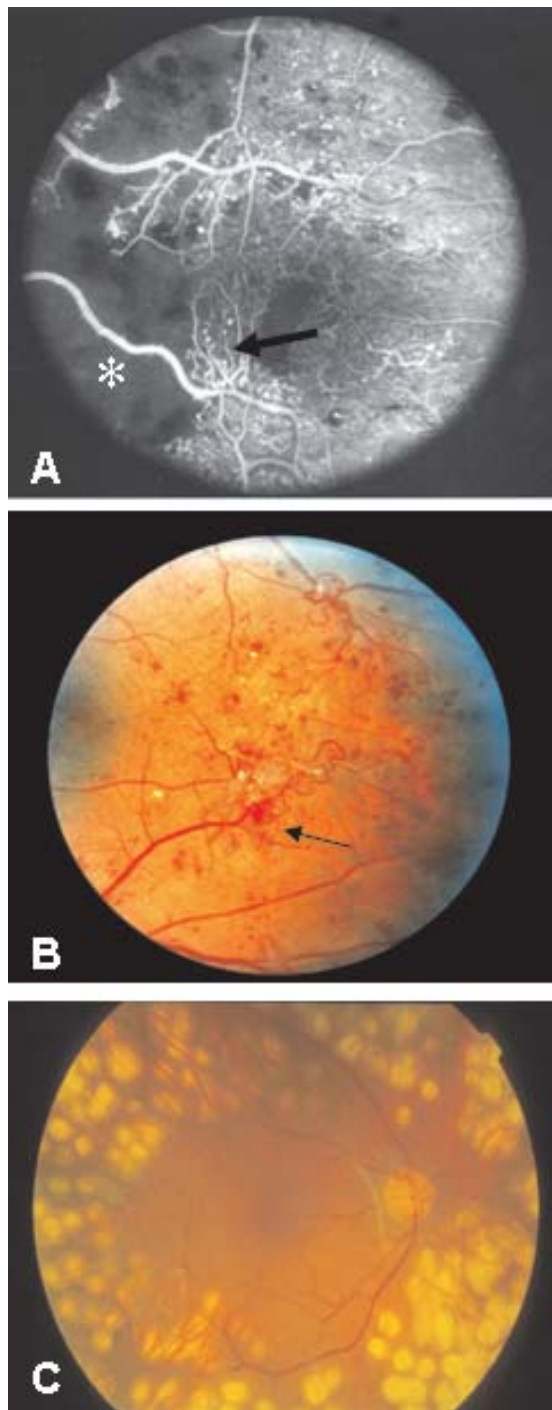


Fig. 1. Clinicopathological hallmarks of diabetic retinopathy. **(A)** Fluorescein angiogram from a patient with non-proliferative diabetic retinopathy. The retinal microvascular tree is damaged with large segments of capillary beds lost. Subsequently there are areas of ischaemia, exemplified by large areas of retina which are non-perfused (*). Arrows indicate microaneurysms on the arteriolar side of the circulation. **(B)** Fundus image from a patient with proliferative diabetic retinopathy showing areas of neovascularisation (arrow) in the pre-retinal space. **(C)** Pan-retinal laser photocoagulation (PRP) has been conducted on a patient with proliferative diabetic retinopathy. This fundus demonstrates the typical scarred appearance of the peripheral retina, although the macular region in the centre of the image is clear of neovascularisation

concurrent retinal neuropathy characterised by early electroretinogram (ERG) defects [96], decreased colour contrast sensitivity, neuronal/glial abnormalities and eventual depletion of ganglion cells [23, 46]. Choroidal vascular changes may also occur, however, it remains uncertain how this influences vision. Diabetic retinopathy is traditionally classified into two clinical forms; non-proliferative and proliferative. The non-proliferative form of the disease is by far the most common and in a significant number of cases it progresses to sight-threatening proliferative diabetic retinopathy [40]. The greatest risk of visual loss occurs in the later phases of diabetic retinopathy with the development of macular edema and retinal neovascularisation, the former being a direct consequence of blood retinal barrier breakdown and the later to widespread retinal ischemia [2].

The histopathology characteristic of non-proliferative diabetic retinopathy is a focus of this review and it is important to note that many of these lesions are temporally separated from more subtle physiological changes in the retinal vessels. It is apparent that diabetes-related retinal vascular dysfunction in humans and animal models commences within weeks of diabetes onset and is characterised by increased blood flow, impaired autoregulation, and abnormal permeability to plasma proteins [50, 70]. Non-proliferative diabetic retinopathy is manifest by excessive capillary permeability leading to inner blood retinal barrier (iBRB) dysfunction [4], capillary basement membrane (BM) thickening [76], pericyte and smooth muscle depletion [22, 36], microaneurysm formation [80], and thereby widespread non-perfusion (Fig. 1A). In the proliferative phase, ischemia drives pre-retinal neovascularisation in a significant proportion of diabetic patients (Fig. 1B) with risk of vision loss through vitreal haemorrhage, fibroglisis and tractional retinal detachment. Sight-threatening diabetic retinopathy and its sequelae can be treated or contained to some extent by laser photocoagulation or vitreoretinal surgery, but at the expense of large areas of functional retina and associated visual field loss (Fig. 1C).

