

Advanced Glycation End Products (AGEs) and Diabetic Vascular Complications

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Abstract: Diabetic vascular complication is a leading cause of acquired blindness, end-stage renal failure, a variety of neuropathies and accelerated atherosclerosis, which could account for disabilities and high mortality rates in patients with diabetes. Chronic hyperglycemia is essentially involved in the development and progression of diabetic micro- and macroangiopathy. Among various metabolic derangements implicated in the pathogenesis of diabetic vascular complication, advanced glycation end product (AGE) hypothesis is most compatible with the theory of 'hyperglycemic memory'. In this review, we discuss the molecular mechanisms of diabetic vascular complication, specially focusing on AGEs and their receptor (RAGE) system. Several types of AGE inhibitors and their therapeutic implications in this devastating disorder are also discussed here.

Keywords: Diabetic vascular complications, atherosclerosis, AGEs, oxidative stress, RAGE, renin-angiotensin system, insulin resistance, pigment epithelium-derived factor (PEDF).

INTRODUCTION

Diabetic vascular complication is a leading cause of end-stage renal failure, acquired blindness, a variety of neuropathies and accelerated atherosclerosis, which could account for disabilities and high mortality rates in diabetic patients. Indeed, cardiovascular diseases (CVD) account for about 70 % of total mortality, and all manifestations of them such as coronary heart disease, stroke, and peripheral vascular disease are substantially more common in patients with diabetes [1]. Chronic hyperglycemia is a major initiator of vascular complications of diabetes. Various hyperglycemia-induced metabolic and hemodynamic derangements, including increased advanced glycation end product (AGE) formation, enhanced production of reactive oxygen species (ROS), activation of protein kinase C (PKC), stimulation of the polyol pathway and the renin-angiotensin system (RAS), contribute to the characteristic histopathological changes observed in diabetic vascular complications [2].

A recent clinical study, the Diabetes Control and Complications Trial-Epidemiology of Diabetes Interventions and Complications (DCCT-EDIC) Research, has shown that the reduction in the risk of progressive retinopathy and nephropathy resulting from intensive therapy in patients with type 1 diabetes persisted for at least four years, despite increasing hyperglycemia [3,4]. Intensive therapy during the DCCT resulted in decreased progression of intima-media thickness six years after the end of the trial as well [5]. These clinical studies strongly suggest that so-called 'hyperglycemic memory' causes chronic abnormalities in diabetic vessels that are not easily reversed, even by subsequent, relatively good control of blood glucose. Among the various

hypotheses implicated in the pathogenesis of diabetic vascular complications, AGE hypothesis seems to be most compatible with this theory [6].

In this review, we discuss the molecular mechanisms of diabetic vascular complication, specially focusing on AGEs and their receptor (RAGE) system. Several types of AGE inhibitors and their therapeutic implications in this devastating disorder are also reviewed here.

AGES

Reactive derivatives from non-enzymatic glucose-protein condensation reactions, as well as lipids and nucleic acids exposed to reducing sugars, form a heterogeneous group of irreversible adducts called "AGEs". AGEs were originally characterized by a yellow-brown fluorescent color and by an ability to form cross-links with and between amino groups [7], but the term is now used for a broad range of advanced products of the glycation process (also called the "Maillard reaction"), including N-carboxymethyllysine (CML) and pyrraline, which show neither color nor fluorescence and do not cross-link proteins [8-10]. CML can be formed from the precursors glyoxal and glycolaldehyde by an intra-molecular Cannizzaro reaction, a process that is largely independent of glucose autoxidation [11]. The concept that CML is a marker of oxidation rather than glycation has recently attracted support.

The formation of AGEs *in vitro* and *in vivo* is dependent on the turnover rate of the chemically modified target, the time available, and the sugar concentration. The structures of the various cross-linked AGEs that are generated *in vivo* have not yet been completely determined. Because of their heterogeneity and the complexity of the chemical reactions involved, only some AGEs have been structurally characterized *in vivo*. The structural identity of AGEs with cytotoxic properties remains unknown.

AGEs are formed by the Maillard process, a non-enzymatic reaction between ketone group of the glucose

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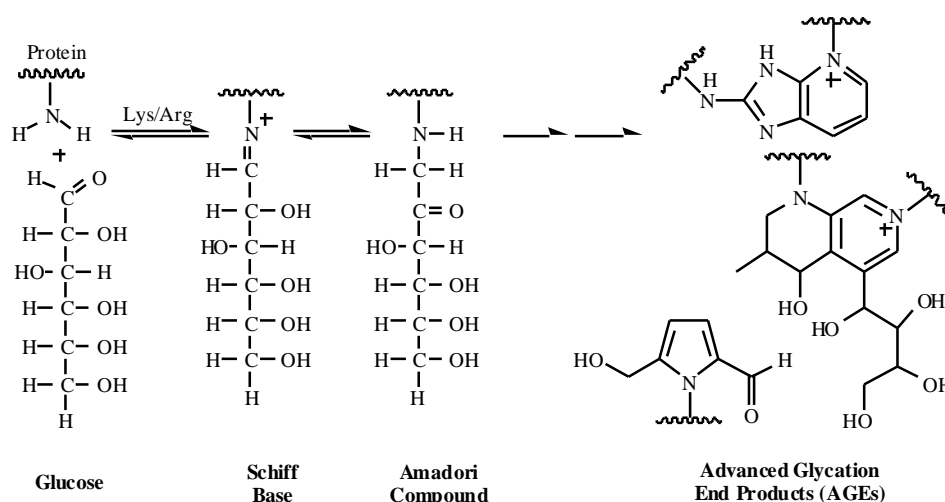


Fig. (1). Formation of AGEs.

molecule or aldehydes and the amino groups of proteins that contributes to the aging of proteins and to the pathological complications of diabetes [12-16]. In the hyperglycemia elicited by diabetes, this process begins with the conversion of reversible Schiff base adducts to more stable, covalently bound Amadori rearrangement products. Over the course of days to weeks, these Amadori products undergo further rearrangement reactions to form the irreversibly bound moieties known as AGEs. Recent studies have suggested that AGEs can arise not only from sugars, but also from carbonyl compounds derived from the autoxidation of sugars and other metabolic pathways [17] (Figure 1).

