The Role of Advanced Glycation End Products in Progression and Complications of Diabetes

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Context: Diabetic complications appear to be multifactorial in origin, but in particular, the biochemical process of advanced glycation, which is accelerated in diabetes as a result of chronic hyperglycemia and increased oxidative stress, has been postulated to play a central role in these disorders. Advanced glycation involves the generation of a heterogenous group of chemical moieties known as advanced glycated end products (AGEs), this reaction occurring as a result of a nonenzymatic reaction with glucose interacting with proteins, lipids, and nucleic acids, and involves key intermediates such as methylglyoxal.

Evidence Synthesis: In this review we report on how these AGEs may exert deleterious effects in diabetes, as well as address current strategies to interrupt the formation or action of AGEs. First, AGEs act directly to induce cross-linking of long-lived proteins such as collagen to promote vascular stiffness, and, thus, alter vascular structure and function. Second, AGEs can interact with certain receptors, such as the receptor for AGE, to induce intracellular signaling that leads to enhanced oxidative stress and elaboration of key proinflammatory and prosclerotic cytokines. Over the last decade, a large number of preclinical studies have been performed, targeting the formation and degradation of AGEs, as well as the interaction of these AGEs with receptors such as the receptor for AGE.

Conclusion: It is hoped that over the next few years, some of these promising therapies will be fully evaluated in the clinical context with the ultimate aim to reduce the major economical and medical burden of diabetes, its vascular complications. **(*J Clin Endocrinol Metab* 93: 1143–1152, 2008)**

Diabetic complications are considered to be multifactorial in origin with increasing evidence that one of the major pathways involved in the development and progression of both microvascular and macrovascular disease as a result of chronic hyperglycemia is the biochemical process of advanced glycation. In this review the importance of this biochemical pathway is reassessed, as well as the increasing body of evidence that targeting accumulation of advanced glycated end products (AGEs) and/or the receptors that mediate their biological actions could potentially confer benefits on diabetes-related end-organ injury.

Biochemistry

AGEs are a complex group of compounds formed via a nonenzymatic reaction between reducing sugars and amine residues on proteins, lipids, or nucleic acids. The major AGEs *in vivo* appear to be formed from highly reactive intermediate carbonyl groups, known as α-dicarbonyls or oxoaldehydes, including 3-deoxyglucosone, glyoxal, and methylglyoxal (1, 2). Some of the best chemically characterized AGEs in humans include pentosidine and N(carboxymethyl)lysine (CML). Some AGEs like pentosidine have intrinsic fluorescence, and as such, tissue and plasma fluorescence can be used as markers of AGE accumulation.

0021-972X/08/$15.00/0

Printed in U.S.A.

doi: 10.1210/jc.2007-1817 Received August 15, 2007. Accepted January 2, 2008.

First Published Online January 8, 2008

Abbreviations: ACE, Angiotensin-converting enzyme; AGE, advanced glycated end product; AGE-R, AGE receptor; apo, apolipoprotein; CML, N(carboxymethyl)lysine; ECM, extracellular matrix; ERM, ezrin, radixin, and moesin; HbA_{1c}, glycosylated hemoglobin; LDL, low-density lipoprotein; LR-90, 4,4′-(-2 chlorophenylureido phenoxyisobutyric acid; NF-κB, nuclear factor-κB; OPB-9195, ($\pm$)-2-isopropylidenehydrazono-4-oxo-thiazolidin-5-ylacetanidilide; PAD, peripheral arterial disease; PARP, poly(ADP ribose) polymerase; PTB, N-phenacylthiazolium bromide; RAGE, receptor for AGE; sRAGE, soluble RAGE; VCAM, vascular cell adhesion molecule; VEGF, vascular endothelial growth factor.

Other AGEs such as CML are nonfluorescent and may be detected by approaches such as ELISA.

Apart from endogenously formed products, AGEs can also originate from exogenous sources such as tobacco smoke and diet (3–6). Food processing, especially prolonged heating, has an accelerating effect in the generation of glyco-oxidation and lipo-oxidation products, and a significant proportion of ingested AGEs is absorbed with food. Tissue and circulating AGE levels are higher in smokers and in patients on high AGE diets, with concurrent increases in inflammatory markers (3–5). Furthermore, there is evidence from animal studies that exposure to high levels of exogenous AGEs contributes to renal and vascular complications (6, 7). Nevertheless, it remains to be determined as to the relative importance of these exogenous sources of AGEs in the pathogenesis of diabetic complications.