The pathogenic basis of diabetic retinopathy is not wholly understood at a cellular and molecular level and the options for effective therapeutic intervention early in the disease process remain extremely limited. Major international epidemiological trials have established that hyperglycemia is a principal and underlying cause of this disease in both Type 1 and Type 2,

Diabetes [16, 97]. Failure to regulate blood glucose leads to biochemical abnormalities in diabetic cells and tissues and the range of pathologic lesions in retina and other vascular beds are indicative of a complex interplay between hyperglycemia-induced metabolic and haemodynamic pathways. Within this context it has been shown in diabetic dogs that good glycemic control following an initial 2.5 year period of poor control is not protective against retinopathy [19]. Similar studies have been demonstrated in long-term diabetic rats that gradually recovered from early diabetes [87]. This strongly suggests that so-called “hyperglycemic memory” produces a poorly understood, chronic abnormality in the retinal microvasculature of diabetics that is not easily reversed, even by subsequent, relative normoglycemia.

It is clear that the pathogenesis of diabetic retinopathy is highly complex and multifactorial. Mechanistic studies have shown that short or long-term exposure to the diabetic milieu may result in a raft of biochemical and metabolic abnormalities occurring over many years, however it remains uncertain how much each contributes to retinal pathophysiology over long-term diabetes. In addition to formation of advanced glycation endproducts (AGEs) (the focus of this review), hyperglycemia can cause increased flux through the polyol or hexosamine pathways and associated alterations in the redox state of pyridine nucleotides [65, 93], the *de novo* synthesis of diacylglycerol (DAG) leading to the over-activation of several isoforms of protein kinase C [44, 104], excessive production of reactive free radicals perhaps causing oxidative stress [14, 47], changes in blood rheology and haemodynamics [24, 34, 94] and over-activation of the renin-angiotensin system (RAS) [24, 105]. Inhibition of many of these pathways can show protection against multiple or specific microvascular complications in diabetic models, including retinopathy. While discussion of these important pathways is beyond the scope of this review, it is important to stress that these mechanisms should not necessarily be viewed as independent phenomena. Indeed, a recent unifying concept has been proposed whereby hyperglycemia increases superoxide production (*via* the mitochondrial electron transport chain) which in turn initiates accelerated AGE formation and also exacerbates many of the aforementioned pathogenic pathways [56]. Indeed, studies using the transketolase activator benfotiamine can inhibit a common conver-

gent pathway and effectively prevent retinopathy in diabetic animals [28].

AGE formation in the body

Cell or tissue exposure to uncontrolled glucose concentrations can be inherently damaging to cells. Indeed, bouts of hyperglycemia, as occurs in diabetes mellitus, can lead to a range of pathogenic events that cause cell damage and, ultimately, organ dysfunction. Excess glucose in mammalian cell systems or impaired glucose handling leads to enhanced non-enzymatic glycation reactions between reducing sugars and the free amino groups on proteins, lipids and DNA. This reaction is an inevitable consequence of the reactivity of aldehydes with primary amino groups and as a consequence nearly all body proteins carry some “burden” of chemically attached carbohydrate. The reaction begins with the formation of a Schiff base between glucose and the ϵ -amino group of lysine that slowly rearranges to relatively stable Amadori adducts (Fig. 2). Both the Schiff base and the Amadori compound can undergo further oxidation and dehydration so that their concentrations ultimately depend on both forward and reverse reactions. The forward reactions give rise to additional protein-bound compounds collectively termed AGEs. These adducts are irreversible and their rate of accumulation in a tissue depends on a number of factors including availability of metal ions and changes in oxidative stress, generally thought to increase in conditions such as ageing and diabetes. AGEs increase the chemical modification, cross-linking, pigmentation and fluorescence of proteins, and their origin from an array of precursor molecules contributes to the heterogeneity of AGE structures [92].

AGEs can form directly from reaction of glucose with amino groups but it has become increasingly appreciated that this sugar is much less reactive than α -oxoaldehydes such as glyoxal, (GO), methylglyoxal (MGO) and 3-deoxyglucosone (3-DG). The concentrations of these dicarbonyl compounds are increased in plasma during hyperglycemia [57] and arise from both chemical and metabolic pathways [43, 91, 92]. Dicarbonyls are an important source of intra- and extracellular AGEs and, because they are highly reactive they can lead to rapid adduct formation [91].