DIABETIC RETINOPATHY

(1) Pathophysiology of Diabetic Retinopathy

Diabetic retinopathy is one of the most important microvascular complications in diabetes and is a leading cause of acquired blindness among the people of occupational age [18]. In a large population-based study, prevalence of any degree or proliferative retinopathy was highest in the younger-onset, insulin-taking diabetic patients and lowest in older-onset group not taking insulin [19,20]. The prevalence of diabetic retinopathy increases with duration of diabetes. After 30 years of diabetes, nearly all patients in the former group have some degree of retinopathy and the prevalence of proliferative retinopathy is about 60 %.

The lesions of diabetic retinopathy can be grouped into those associated with background, preproliferative and proliferative retinopathy. The earliest histopathological hallmark of diabetic retinopathy is loss of pericytes [21]. Normally, the ratio of endothelial cells (ECs) to pericytes in the retinal capillaries is 1:1, but its levels are reported to decrease to 1:4 after several years of diabetes and eventually to 1:10 with even longer diabetic exposure [21,22]. In parallel with loss of pericytes, several characteristic changes including thickening of the basement membrane, hyperpermeability, and formation of microaneurysm are observed [23,24]. These structural and functional abnormalities are followed by microvascular occlusion in the retinas, which ultimately progresses to proliferative changes

associated with neovascularization [23,24]. It has been postulated that many of these changes are the consequent of the loss of pericytes.

Pericytes are elongated cells of the mesodermal origin, wrapping around and along ECs of small vessels [25]. As pericytes contain contractile muscle filaments on their EC side, they have been regarded for a long time just as microvascular counterparts of smooth muscle cells, and implicated in the maintenance of capillary tone [26,27]. In 1983, D'Amore developed a procedure for isolating pericytes from small vessels, and enabled us to elucidate the functional roles and biological characteristics of pericytes [28]. By using pericyte-EC co-culture systems, we have found that pericytes not only regulate the growth, but also preserve the prostacyclin-producing ability and protect against lipid-peroxide-induced injury of ECs, thus playing an important role in the maintenance of microvascular homeostasis [29,30]. Therefore, the loss of pericytes could predispose the vessels to angiogenesis, thrombogenesis, and EC injury, leading to full blown clinical expression of diabetic retinopathy. D'Amore *et al.* demonstrated that an active form of transforming growth factor- (TGF-) was produced by co-cultures of ECs and pericytes and that antibodies against TGF- added to the co-culture systems abolished the growth inhibitory effects of pericytes on neighboring ECs [31]. These observations suggest that a candidate molecule that would mediate the functional interactions between pericytes and ECs is TGF- .

Recently, Hammes *et al.* investigated the role of capillary coverage with pericytes in early diabetic retinopathy and the contribution to proliferative retinopathy using mice with a single functional allele of platelet-derived growth factor-B (PDGF-B(+/-) mice) [32]. They demonstrated in their studies that retinal capillary coverage with pericytes is crucial for the survival of ECs, particularly under stress conditions such as diabetes, and that pericyte deficiency leads to reduced inhibition of EC proliferation, thus promoting angiogenesis in the retinopathy of premature model. Our *in vitro* and their recent *in vivo* observations provide a basis for understanding why diabetic retinopathy develops consequent to pericyte

loss, the earliest histopathological hallmarks of diabetic retinopathy.

(2) Role of AGEs in Diabetic Retinopathy

(i) Involvement of AGEs in Pericyte Loss and Dysfunction

Retinal pericytes accumulate AGEs during diabetes [33], which would be expected to have a detrimental influence on pericyte survival and function [34]. AGEs are toxic to retinal pericytes *in vitro*. We have found that AGEs not only induce growth retardation and apoptotic cell death of, but also exert an immediate toxicity to cultured retinal pericytes [35,36]. Antisense DNA complementary mRNA coding for RAGE reversed the AGE-induced decrease in viable cell number of pericytes, while overexpression of RAGE potentiated the deleterious effects of AGEs. Therefore, the AGE effects on pericytes could be mediated through the interactions with RAGE.

We also found that ROS generation induced by AGE-RAGE interaction mediated these deleterious effects of AGEs [37]. Moreover, AGEs up-regulated RAGE mRNA levels in pericytes through the intracellular ROS generation [38]. Therefore, these positive feedback loops further transduced the AGE signals, thus exacerbating the cytopathic effects of AGEs on pericytes in diabetic retinopathy. With regard to a source of ROS generation elicited by AGEs, we have recently found that beraprost sodium, a prostacyclin analogue, or forskolin, an activator of adenylate cyclase, protects against the AGE-induced pericyte apoptosis by suppressing ROS generation and subsequent RAGE overexpression [39]. Since cyclic AMP elevating agents were known to block ROS generation in neutrophils by inhibiting reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity [40], NADPH oxidase might be a source of ROS production elicited by AGEs and also be a target of beraprost sodium.

Pericyte dysfunction has been considered one of the characteristic changes of early phase of diabetic retinopathy as well. AGEs act on pericytes to stimulate vascular endothelial growth factor (VEGF) expression [36]. VEGF is a specific mitogen to ECs, also known as vascular permeability factor, and is thought a pivotal factor in the pathogenesis of proliferative diabetic retinopathy. Indeed, some clinical observations have demonstrated that VEGF level in ocular fluid is positively correlated with the activity of neovascularization in diabetic retinopathy [41,42]. Recently, VEGF level was also found to be associated with the breakdown of the blood-retinal barrier, thus being involved in microvascular hyperpermeability in background retinopathy [43]. These observations suggest that AGEs might be involved in the development of diabetic retinopathy by inducing VEGF overexpression in pericytes as well.