AGEs often accumulate intracellularly (8) as a result of their generation from glucose-derived dicarbonyl precursors (1). It is likely that these intracellular AGEs play important roles as stimuli for activating intracellular signaling pathways as well as modifying the function of intracellular proteins (1, 9).

In diabetes, AGE accumulation may result from chronic hyperglycemia promoting the generation of AGEs, and also with concomitant impaired renal function because the kidney is the major site of AGE clearance. AGE modified proteins may be more resistant to enzymatic degradation (9), and it is likely that this further promotes local tissue AGE accumulation.

Effect of AGEs

The effects of AGEs (Fig. 1) may be classified as receptor-independent or -dependent, and can act intracellularly or circulate and act on cell surface receptors such as the receptor for AGEs (RAGEs). Advanced glycation occurs over a prolonged period, affecting long-lived proteins. The structural components of the connective tissue matrix and, in particular, basement membrane components such as type IV collagen are prime targets, but other long-lived proteins can also undergo advanced glycation, including myelin, tubulin, plasminogen activator 1, and fibrinogen (10).

Extracellular matrix (ECM) proteins are susceptible to AGE modification because of their slow turnover rate. The formation of intermolecular and intramolecular cross-links with collagen as a result of the glycation process leads to structural alterations, leading to increased stiffness and resistance to proteolytic digestion. For example, AGE cross-linking on type I collagen and elastin leads to increased stiffness of blood vessels (11, 12). The composition of ECM is also modified by AGE, with increased expression of ECM proteins, including fibronectin, types III, IV, and VI collagen and laminin, possibly mediated through up-regulation of key profibrotic cytokines such as TGF-β (13, 14) and connective tissue growth factor (15).

RAGE and Other Receptors

The receptor-dependent effects of AGE are mediated via interactions with various proteins that have been shown to bind to these chemical moieties. The most extensively studied is RAGE, but other binding proteins include AGE receptors (Rs) 1, 2, and 3 (AGE-R1, AGE-R2, and AGE-R3/galactin-3, respectively), and the ezrin, radixin, and moesin (ERM) family (16).

RAGE is a member of the Ig superfamily of receptors. Through the interaction with RAGE, AGEs trigger the activation of secondary messenger pathways such as protein kinase C. A key target of RAGE signaling is nuclear factor-κB (NF-κB), which is translocated to the nucleus where it increases transcription of a number of proteins, including intercellular adhesion molecule-1,