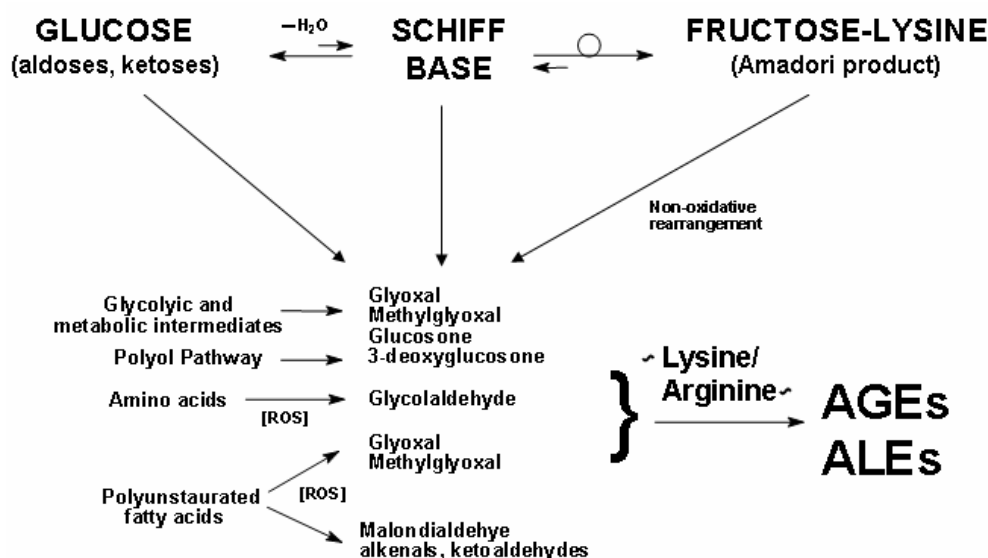


Fig. 2. Scheme showing sources of reactive carbonyl intermediates in the formation of AGE/ALEs. Carbohydrates (aldoses, ketoses, ascorbate) react with protein amino groups, illustrated here for the reaction of glucose with lysine residues in protein (~), to first form the unfavored Schiff base, followed by an Amadori rearrangement, to a stable ketoamine adduct, shown as fructose-lysine. ROS and RNS, reactive oxygen and nitrogen species, oxidize both free and protein-bound carbohydrates producing a variety of dicarbonyl compounds (glyoxal, methylglyoxal, glucosone and 3-deoxyglucosone) that react with lysine and arginine residues on protein to form AGE/ALEs. Oxidation of amino acids and polyunsaturated fatty acid also give rise to many of the same reactive dicarbonyl compounds. However, some reactive dicarbonyl intermediates arise from non-oxidative rearrangements of sugars or from spontaneous decomposition of products of glycolysis and the polyol pathway

Dicarbonyls can react directly with protein to yield many of the same structures derived from the Amadori product. For example, fructose-lysine can undergo metal-catalysed oxidative cleavage giving rise to the irreversible “glycooxidation” product, N-(carboxy-methyl)lysine (CML). However, CML can also be formed from direct reaction of GO with lysine, independent of the presence of glucose; GO also reacts with arginine residues on protein to form carboxy-methyl-arginine (CMA) [25]. Similarly, MGO can be formed on oxidation of the Schiff base and on reaction with protein to give rise to the AGEs N-(carboxyethyl)lysine (CEL) and arginine-hydroimidazolone [91, 92]. Further, MGO can arise by spontaneous β -elimination of phosphate from triose phosphates, the concentrations of which are increased during hyperglycemia because of the increased flux of glucose through glycolysis. Lipid peroxidation reactions can also form a class of Maillard products called advanced lipoxidation endproducts (ALEs) [60] (Fig. 2). Indeed, lipids are important sources of chemical modifications of proteins especially in lipid rich, highly oxidative environments, such as in the retina, and dyslipidemia may be an important pathogenic force in retinopathies.

Beyond non-enzymatic glycation, it is now appreciated that auto-oxidation of free sugars, superoxide production and metabolism of glucose can lead to high levels of reactive dicarbonyls such as MGO, GO, and 3-DG. These dicarbonyls can lead to very rapid AGE formation especially in circumstances of enhanced glycolytic activity (such as in hyperglycemia) [92], although the net importance of AGEs such as imidazolones are not fully understood. Under normal circumstances the cell can protect itself against these dicarbonyls through a range of intracellular detoxifying enzymes which serve to limit adduct formation of important structural and functional proteins. Alterations in these enzymes during disease may have implications for AGE accumulation and pathogenic effects in cells and tissues [52, 89, 90]. Indeed, it has been demonstrated that up-regulation of glyoxalase-1 can reverse high-glucose mediated AGE formation over a short, 10-day period and prevent AGE-mediated cell abnormalities [75].