(ii) Involvement of AGEs in Retinal Inflammation, Thrombosis and Angiogenesis

There has been an increasing interest in the role of inflammatory reaction in diabetic retinopathy [44]. AGEs are implicated in the process of vascular inflammation as well. Indeed, AGEs have been recently shown to increase leukocyte adhesion to cultured retinal microvascular ECs by

inducing intracellular cell adhesion molecule-1 (ICAM-1) expression [45]. This phenomenon is also apparent in non-diabetic mice infused with preformed AGEs, which results in significant leukostasis and blood-retinal barrier dysfunction in these mice [45]. Recently, retinal VEGF has been found to induce ICAM-1 expression, thus leading to leukostasis and breakdown of blood-retinal barrier *in vivo* [46-48]. It is not known at present whether the AGE-elicited pro-inflammation is modulated by blockage of VEGF. AGEs are also known to induce monocyte chemoattractant protein-1 (MCP-1) in microvascular ECs through intracellular ROS generation [49]. Since the levels of MCP-1 in vitreous fluids are correlated with the severity of proliferative diabetic retinopathy [50], AGEs would be one of the key pro-inflammatory factors for progression of diabetic retinopathy.

Microthrombosis formation contributes to capillary obliteration and retinal ischemia, thus being involved in the progression of diabetic retinopathy [51]. AGEs inhibit prostacyclin production and induce plasminogen activator inhibitor-1 (PAI-1) in microvascular ECs through an interaction with RAGE [52,53]. These observations suggest that AGE have the ability to cause platelet aggregation and fibrin stabilization, resulting in a predisposition to thrombogenesis and thereby contributing to the promotion of diabetic retinopathy. Retinal ischemia due to microthrombus formation may trigger VEGF expression in retinal cells, thus further promoting diabetic retinopathy [54-56]. Since AGEs decrease the intracellular cyclic AMP concentrations in ECs and that cyclic AMP agonists such as beraprost sodium and forskolin reduce the AGE-induced PAI-1 production, cyclic AMP elevating agents may have a therapeutic potential in the treatment of diabetic retinopathy.

AGEs also directly stimulate growth and tube formation of microvascular ECs, the key steps of angiogenesis [57,58]. The angiogenic activity of AGEs was significantly enhanced in RAGE-overexpressed ECs, while it was completely inhibited by treatment of antisense DNA against RAGE mRNA. The observations suggest that AGEs could elicit angiogenesis through the interaction with RAGE. We found that it was autocrine VEGF production in ECs that mainly mediated the angiogenic activity of AGEs. Although the molecular mechanisms of VEGF overexpression elicited by AGEs are not fully understood, our recent investigation has shown that the AGE-RAGE interaction might increase VEGF gene transcription in microvascular ECs by NADPH oxidase-mediated ROS generation and the subsequent nuclear factor- κ B (NF- κ B) activation via Ras-mitogen activated protein kinase (MAPK) pathway [59,60].

Angiopoietin (ang)-Tie receptor interaction plays an important role in both physiological and pathological angiogenesis as well [61]. Engagement of tie-2 by ang-1 has been known to promote recruitment of pericytes, thereby supporting the establishment and maintenance of vascular integrity; while ang-2 is a naturally occurring antagonist of ang-1, and induces the loosening of contacts between ECs and pericytes [62-64]. AGEs increase the ratio of ang-2 to ang-1 mRNA level and simultaneously up-regulate VEGF mRNA levels in microvascular ECs. A plastic window for blood vessel remodeling is defined by pericyte coverage of the preformed endothelial network [65]. Therefore, the AGE-

induced pericyte apoptosis and increased ratio of ang-2/ang-1 in ECs could disrupt the pericyte-EC interactions, thus promoting angiogenesis by acting in concert with VEGF.

(iii) *Cross-Talk between AGEs and RAS in Diabetic Retinopathy*

The local RAS is activated under diabetes [66]. We have recently found that angiotensin II (AII) stimulates intracellular ROS generation in retinal pericytes through an interaction with type 1 receptor. Further, AII decreased DNA synthesis and simultaneously up-regulated VEGF mRNA levels in pericytes, both of which were blocked by treatment with telmisartan, a newly developed AII type 1 receptor antagonist, or *N*-acetylcysteine (NAC), an anti-oxidant [67,68]. These results suggest that AII-type 1 receptor interaction could induce pericyte loss and dysfunction through intracellular ROS generation, thus being involved in diabetic retinopathy. Furthermore, we have very recently found that AII potentiates the deleterious effects of AGEs on pericytes by inducing RAGE protein expression (unpublished data). The observations suggest the functional interaction between the AGE-RAGE system and the RAS in the pathogenesis of pericyte loss and dysfunction in diabetic retinopathy. AII induced the VEGF receptor expression in retinal microvascular ECs. The retinal RAS might also augment the permeability- and angiogenesis-inducing activity of VEGF, thus being involved in the progression of diabetic retinopathy [69]. In support of this, blockade of the RAS by inhibitors of angiotensin converting enzyme or AII type 1 receptor antagonists have recently been found to reduce retinal overexpression of VEGF and hyperpermeability and neovascularization in experimental diabetes [70-72]. In the EUCLID Study, the angiotensin converting enzyme inhibitor, lisinopril, reduced the risk of progression of retinopathy by approximately 50%, and also significantly reduced the risk of progression to proliferative retinopathy although retinopathy was not a primary end-point and the study was not sufficiently powered for the eye-related outcomes [73].

(3) *Prevention of Diabetic Retinopathy by AGE Inhibitors*

The above-discussed *in vitro* and *in vivo* effects of AGEs strongly suggest a pathological role for these senescent macromolecules in diabetic retinopathy. Furthermore, serum levels of AGEs are found to be correlated to severity of diabetic retinopathy with both type 1 and type 2 diabetes [74,75]. Therefore, inhibition of AGEs formation, blockade of AGE-RAGE interactions, or the downstream signaling pathways has been supposed to be potential therapeutic strategies in the prevention of diabetic retinopathy.