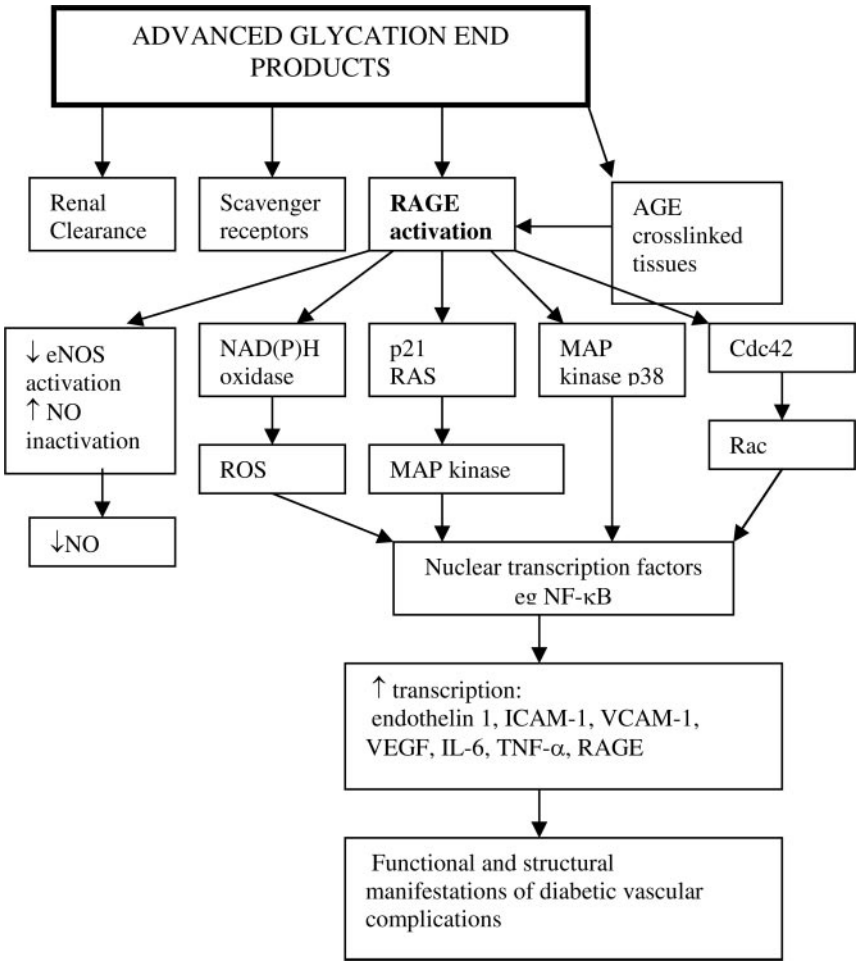

FIG. 1. Effects of AGEs. Cdc42, Cell division cycle 42 protein; eNOS, endothelial nitric oxide synthase; ICAM-1, intercellular adhesion molecule-1; MAP, mitogen-activated protein; NAD(P)H nicotinamide dinucleotide phosphate; NO, nitric oxide; ROS, reactive oxygen species. [Adapted from A. Goldin, J. A. Beckman, A. M. Schmidt, and M. A. Creager: *Circulation* 114:597–605, 2006 (17).

E-selectin, endothelin-1, tissue factor, vascular endothelial growth factor (VEGF), and proinflammatory cytokines (17–19).

The promoter region of RAGE contains functional binding elements for NF-κB (20), and one consequence of NF-κB translocation is the up-regulation of RAGE itself.

Although originally RAGE was discovered in the context of a strategy to identify binding sites to AGEs (21, 22), it is now considered that RAGE may indeed be an accidental receptor to AGE ligands. Various AGEs have been reported to bind to RAGE, including CML and hydroimidazolones.

Furthermore, RAGE has been shown to bind to a range of other peptides and proteins that are not AGEs, including certain S100 isoforms (23) and amphoterin (also known as high mobility group box 1) (24), ligands that are also implicated in progressive vascular injury.

In endothelial cells the AGE-RAGE interaction can lead to disturbances in cellular function through additional pathways involving nicotinamide dinucleotide phosphate oxidase and MAPKs (25).

Endogenous soluble RAGE (sRAGE) is a splice variant of the full-length receptor and has been detected in plasma. sRAGE lacks the COOH terminal and transmembrane domains, and can bind extracellular ligands. Excess sRAGE may competitively bind RAGE ligands, preventing their interaction with the cell-surface RAGE receptor and, thus, hindering cellular signaling. Indeed, a positive correlation of plasma sRAGE concentrations with urinary albumin excretion has been demonstrated in type 2 diabetic patients (26), suggesting that sRAGE levels may represent an early marker of microvascular dysfunction and possibly nephropathy in type 2 diabetes. The potential therapeutic role of sRAGE has been observed in both experimental models of diabetic nephropathy (27) and atherosclerosis (28).

AGE-R1 facilitates clearance of AGE, and appears to be involved in AGE-specific ligand binding and degradation. Interruption of AGE-R1 dependent uptake of AGEs and subsequent degradation is associated with accelerated glomerular renal pathology in the spontaneously nonobese diabetic strain of mice (29). Furthermore, diminished expression of AGE-R1 in circulating mononuclear cells and a corresponding elevation in serum AGE levels are seen human subjects with severe diabetic complications (30).

AGE-R3 (galectin-3) belongs to the lectin family of carbohydrate-binding proteins. AGE-R3 is up-regulated in hyperglycemia and after exposure to AGEs. Galectin-3 knockout mice have developed accelerated glomerulopathy in response to diabetes, with increased renal glomerular AGE accumulation and diminished scavenger receptor expression (31, 32).

The ERM family of proteins have been reported to bind to AGEs with similar affinity to other receptors such as RAGE (16), with the binding site located at the N-terminal end of the ERM. *In vitro* studies have identified that AGEs can modulate renal tubular growth and migration via an ezrin-dependent pathway (33). The relevance of these findings to diabetic nephropathy remains to be clarified.

Complications of Diabetes

AGEs accumulate within the various organs that are damaged in diabetes, with the accumulation rate of these AGEs accelerated by hyperglycemia. The intermolecular collagen cross-linking caused by AGEs leads to diminished arterial and myocardial compliance and increased vascular stiffness, phenomena that are considered to explain partly the increase in diastolic dysfunction and systolic hypertension seen in diabetic subjects (34). AGEs accumulate in most sites of diabetes complications, including the kidney, retina, and atherosclerotic plaques (35–37). AGEs have been measured and reported to be linked to the sustained effects of prior glycemic control on the subsequent development of vascular complications.

In the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications trial (38), subjects treated previously with intensive glucose control displayed decreased carotid intima-media thickness, many years after the levels of glycosylated hemoglobin (HbA_{1c}) between the intensively and conventionally treated groups became indistinguishable (39, 40). It has been postulated that these effects have occurred as a result of "hyperglycemic memory" and may involve AGEs.