Since the products of advanced glycation/lipoxidation reactions are constantly forming under physiological conditions it has been suggested that complex receptor systems may have evolved to remove senescent, glycation-modified molecules and/or degrade existing AGE/ALE-crosslinks from tissues thereby

limiting their deleterious effects. Such receptors could play a critical role in AGE-related biology and the pathology associated with diabetes and aging disorders [67, 72, 100]. Several AGE-binding molecules have been described and it is thought that many of the adverse effects caused by advanced glycation are mediated *via* AGE-receptors such as the receptor for AGEs (RAGE) [71], AGE-R1 [45, 82], galectin-3 [64], CD36 [58] and the type I and II scavenger receptor [31]. The relative pathogenic contribution of these receptors in instigating diabetic complications is poorly defined, although RAGE is by far the best characterised. Mechanistic *in vitro* and *in vivo* studies on RAGE and its regulatory fragments such as soluble RAGE (sRAGE) indicate an important role in pathobiology [7, 32].

Pathogenic role of AGEs in diabetic retinopathy

Over the last twenty years it has become evident that AGE-modification represents a major pathogenic factor in ageing and a spectrum of human diseases such as diabetic complications [84, 101], neurodegeneration (including Alzheimer's disease) [54], ischemic heart disease and atherosclerosis [78, 81]. The mammalian eye is configured to optimise transmittance of light photons to the neural retina and it is important to maintain structural integrity, optical clarity and nourishment for the highly specialised cells of the eye. For example, an opaque lens will prevent light penetration

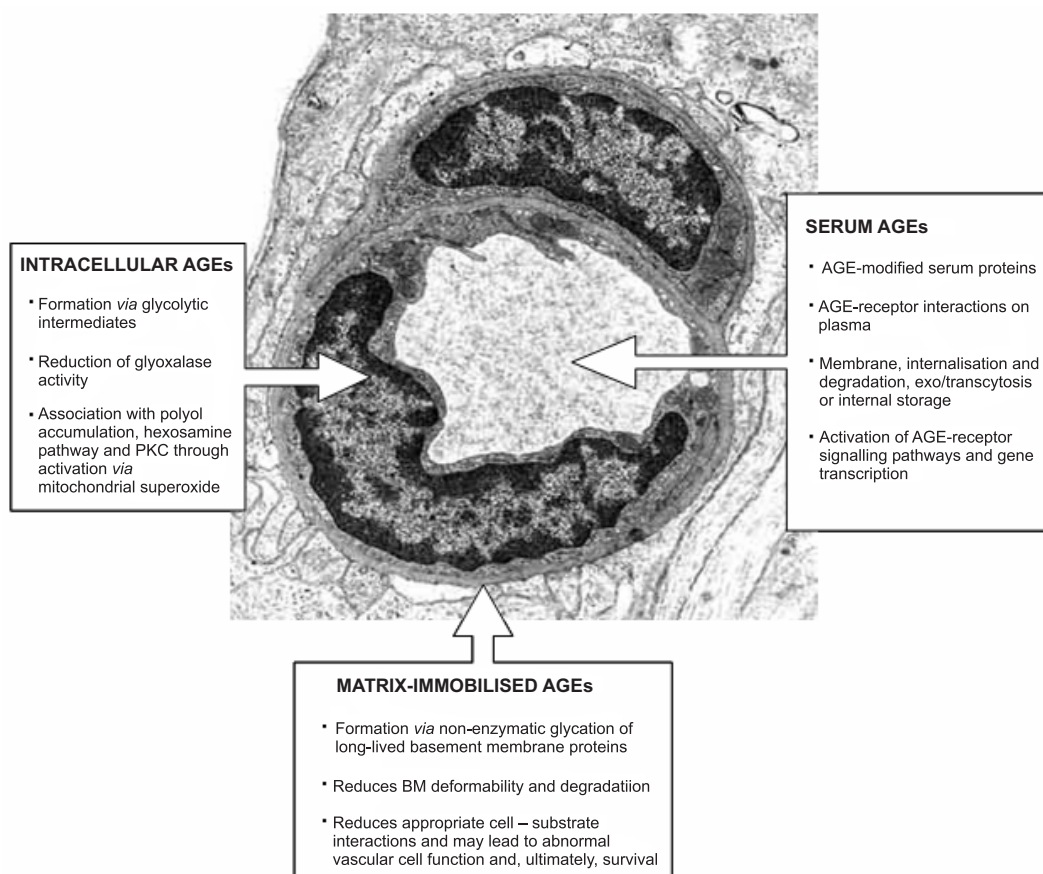


Fig. 3. AGE-exposure to retinal vascular cells. Vascular endothelial cells and pericytes in the retinal capillaries would be expected to encounter AGEs immobilised on the sub-cellular matrix where they may influence cell attachment through disruption of integrin – BM protein interactions. Failure to receive appropriate survival cues from the BM can precipitate death in sedentary cells. Serum-derived AGEs may also interact with vascular endothelium *via* AGE-receptors that are on the apical (and basal) plasma membrane. Serum-derived AGEs may be passed to pericytes *via* the endothelium or as a result of blood retinal barrier dysfunction. These serum AGEs may also interact directly with cell surface glycoproteins with potentially damaging effects on membrane integrity and function. Hyperglycaemia can lead to rapid AGE formation through reaction of cytoplasmic molecules with glycolytic intermediates and dicarbonyls or through complex interplay with superoxide radicals released from the mitochondria. Such “cytoplasmic” AGEs can lead to many dysfunctional responses by vascular endothelium and retinal pericytes