The hydrazine compound aminoguanidine is the first AGE inhibitor discovered [76]. Aminoguanidine can trap -dicarbonyl compounds, thus preventing their further reactions with amino groups of proteins [76]. Treatment of diabetic rats for 26 weeks with aminoguanidine prevented a 2.6-fold accumulation of these products at branching sites of pre-capillary arterioles and thereby prevented abnormal EC proliferation and significantly diminished pericyte dropout

[77]. A multicenter clinical trial revealed that pimagedine^R (aminoguanidine) slowed the progression of diabetic retinopathy, although it was terminated early due to safety concerns [78,79].

Amadorins have an ability to scavenge dicarbonyls and therefore inhibit the conversion of Amadori intermediates to AGEs [80,81]. The derivative of vitamin B₆, pyridoxamine, has been shown to an efficacious and specific post-Amadori inhibitor, with the ability to prevent up-regulation of retinal basement membrane-associated genes and capillary dropout [82].

Compared to the strategy of preventing AGE formation, the manipulation of the AGE signaling pathways as a therapeutic option in diabetic retinopathy remains much less developed. However, recently, we have found that pigment epithelium-derived factor (PEDF), one of the superfamily of serine protease inhibitors with potent neuronal differentiating activity in human retinoblastoma cells [83], inhibits the AGE-induced pericyte apoptosis and EC proliferation and activation through its anti-oxidative properties [37,49,84-86]. Since the levels of vitreal PEDF are decreased in angiogenic eye diseases such as proliferative diabetic retinopathy [87], substitution of PEDF may disrupt inappropriate retinal cell responses to AGEs, thus being a promising strategy for treatment of patients with diabetic retinopathy. We also found that cerivastatin, a 3-hydroxy-3-methylglutaryl co-enzyme A (HMG-CoA) reductase inhibitor, or incadronate disodium, a nitrogen-containing bisphosphonates inhibited the AGE-signaling to angiogenesis by blocking autocrine production of VEGF via suppression of protein farnesylation [59,60].

We have previously found that high glucose stimulated AGEs formation as well as NF- κ B activity in ECs through overproduction of mitochondria-derived superoxide [88]. Blockade of this ROS generation by benfotiamine, a lipid-soluble thiamine derivative, has been recently reported to inhibit AGE formation and NF- κ B activation in retinas of diabetic animals and prevent experimental diabetic retinopathy [89].

As a summary of this section, we can posit an overall scheme concerning the molecular mechanisms and potential therapeutic strategies for diabetic retinopathy (Figures 2 and 3).

DIABETIC NEPHROPATHY

(1) *Pathophysiology of Diabetic Nephropathy*

The earliest clinical manifestation for incipient diabetic nephropathy is the development of the persistent microalbuminuria (urinary albumin excretion rate (UAER), 20-200 μ g/min), which is Albustix-negative. If untreated, approximately 80 % of type 1 diabetic patients will develop overt albuminuria (UAER>200 μ g/min) over a 15-year period [90]. Of these patients, 50 % will develop end-stage renal disease (ESRD) over the ensuing 10 years. In type 2 diabetes, if no treatment is initiated, up to 20-40 % of patients will progress to overt albuminuria and 20 % of those with overt albuminuria will develop ESRD over the next 20 years.

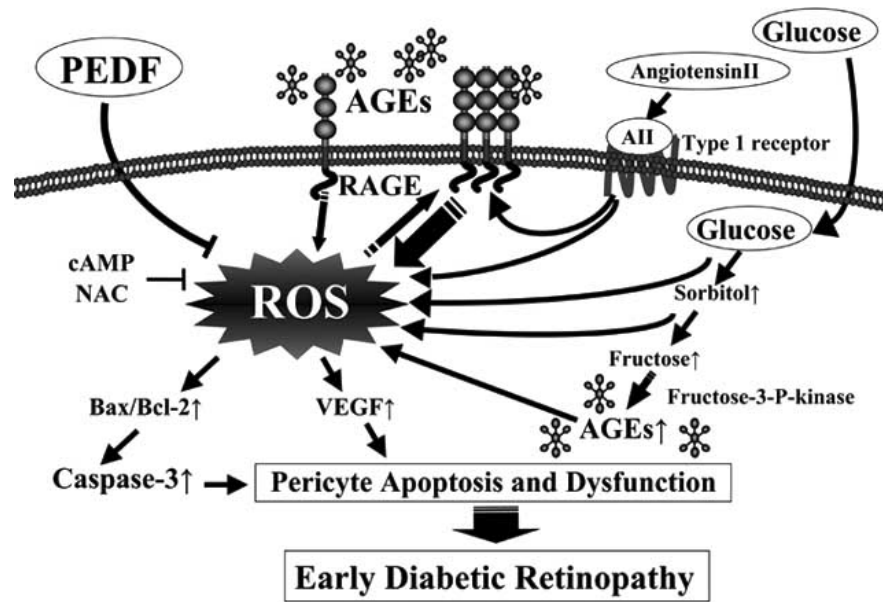


Fig. (2). Possible involvement of AGEs in early diabetic retinopathy.

Diabetic nephropathy is a leading cause of ESRD, and accounts for disabilities and the high mortality rate in patients with diabetes [91,92]. Development of diabetic nephropathy is characterized by glomerular hyperfiltration and thickening of glomerular basement membranes, followed by an expansion of extracellular matrix in mesangial areas and increased UAER. Diabetic nephropathy ultimately progresses to glomerular sclerosis associated with renal dysfunction [93]. Further, it has recently been recognized that changes within tubulointerstitium, including proximal tubular cell atrophy and tubulointerstitial fibrosis, are also

important in terms of renal prognosis in diabetic nephropathy [94-98]. Such tubular changes have been reported to be the dominant lesion in about one third of patients with type 2 diabetes [99]. It appears that both metabolic and hemodynamic factors interact to stimulate the expression of cytokines and growth factors in glomeruli and tubules from the diabetic kidney [100]. Evidence has implicated the TGF- β system as a major etiologic agent in the pathogenesis of glomerulosclerosis and tubulointerstitial fibrosis in diabetic nephropathy [93,101,102].