Diabetic nephropathy

The kidney is a target for AGE-mediated damage and is also a contributor to circulating AGE concentrations as seen in settings such as diabetes because the kidney is the major site of clearance of AGEs (41).

Animal studies have clearly demonstrated a pathogenic role for AGEs and RAGE in diabetic nephropathy. Diabetic animals have significant increases in renal AGEs (42), and these abnormalities have been linked to various structural aspects of diabetic nephropathy, including glomerular basement membrane thickening, mesangial expansion, glomerulosclerosis, and tubulointerstitial fibrosis (43). Administration of AGE albumin in murine models has resulted in changes similar to that observed in diabetic nephropathy, including glomerular basement membrane thickening, mesangial matrix expansion, and increased collagen IV and TGF-β expression (44). The strongest evidence of a role for AGEs in the development of diabetic nephropathy has come from studies targeting the AGE-RAGE pathway. Specifically, renal pathological changes are reduced by AGE formation inhibitors such as aminoguanidine (42), (±)-2-isopropylidenehydrazono-4-oxo-thiazolidin-5-ylacetanidilide (OPB-9195) (45), and ALT-946 (46), as well as with agents that are postulated to reduce AGE accumulation such as the putative cross-link breaker ALT-711, also known as alagebrium (47). Treatments targeting RAGE such as sRAGE, which acts as an endogenous antagonist to the full-length receptor (27) and a RAGE-specific neutralizing antibody (48), have also been shown to attenuate nephropathy. Furthermore, RAGE knockout mice rendered diabetic have less renal functional and structural injury (27).

Diabetic ocular disease

CML and other AGEs have been localized to retinal blood vessels in patients with type 2 diabetes and found to correlate

with the degree of retinopathy (49, 50). When nondiabetic animals are infused with preformed AGE albumin, the adducts accumulate around and within the pericytes, colocalize with AGE-Rs, induce basement membrane thickening, and contribute to the breakdown of the inner blood-retinal barrier (51, 52). Furthermore, retinal vascular endothelial cells exposed to AGEs show abnormal endothelial nitric oxide synthase expression (53), which may account for some of the vasoregulatory abnormalities seen in the retinal microcirculation in diabetes. *In vitro* studies have also demonstrated the up-regulation of VEGF in retinal cells after exposure to AGEs (54), potentially promoting retinal neovascularization and increasing permeability to proteins across the retinal barrier.

Diabetic peripheral neuropathy

Elevated levels of AGEs have been documented in the peripheral nerves of subjects with diabetes. The AGE-RAGE axis is a likely mechanism linking microangiopathy and neuropathy, and is supported by the colocalization of CML, RAGE, NF-κB, and IL-6 to epineurial vessels, perineurium and endoneurial vessels. It has been demonstrated in murine models (55) that AGEs worsen diabetic neuropathy by reducing sensorimotor conduction velocity and decreasing blood flow to peripheral nerves.

Administration of AGEs and subsequent RAGE activation replicates the effects of hyperglycemia, whereas RAGE antibody administration suppresses both NF-κB activation and expression of IL-6 transcripts in sciatic nerve studies (56, 57). A series of experiments on RAGE-null mice have demonstrated that CML and AGE effects are not seen in the dorsal root ganglia of such animals, and neither is diabetes-induced NF-κB activation. Interestingly, despite inactivation of RAGE (through deletion of RAGE gene or blockade with sRAGE or receptor antibodies), the activation of NF-κB and expression of IL-6 mRNA still occurred at a low level, and nociception was not fully maintained (58). The RAGE-null status also did not protect from the diabetes-induced loss of PGP9.5-positive small fibers. This confirms that AGE-RAGE has a substantial role in mechanisms leading to neuropathy, especially sensory deficits, but is unlikely to be the sole factor responsible for progressive neurological damage in diabetes.

Atherosclerotic disease

AGEs are likely to be linked to atherosclerosis in multiple ways, including enhancing endothelial dysfunction, elevating vascular low-density lipoprotein (LDL) levels by reducing LDL uptake, promoting plaque destabilization via effects on matrix metalloproteinases, inducing neointimal proliferation, and inhibiting vascular repair in response to injury. Serum levels of AGEs have increased in patients with type 2 diabetes and coronary heart disease (59). Furthermore, AGEs have been localized to atherosclerotic lesions, fatty streaks, lipid-containing smooth muscle cells, and macrophages in individuals with diabetes (60–62). A correlation between tissue AGE concentration and the severity of atherosclerotic lesions has also been demonstrated (62). There appear to be multiple potential mechanisms whereby AGEs may enhance atherosclerosis. AGEs quench nitric oxide and impair LDL removal by trapping LDL in the subendothelium and decreasing LDL receptor recognition of AGE-modified LDL (63). AGE binding to apolipoprotein (apo) B impairs its hepatic clearance, and induces increased retention of LDL in the aortic wall and increased recognition by macrophages at this site. Consequently, there is increased localization of AGE-LDL in vessels and increased production of foam cells, accelerating atheroma formation (64).