to the retina and reduce visual acuity. Unfortunately, many of the post-mitotic, differentiated cells of the eye have little or no regenerative capacity. This makes these cell structures highly susceptible to aging processes and systemic diseases which accelerate AGE-modification of macromolecules and alter structural proteins. Indeed, ophthalmologists and vision scientists have long recognised that the eye is profoundly influenced by diseases such as diabetes and age-related dysfunction which, together, account for the leading causes of visual impairment world-wide. It is anticipated that AGEs form in the extracellular space as well as within the cytoplasmic compartment of retinal cells. In particular sedentary cells resting in direct contact with extracellular matrix and/or basement membranes will encounter AGE crosslinks that have accumulated within these long-lived proteins (Fig. 3).

AGE quantification and associations with retinopathy

Such is the heterogeneity of AGE chemical adducts and the diversity of macromolecules that are modified by these adducts, the methods for AGE quantification remain variable and somewhat controversial, according to the basis of analytical and/or immunocytochemical analysis. With this proviso, clinical studies have demonstrated that the levels of AGEs in serum [18, 59, 103], skin [73] or cornea [68] correlate with the onset or grade of diabetic retinopathy. AGEs are significantly elevated in diabetic pre-pubescent children and adolescents who have background or pre-proliferative retinopathy compared to counterparts who are free from clinical signs of the disease [11]. While many of the reported studies measured a range of ill-defined AGE moieties, others evaluated defined adducts such as CML, pentosidine or crossline [88, 109] in association with diabetic retinopathy. At the same time, some studies have reported no correlation between AGE levels and retinopathy in diabetic patients [88, 103], although the apparent disparity with other studies may be related to variations in patient populations and/or the non-uniform assays for plasma AGE-quantification.

As demonstrated in other microvascular beds, AGEs and/or late Amadori products have been localised to retinal vessels and neuroglia of diabetic patients [21, 26, 27, 55, 69, 85] where they would be expected to have a range of deleterious effects on cell function. *In vivo* and *in vitro* studies suggest that ele-

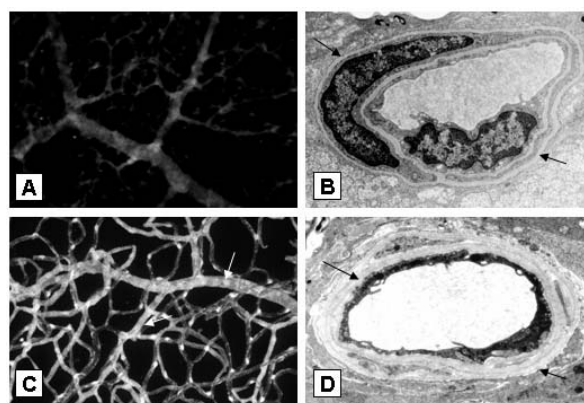


Fig. 4. Vascular BM thickening and AGE-immunoreactivity in diabetic rat retina. Trypsin digests from non-diabetic rat retina (**A&B**), 12 month diabetic retina (**C&D**) were evaluated for AGE- immunoreactivity and BM thickening. The retinal microvasculature of 12 month old non-diabetic rats shows some AGE-immunoreactivity which is largely localised to the vascular wall, as indicated by diffuse fluorescence (**A**). By contrast 12 month diabetic rat retinal vessels show intense AGE-immunoreactivity, localised particularly in the retinal arteries and arterioles (arrows) (**C**). Within the capillary bed the vessel walls and the cell bodies of the retinal pericytes show high levels of AGEs when compared to controls. The BM of the diabetic capillaries are significantly thicker than non-diabetic counterparts (compare **C** with **D**); arrows show BM

vated AGE level occurring in diabetes may be an important factor the initiation and progression of retinopathy (Fig. 4), where immunoreactivity is associated with lesions such as basement membrane (BM) thickening. Retinal pericytes accumulate AGEs during experimental diabetes in animal models [85] which would be expected to have a detrimental influence on cell function and survival, especially since these cells have a much lower replicative capacity when compared to retinal microvascular endothelium [74]. Indeed, studies have shown dysfunctional effects on retinal pericytes, such as impaired phospholipid hydrolysis and phospholipid enzyme inhibition [6] or modification of the antioxidant enzymes catalase and superoxide dismutase [61].