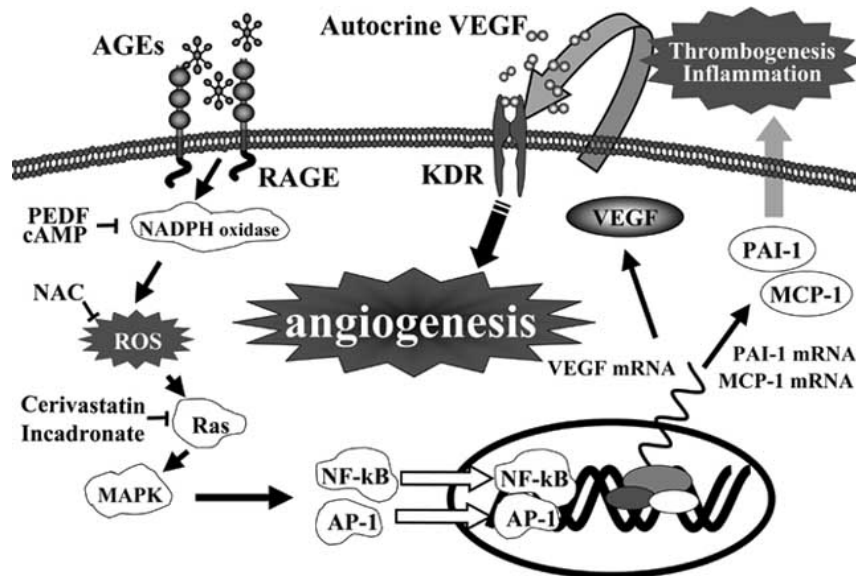


Fig. (3). Possible involvement of AGEs in proliferative diabetic retinopathy.

(2) Role of AGEs in Diabetic Nephropathy

(i) Involvement of AGEs in Mesangial Cell Dysfunction

AGEs induce apoptotic cell death and VEGF expression in human cultured mesangial cells, as the case in pericytes [103]. Mesangial cells occupy a central anatomical position in the glomerulus, playing crucial roles in maintaining structure and function of glomerular capillary tufts [104]. They actually provide structural support for capillary loops and modulate glomerular filtration by its smooth muscle activity [104-106]. Therefore, the AGE-induced mesangial apoptosis and dysfunction may contribute in part to glomerular hyperfiltration, an early renal dysfunction in diabetes. Recently, antibodies against VEGF have been found to improve hyperfiltration and albuminuria in experimental diabetes, supporting our speculation [107]. Furthermore, we have recently found that AGEs stimulate MCP-1 expression in mesangial cells [103]. Increased MCP-1 expression associated with monocyte infiltration in mesangium has been observed in the early phase of diabetic nephropathy [108]. AGE accumulation in glomerulus could also be implicated in the initiation of diabetic nephropathy by promoting the secretion of MCP-1 in mesangial cells.

(ii) Involvement of AGEs in Glomerulosclerosis

AGEs formation on extracellular matrix proteins alters both matrix-matrix and cell-matrix interactions, involved in the pathogenesis of diabetic glomerulosclerosis. For example, non-enzymatic glycations of type IV collagen and laminin reduce their ability to interact with negatively charged proteoglycans, increasing vascular permeability to albumin [109]. Furthermore, AGEs formation on various types of matrix proteins impairs their degradation by matrix metalloproteinases, contributing to basement membrane thickening and mesangial expansion, hallmarks of diabetic nephropathy [110,111]. AGEs formed on the matrix components can trap and covalently cross-link with the extravasated plasma proteins such as lipoproteins, thereby exacerbating diabetic glomerulosclerosis [111].

AGEs stimulate insulin-like growth factor-I, -II, PDGF and TGF- β in mesangial cells, which in turn mediate production of type IV collagen, laminin and fibronectin [112]. Recently, Ziyadeh *et al.* reported that long-term treatment of type 2 diabetic model mice with blocking antibodies against TGF- β suppressed excess matrix gene expression, glomerulosclerosis, and prevented the development of renal insufficiency [113]. These observations suggest that AGE-induced TGF- β expression plays an important role in the pathogenesis of glomerulosclerosis in diabetic nephropathy [114,115].

In vivo, the administration of AGE-albumin to normal healthy mice for 4 weeks has been found to induce glomerular hypertrophy with overexpression of type IV collagen, laminin B1 and TGF- β genes [116]. Chronic infusion of AGE-albumin to otherwise healthy rats leads to focal glomerulosclerosis, mesangial expansion, and albuminuria [117]. Recently, RAGE-overexpressing diabetic mice have been found to show progressive glomerulosclerosis with renal dysfunction, compared with diabetic littermates lacking the RAGE transgene [118].

Furthermore, Schmidt *et al.* reported that diabetic homozygous RAGE null mice failed to develop mesangial matrix expansion or thickening of the glomerular basement membrane. They also claimed in their report that activation of RAGE in podocytes could contribute to expression of VEGF and enhanced attraction/activation of inflammatory cells in the diabetic glomerulus, causing albuminuria and glomerulosclerosis in diabetes [119]. AGEs including glycoxidation or lipoxidation products such as N-(carboxymethyl)lysine, pentosidine, malondialdehyde-lysine accumulate in the expanded mesangial matrix and thickened glomerular basement membranes of early diabetic nephropathy, and in nodular lesions of advanced disease, further suggesting the active role of AGEs for diabetic nephropathy [120].

(iii) Role of AGEs in Proximal Tubular Cell Injury

AGEs stimulated ROS generation and subsequently inhibited *de novo* protein synthesis in proximal tubular cells [121]. Since AGEs did not affect DNA synthesis and viable cell numbers in these cells, AGEs might be involved in the pathogenesis of tubular atrophy, one of the representative tubulointerstitial changes in diabetic nephropathy. AGEs also induced TGF- β in proximal tubular cells, both at mRNA and protein levels. TGF- β is a major etiologic agent in the pathogenesis of tubulointerstitial fibrosis in diabetic nephropathy [93]. These observations suggest the involvement of AGEs in proximal tubular cell injury in diabetes as well.