In vitro responses of retinal cells to AGE exposure

AGEs are toxic to retinal pericytes [12, 35, 66], a response that is mediated by AGE-receptor activation [12]. Indeed, recent evidence also suggests that these adducts can induce osteoblastic differentiation and calcification in pericytes [108] and a potent apoptotic death response [107]. *In vivo*, retinal pericytes are surrounded by vascular BM and lie outside the blood

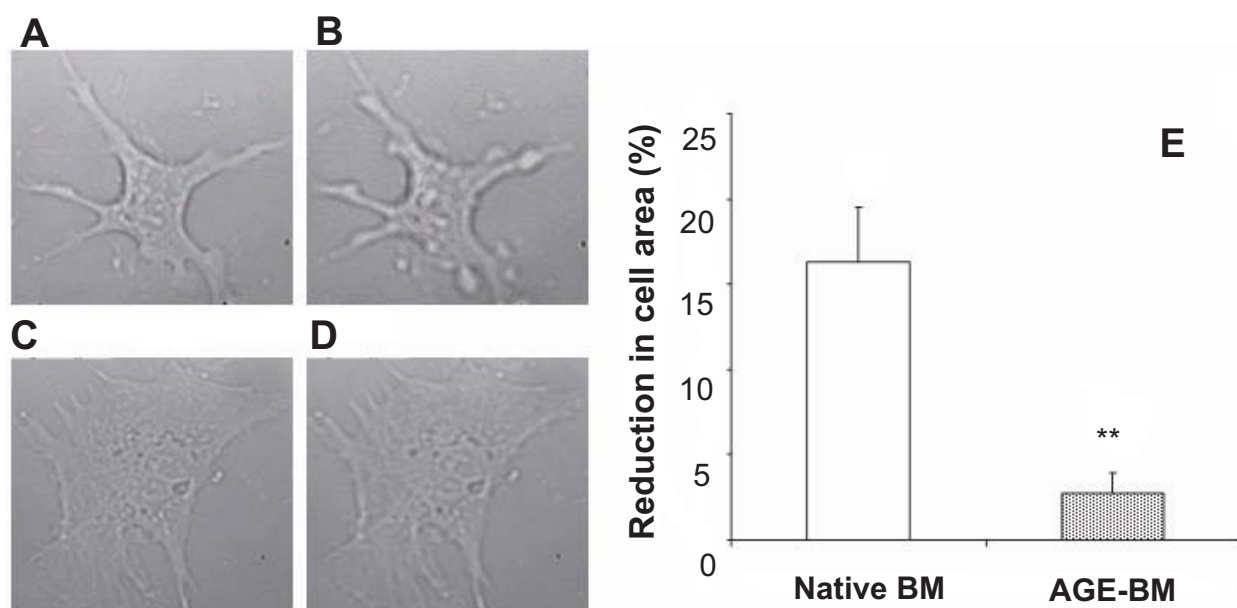


Fig. 5. Functional integrity of pericytes is altered by AGE-exposure. The functional integrity of the contractile machinery in retinal pericytes cultured on native BM (Matrigel) and AGE-modified BM. A,B Photomicrographs showing a bovine retinal pericyte grown on native BM prior to (A) and following (B) 5 minutes permeabilisation to Ca^{2+} with 1 μM Ca^{2+} Hanks solution (Ca^{2+} clamped with EGTA) containing ionomycin (1 μM). (C,D) Light micrographs showing a retinal pericyte cultured on AGE-BM before (C) and after (D) permeabilisation to Ca^{2+} . (E). Quantification using image analysis (2-dimensional surface area) confirmed that pericytes cultured on AGE-BM exhibited a reduced ability to contract. Values above the histograms indicate the number of pericytes tested per treatment group; ** $p < 0.01$ (unpaired t -test on arcsine transformed data)

retinal barrier. Our own group has developed an *in vitro* system whereby pericytes are grown on a diabetic-like AGE-modified BM. Short-term exposure to these “AGE-substrates” causes subtle physiological alterations to pericytes consistent with those seen in the diabetic retina. In diabetes the retinal blood vessels become resistant to the actions of the vasoconstrictive peptide endothelin-1 (ET-1), and this is thought to directly contribute to the abnormal retinal haemodynamics observed [10, 15]. Likewise, when retinal pericytes are propagated on AGE-modified BM they exhibit impaired calcium signalling and contractile responses to ET-1 [33]. In diabetes, the most probable explanation for the attenuated ET-1 responses is a reduction in the number of functional ET_A receptors [15]. While ET_A gene transcription is unaffected in retinal pericytes by AGE modification of basement membrane, ET_A receptor protein expression is reduced [33]. This implies that AGE-modification of BM may profoundly influence mRNA translation and/or post-translational degradative pathways in retinal pericytes. Recent unpublished data from our laboratory also suggests that AGE-modified BM leads to a marked deterioration in the functioning of the contractile apparatus in retinal pericytes (Fig.

5). It has been previously demonstrated that attachment-dependent cells grown on AGE-modified matrices exhibit a marked alteration in the organization of their actin cytoskeleton [30], a response that could account for the impaired pericyte contraction observed in our experiments. In light of the findings above, it may be surmised that during diabetes, accumulation of AGEs in the BMs of retinal pericytes could compromise their ability to appropriately modulate retinal capillary blood flow in response to a range of locally derived vasoactive agents. This is significant since retinal haemodynamic abnormalities are a pathological hallmark signifying the onset of diabetic retinopathy and these changes appear to coincide with the accumulation of AGEs in the extracellular domains of the retinal capillaries [70, 85]. Long-term exposure of retinal pericytes to AGE-BM also causes apoptotic death, a response that can be prevented by immobilising platelet-derived growth factor-BB (PDGF-BB) on the matrix [83] (Fig. 6).

***In vivo* responses of retina to AGE-exposure**

Exposure of retinal cells to pre-formed AGEs *in vivo* are known to cause significant up-regulation of vascu-

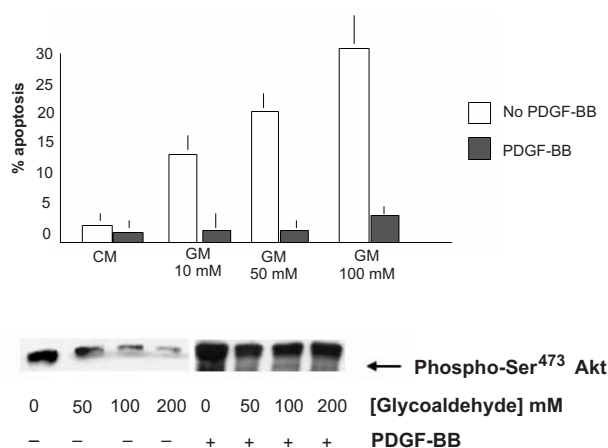


Fig. 6. AGE-modification of basement membrane (BM) induces pericyte apoptosis. When apoptotic pericytes are quantified there is a clear increase in this form of death when cells are exposed to AGE-BM, which is in accordance with the degree of AGE-modification (10–100mmol/l glycoaldehyde). When the substrates are supplemented with PDGF-BB peptide there is a significant reduction in apoptosis, in many cases, bringing levels down to native-BM levels. Clear bars represent non-PDGF-exposed BM; black bars represent PDGF-supplemented BM. Error bars represent SD \pm mean. Supplementation of AGE-BM with PDGF-BB restores Akt phosphorylation (an important cell survival signalling molecule) in retinal pericytes. Retinal pericytes grown on AGE-BM for 24 h show reduced phosphorylation of Akt, which reduces with increased degree of AGE-modification. When PDGF-BB is added to the substrate prior to cell seeding, Akt phosphorylation levels are restored to native-BM levels.

lar endothelial growth factor (VEGF) [48, 77, 95, 107]. In addition to its importance in neovascularisation during proliferative diabetic retinopathy, VEGF is also a potent vasopermeability factor in the retinal microvasculature, with a role in inner blood retinal barrier (iBRB) dysfunction [3]. Excessive vasopermeability is a pathophysiological hallmark of diabetic retinopathy and there is evidence to suggest that AGEs could play a role in compromising of the capillary unit leading to subtle and overt breakdown of the iBRB. Loss of iBRB integrity is observed in non-diabetic rats infused with AGE-modified proteins [77] with a concomitant increase in intracellular adhesion molecule-1 (ICAM-1) [53]. Indeed, it is recognised that pro-inflammatory pathways may be active during diabetic retinopathy, manifested by increased levels of adhesion molecules such as ICAM-1 on the surface of retinal microvascular endothelial cells. In combination with an enhanced stickiness and reduced deformability of blood-borne leukocytes in the diabetic state, this can lead to a marked leukocyte adhesion to endothelium that precipitates capillary occlusion and vascular cell death [51]. AGEs are a possible pathogenic factor in pro-inflammatory responses and they can en-

hance ICAM-1 expression in macrovessels [42] and have now been shown to evoke similar responses in the retinal microvascular endothelium both *in vitro* and *in vivo* [49, 53].

We have also recently demonstrated that AGE-albumin can impair angiogenic potential in retinal microvascular endothelial cells – an effect that is replicated in an *in vivo* model of retinal neovascularisation (Fig. 7). This has important implications for vasodegeneration during diabetic retinopathy and suggests that these adducts could play a hitherto unrecognised role in lack of vascular repair in the retinal microvasculature during diabetes [86].

Prevention of AGE formation in animal models

Inhibition of AGEs has exciting possibilities for preventing retinal pathology during diabetes. To date, there have been many approaches to either prevent AGE-formation, reduce AGE receptor – ligand interactions/signalling pathways effects or break established AGE crosslinks. These treatments not only offer an important insight into the pathogenic role of AGEs in diabetic retinopathy but may have clear applicability to the treatment of patients with other ocular diseases.