(iv) Cross-Talk Between AGEs and RAS in Diabetic Nephropathy

There is a growing body of evidence to suggest that the RAS plays an important role in the regulation of glomerular hemodynamics and renal expression of cytokines, thus being involved in glomerular hyperfiltration and mesangial expansion in diabetic nephropathy [122,123]. Several reports suggest an interaction between AGEs and the RAS in diabetic nephropathy. Angiotensin converting enzyme inhibition reduces the accumulation of renal and serum AGEs, probably via effects on oxidative pathways [124]. Long-term treatment with AII receptor 1 antagonist may exert salutary effects on AGEs levels in the rat remnant kidney model, probably due to improved renal function [125]. Administration of ramipril, an angiotensin converting enzyme inhibitor, has been recently shown to result in a mild decline of fluorescent non-carboxymethyllysine-AGEs and malondialdehyde concentrations in non-diabetic nephropathy patients [126]. In addition, we have very recently found that AGE-RAGE-mediated ROS generation activates TGF- β - Smad signaling and subsequently induces mesangial cell hypertrophy and fibronectin synthesis by autocrine production of AII. Taken together, these data may provide an important mechanistic link between metabolic and hemodynamic factors in promoting the development and progression of diabetic nephropathy.

(3) Prevention of Diabetic Nephropathy by AGE Inhibitors

A number of studies have demonstrated that aminoguanidine decreased AGE accumulation and plasma

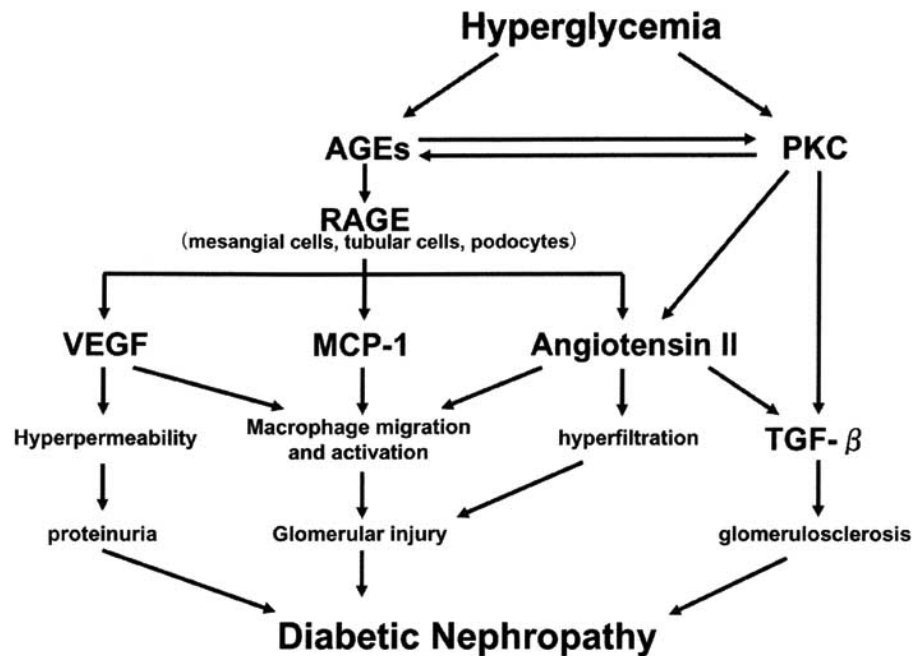


Fig. (4). Possible involvement of AGEs in diabetic nephropathy.

protein trapping in the glomerular basement membrane [16,115]. In streptozocin-induced diabetic rats, aminoguanidine treatment for 32 weeks dramatically reduced the level of albumin excretion and prevented the development of mesangial expansion [127]. Furthermore, aminoguanidine treatment was found to prevent albuminuria in diabetic hypertensive rats without affecting blood pressure [115]. Whether inhibition by aminoguanidine of inducible nitric oxide synthase (iNOS) could contribute to these renoprotective effects remains to be elucidated. However, methylguanidine, which inhibits iNOS but not AGE formation, was reported not to retard the development of albuminuria in diabetic rats [128]. These observations suggest that the beneficial effects of aminoguanidine could be mediated predominantly by decreased AGE formation rather than via iNOS inhibition. A recent randomized, double-masked, placebo-controlled study (ACTION I trial) revealed that pimagedine^R (aminoguanidine) reduced the decrease in glomerular filtration rate and 24-hour total proteinuria in type 1 diabetic patients [129]. Although the time to doubling of serum creatinine, a primary end point of this study, was not significantly improved by pimagedine^R treatment ($p=0.099$), the trial provided the first clinical proof of the concept that blockade of AGE formation could result in a significant attenuation of diabetic nephropathy.

We have found that OPB-9195 ((±)-2-isopropylidene-hydrazone-4-oxo-thiazolidine-5-ylacetanilide), a synthetic thiazolidine derivative and novel inhibitor of AGEs, prevented the progression of diabetic nephropathy by lowering serum concentrations of AGEs and their deposition of glomeruli in Otsuka-Long-Evans-Tokushima-Fatty rats, a type II diabetes mellitus model animal [130]. OPB-9195 was also found to retard the progression of diabetic nephropathy by blocking type IV collagen production and suppressing overproduction of two growth factors, TGF- and VEGF.

Recently, Baynes *et al.* reported that pyridoxamine inhibited the progression of renal disease, and decreased hyperlipidemia and apparent redox imbalances in diabetic rats [131]. Pyridoxamine and aminoguanidine had similar effects on parameters measured, supporting a mechanism of action involving AGE inhibition [131].

As a summary of this section, we can posit an overall scheme concerning the molecular mechanisms for diabetic nephropathy (Figure 4).

DIABETIC NEUROPATHY

(1) Pathophysiology of Diabetic Neuropathy

Diabetes mellitus is a major cause of peripheral neuropathy, commonly manifested as distal symmetrical polyneuropathy [132]. The epidemiology of diabetic neuropathy is unclear because of inconsistency in defining neuropathy. In a large prospective study of diabetic outpatients, the prevalence rose from 7.5 % at the time of diagnosis of diabetes to 50 % after 25 years [133]. The prevalence after 20 years was over 40 %, but in the other prospective study, it was only half of this value [134]. Diabetic neuropathy is associated with risk factors for other vascular complications such as poor metabolic control, dyslipidemia, hypertension, body mass index, smoking, microalbuminuria and retinopathy [135-137]. However, a report from the Steno type-2 randomized study showed the effectiveness of aggressive risk factor control on retinopathy, nephropathy and autonomic neuropathy, but not peripheral neuropathy [138].

Both vascular and metabolic factors have been involved in the pathogenesis of diabetic neuropathy. Studies in human and animal models have shown reduced nerve perfusion and endoneurial hypoxia, which might play a role in nerve dysfunction [139,140]. Retinopathy-like microvasculature changes including basement membrane thickening and

pericyte degeneration have been observed in diabetic neuropathy [141,142], and arterio-venous shunting contributes to reduced endoneurial perfusion as well [143]. Hyperglycemia, via various metabolic derangements, contributes to the etiology of diabetic neuropathy as well.

(2) Role of AGEs in Diabetic Neuropathy

Sural, peroneal, and saphenous nerves of human diabetic subjects contain AGEs in the perineurium, ECs, and pericytes of endoneurial microvessels and in myelinated and unmyelinated fibers [144]. Tubulin glycation profoundly inhibits GTP-dependent tubulin polymerization, thus impairing axonal transport [145,146]. Excessive glycation of both peripheral and central nervous system myelin components has also been observed in diabetic rats [147]. An interaction between AGE-myelin and macrophages may initiate or contribute to the segmental demyelination associated with diabetic neuropathy [148].

(3) Prevention of Diabetic Neuropathy by AGE Inhibitors

Aminoguanidine treatment inhibits an accumulation of fluorescent AGE in diabetic nerves, and partially prevents demyelination and axonal atrophy probably through the correction of endoneurial microcirculation [149,150]. Recently, Yagihashi *et al.* reported that OPB-9195 treatment improved tibial motor nerve conduction velocity and restored the decrease in sciatic nerve Na⁺-K⁺-ATPase activity in diabetic rats, which was in parallel with suppression of oxidative stress-induced DNA damage [151]. ROS production induced by AGE-RAGE interactions might be involved in endoneurial vascular dysfunction and nerve injury in diabetic neuropathy [152].

CVD

(1) Pathophysiology of CVD in Diabetes

Atherosclerotic arterial disease may be manifested clinically as CVD. CVD is responsible for about 70 % of all causes of death in patients with type 2 diabetes [1]. In Framingham study, the incidence of CVD was 2-4 times greater in diabetic patients than in general population [153]. Conventional risk factors, including hyperlipidemia, hypertension, smoking, obesity, lack of exercise, and a positive family history, contribute similarly to macrovascular complications in type 2 diabetic patients and non-diabetic subjects [1]. The levels of these factors in diabetic patients were certainly increased, but not enough to explain the exaggerated risk for macrovascular complications in diabetic population [154]. Therefore, specific diabetes-related risk factors should be involved in the excess risk in diabetic patients.

In the last decade, several prospective studies have shown that hyperglycemia itself is clearly involved in predicting CVD [155]. In newly diagnosed type 2 diabetes, 10-year cardiovascular mortality increased threefold by tertiles of blood glucose and HbA_{1c} [156]. There was a significant increase in the risk of CVD death and all CVD events in type 2 diabetic subjects with HbA_{1c} levels higher than 7.0 % compared with diabetic subjects with lower

HbA_{1c} [157,158]. More recently, the incidence of myocardial infarction and coronary artery disease mortality have been found to be correlated to postprandial hyperglycemia but not fasting hyperglycemia (Diabetes Intervention Study and DECODE Study) [159,160]. Postprandial hyperglycemia-induced AGEs formation and ROS generation may play an important role in the poor cardiovascular outcome of diabetic patients [161]. An α -glucosidase inhibitor, acarbose treatment has shown to improve postprandial hyperglycemia and reduce the risk of CVD and hypertension in patients with impaired glucose tolerance, further suggesting the importance of control of postprandial hyperglycemia in preventing CVD [162].

The conclusive answer to the question on the existence of cause-effect relationship between hyperglycemia and CVD derives from intervention studies. In United Kingdom Prospective Diabetes Study (UKPDS), intensive blood glucose control has effectively reduced microvascular complications in type 2 diabetic patients [163]. However, the risk of myocardial infarction reduced slightly but not significantly by about 15 %, and less than treatment of hypertension (21 %) or hypercholesterolemia (31 %). Since the reduction of hyperglycemia is small in this trial, it may be an underestimation of the role of hyperglycemia in preventing CVD.

It is believed that macrovascular disease starts before the development of diabetes. Several studies have confirmed the increased risk of CVD in patients with impaired glucose tolerance [164-166]. There is a growing body of evidence that insulin resistance in the absence of overt diabetes has been associated with endothelial dysfunction, an initial process of atherosclerosis [167,168]. Therefore, atherosclerotic process may actually begin earlier in the spectrum of insulin resistance. These observations suggest that aggressive treatment of dyslipidemia and hypertension, even before the onset of type 2 diabetes, would appear prudent in decreasing the progression of the atherosclerotic process. Effectiveness of aggressive treatment for conventional risk factors, including hyperglycemia, has been shown in a recent Steno-2 Study [169]. A long-term, intensified intervention aimed at multiple risk factors in patients with type 2 diabetes and microalbuminuria reduces the risk of cardiovascular and microvascular events by about 50 percent.

(2) Role of AGEs in CVD in Diabetes

A variety of molecular mechanisms underlying the actions of AGEs and their contribution to diabetic macrovascular complications have been proposed [170-174]. AGEs formed on the extracellular matrix results in decreased elasticity of vasculatures, and quench nitric oxide, which could mediate defective endothelium-dependent vasodilatation in diabetes [175]. AGE modification of low-density lipoprotein (LDL) exhibits impaired plasma clearance and contributes significantly to increased LDL *in vivo*, thus being involved in atherosclerosis [176]. Binding of AGEs to RAGE results in generation of intracellular ROS generation and subsequent activation of the redox-sensitive transcription factor NF- κ B in vascular wall cells, which promotes the expression of a variety of atherosclerosis-

related genes, including ICAM-1, vascular cell adhesion molecule-1, MCP-1, PAI-1, tissue factor, VEGF, and RAGE [49,52,57,177-181]. AGEs have the ability to induce osteoblastic differentiation of microvascular pericytes, which would contribute to the development of vascular calcification in accelerated atherosclerosis in diabetes as well [182]. The interaction of the RAS and AGEs in the development of diabetic macrovascular complications has also been proposed. AGE-RAGE interaction augments AII-induced smooth muscle cell proliferation and activation, thus being involved in accelerated atherosclerosis in diabetes [183].