Amadori product formation is an important basis of Maillard chemistry in biological systems because progression to crosslink pathology requires chemical re-

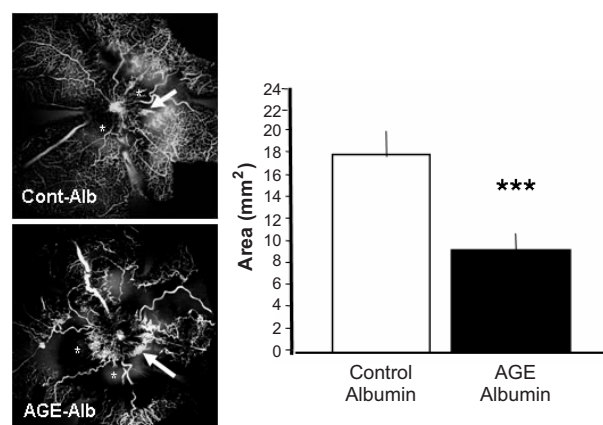


Fig. 7. AGEs reduce neovascularisation in an *in vivo* model. The hyperoxia-induced retinopathy model results in retinal ischemia (*) at post-natal day 20 which drives a neovascular response depicted by hyperfluorescent pre-retinal fronds (arrow). When mice are infused with AGE-modified Mouse serum albumin (AGE-MSA) between P12-P20 the retinal microvasculature shows more ischemia but less neovascularisation (arrow) when compared to native MSA controls. Data are presented as mean values (\pm SD). (***) $p < 0.001$ comparisons between AGE-MSA and native-MSA treatments).

arrangement to create reactive intermediates before the formation of irreversible AGEs. An important pharmacological strategy for the inhibition of this process has utilised the small nucleophilic hydrazine compound aminoguanidine (Pimagedine) [9]. This drug is a potent inhibitor of AGE-mediated crosslinking and has been shown to prevent a range of diabetic vascular complications in experimental animals (reviewed by Vasan et al. [98]), including diabetic retinopathy [1, 21, 29, 37, 38]. Aminoguanidine has been evaluated in a multi-centre clinical trial where it failed to achieve statistically significant lowering of serum creatinine, urinary albumin but showed positive signs towards slowing the progression of overt nephropathy and retinopathy progression [8].

Another anti-AGE strategy is to attack AGE crosslinks formed in biological systems. This constitutes an exciting approach since it would “break” pre-accumulated AGEs and subsequently allow their clearance *via* the kidney. An AGE crosslink “breaker” prototype has been described to attack dicarbonyl-derived crosslinks *in vitro* [99] and there are now at least two such (related) chemical agents with the ability to reduce tissue AGEs in experimental diabetes [13, 106]. The “breaker” ALT-711, has been shown to ameliorate myocardial stiffness in aged dogs [5] and improved the ability of the carotid artery to expand during systole in diabetic rats [106]. In preliminary clinical trials, ALT-711 modestly improved arterial compliance in aged patients with measurable cardiovascular stiffening [98]. The effects of ALT-711 on retinopathy have yet to be evaluated.

Another successful approach has been to screen for compounds with post-Amadori product scavenging potential, since this is an important route for AGE formation *in vivo*. So-called “Amadorins” have an ability to scavenge reactive carbonyls and therefore inhibit the conversion of Amadori intermediates to AGEs and also ALEs [39, 60]. Aminoguanidine possesses no scavenging properties [39, 102], but it has been found that the derivative of vitamin B6, pyridoxamine (Pyridorin) is an efficacious and specific post-Amadori inhibitor [60, 63, 102], with the ability to prevent renal dysfunction in diabetic rats [17]. Also in diabetic rats, pyridoxamine successfully reduced retinal AGE accumulation and also prevented up-regulation of BM-associated genes and diabetes-associated capillary acellularity [79].

Conclusion

AGEs play an important pathogenic role in diabetic retinopathy although it should be appreciated that the onset and progression of retinopathy probably involves a complex interplay between a range of pathogenic mechanisms. This is a reflection of the multifactorial nature of metabolic upset within the diabetic milieu. Long-term management of retinopathy in the ever-growing number of diabetic patients will involve precise regulation of their hyperglycemia and lipidemic profile hopefully in combination with drugs that ameliorate an array of biochemical and metabolic abnormalities. Experience shows that some inhibitors will probably be of more benefit to some complications over others, and indeed, clinical trials are often more difficult and costly between, for example, diabetic retinopathy and nephropathy. Experimental work suggests that inhibition of AGE-mediated pathways present a valid avenue for therapeutic exploitation in diabetic retinopathy and as the chemical reactions and structure of AGE moieties become further understood within *in vitro* and, ultimately, *in vivo* systems it will enable targeting of therapeutic agents to prevent AGE-mediated effects especially associated with lesions in the diabetic retina. An effective drug(s) to prevent the pathogenic effects of AGEs may become a vital component of the ophthalmologist’s future therapeutic armoury to prevent onset and progression of diabetic retinopathy.

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