Smooth muscle cell proliferation, migration, and neointimal expansion upon arterial injury were strikingly suppressed in homozygous RAGE null mice compared with those observed in wild-type littermates [184]. These data highlight key roles for RAGE in modulating smooth muscle cell properties after injury and suggest that RAGE is a logical target for suppression of untoward neointimal expansion consequent to arterial injury.

AGEs have been actually detected within atherosclerotic lesions in both extra- and intracellular locations [185-187]. Furthermore, RAGE overexpression is associated with enhanced inflammatory reaction and matrix metalloproteinase (MMP) expression in plaque macrophages in diabetic patients [188]. The AGE-RAGE interaction could contribute to plaque destabilization by inducing culprit MMP expression.

(3) Prevention of Accelerated Atherosclerosis by AGE Inhibitors

In animal models, Schmidt *et al.* has demonstrated that diabetic apolipoprotein E (apoE) null animals receiving soluble RAGE (sRAGE) display a dose-dependent suppression of accelerated atherosclerosis in these mice [189]. Lesions that formed in animals receiving sRAGE appeared largely arrested at the fatty streak stage; the number of complex atherosclerotic lesions was strikingly reduced in diabetic apoE null mice. The tissue and plasma AGE burden was suppressed in diabetic apoE null mice receiving sRAGE, suggesting that the AGE-RAGE-induced oxidative stress generation might participate in AGEs formation themselves. Treatment with sRAGE did not affect the levels of established risk factors in these mice. These observations suggest the active involvement of AGE-RAGE interaction in the pathogenesis in accelerated atherosclerosis in diabetes. The same group has recently reported that the AGE-RAGE system contributes to atherosclerotic lesion progression as well, and that RAGE blockade stabilizes the lesions in these mice [190]. Other study shows a correlation between AGE levels and the degree of atheroma in cholesterol-fed rabbits, and that aminoguanidine has an anti-atherogenic effect in these rabbits by inhibiting AGEs formation [191].

Recently, food-derived AGEs are reported to induce oxidative stress and promote inflammatory signals [192]. Dietary glycotoxins promote diabetic atherosclerosis in apoE-deficient mice [193]. Furthermore, high levels of dietary AGEs transform low-density lipoprotein into a potent redox-sensitive mitogen-activated protein kinase stimulant in diabetic patients [194]. The marked anti-atherogenic effects

of an AGE-restricted diet in these models and patients may provide the basis for relevant clinical studies.

Taken together, in diabetes, when fueled by hyperglycemia, AGEs and oxidative stress, the AGE-RAGE axis amplifies vascular stress and accelerates atherosclerosis and neointimal expansion [195]. Blockade of the AGE-RAGE interaction may lead to a successful reduction of CVD in diabetes.

CONCLUSION

Two recent large prospective clinical studies, DCCT and UKPDS, have shown that intensive blood glucose control effectively reduces microvascular complications among patients with diabetes [163,196]. Further, there is a growing body of evidence to conclude that tight blood glucose control has no more than a marginal impact on CVD in general, and on coronary heart disease in particular, regardless of the type of diabetes [197]. However, control of hyperglycemia to strict levels is often very difficult to maintain and may increase the risk of severe hypoglycemia in diabetic patients. Therefore, to develop novel therapeutic strategies that specifically target diabetic vascular complications may be desirable for most patients with diabetes.

There are several contradictory results regarding the biological role of AGEs in diabetic vascular complication; trace amounts of redox active metal ions or endotoxin in biological buffers could induce oxidative stress and alternations in cellular functions attributed to AGE-proteins *in vitro* [198,199]. However, as we reviewed here, there is growing evidence to suggest that AGEs are mainly involved in the pathogenesis of diabetic vascular complication. Inhibition of AGEs formation or blockade of their downstream signaling pathway may be a promising therapeutic strategy for treatment of patients with diabetic vascular complication.

ABBREVIATIONS:

AII	=	Angiotensin II
AGEs	=	Advanced glycation end products
Ang	=	Angiopietin
apoE	=	Apolipoprotein E
CML	=	Carboxymethyllysine
CVD	=	Cardiovascular disease
DCCT-EDIC	=	Diabetes Control and Complications Trial-Epidemiology of Diabetes Interventions and Complications; ECs, endothelial cells
ESRD	=	End-stage renal disease
HMG-CoA enzyme A	=	3-hydroxy-3-methylglutaryl co-intracellular adhesion molecule-1
ICAM-1	=	
iNOS	=	Inducible nitric oxide synthase
LDL	=	Low-density lipoprotein
MAPK	=	Mitogen activated protein kinase

MCP-1	=	Monocyte chemoattractant protein-1
MMP	=	Matrix metalloproteinase
NAC	=	<i>N</i> -acetylcysteine
NADPH	=	Reduced nicotinamide adenine dinucleotide phosphate
NF- B	=	Nuclear factor- B
PAI-1	=	Plasminogen activator inhibitor-1
PDGF-B	=	Platelet-derived growth factor-B
PEDF	=	Pigment epithelium-derived factor
PKC	=	Protein kinase C
RAGE	=	Receptor for AGEs
RAS	=	Renin-angiotensin system
ROS	=	Reactive oxygen species
sRAGE	=	Soluble RAGE
TGF-	=	Transforming growth factor-
UAER	=	Urinary albumin excretion rate
UKPDS	=	United Kingdom Prospective Diabetes Study
Study VEGF	=	Vascular endothelial growth factor

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