Endothelial migration of monocytes, one of the first steps in atherogenesis, is dependent upon up-regulation of vascular cell adhesion molecule (VCAM)-1 expression, and AGEs have increased VCAM-1 expression via activation of the key nuclear transcription factor NF-κB (65). Forbes *et al.* (66) have shown that attenuation of the plaque area can be achieved in a murine model of diabetes-associated atherosclerosis with the AGE inhibitor aminoguanidine as well as with the putative AGE cross-link breaker alagebrium. These two disparate pharmacological interventions resulted in reduced accumulation of AGEs within the aortas, reduced expression of RAGE, and diminished expression of prosclerotic growth factors and various collagens. Animal studies using sRAGE, either as a preventative strategy (28) or delayed intervention (67), resulted in suppressed vascular lesion formation.

Diabetic cardiomyopathy and peripheral arterial disease (PAD)

Patients with diabetes are more prone to develop cardiomyopathy and heart failure than nondiabetic subjects (68). Furthermore, cross-linking of collagen is considered to play a role in the development of diabetic cardiomyopathy and PAD. A positive correlation between serum levels of AGEs and the isovolumetric relaxation time, a parameter measured on echocardiography reflecting cardiac function, has been documented in patients with type 1 diabetes (69). High serum levels of the fluorescent AGE pentosidine are also associated with both increased carotid intima media wall thickness and arterial stiffening (70, 71). Increased levels of pentosidine and malondialdehyde (an indicator of lipid peroxidation) have also been observed in diabetic patients with PAD (70).

Measurement of AGEs

Clinical studies have demonstrated that the level of circulating AGEs may be linked to various diabetes complications. However, until recently the sophisticated and expensive laboratory techniques required such as mass spectrometry, gas and/or liquid chromatography, for measurement of specific AGEs have retarded any attempts at widespread use of such measurements in the clinic. Furthermore, there is no universally accepted method to detect or measure AGEs, with no internal standards or an internationally recognized standard unit of measurement, thus making comparison of results between different laboratories very difficult. Blood is more accessible for repeated measurements of AGEs than tissue-requiring biopsies, but plasma AGE assays have not yet been shown to be directly related to tissue AGE content (72). In addition, it remains unclear which circulating AGEs should be measured, and in particular, which chem-

ical moiety is most relevant biologically. A noninvasive tool has been developed using skin autofluorescence, which has been correlated with tissue levels of pentosidine, CML, measures of long-term glycemic control, and the presence of diabetic complications (73). Recent publications have suggested that skin autofluorescence serves as a marker of vascular damage (74), as well as a predictor of cardiac mortality in patients with type 1 and 2 diabetes (75). However, long-term studies validating both the specificity and sensitivity of this investigation, and its link to certain AGEs, remain to be confirmed. Thus, currently there is no evidence that the measurement of AGEs has any clinical use, and importantly, AGE measurements should not be considered a replacement for HbA_{1c} as a marker of overall glycemic control.

Therapeutic Options and Interventions

In this review we describe a number of therapeutic strategies, some of which are currently under experimental and/or clinical evaluation (Table 1). It is important to appreciate that although AGE inhibition strategies have often focused on diabetes-related disorders because advanced glycation occurs normally and may also be implicated in aging disorders, it is possible and, indeed, the focus of some clinical investigators to explore these therapies in the nondiabetic context. Because the AGE pathway appears to be implicated in a range of disorders and has been demonstrated, predominantly *in vitro*, to influence a variety of signaling pathways, a major aim of ongoing clinical research programs continues to be the monitoring of potential and unexpected side effects. Furthermore, without detailed safety data or evidence of clinical efficacy in subjects with early or advanced nephropathy, trials have focused on subjects with renal disease (76) rather than considering such therapeutic strategies as primary prevention.

Agents that reduce/inhibit AGE formation

Aminoguanidine [also known as Pimagedine (Alteon, Ramsey, NJ)] was one of the first inhibitors of AGE formation studied (77), and is thought to act as a nucleophilic trap for carbonyl intermediates. Aminoguanidine has prevented a range of diabetic vascular complications in animal studies (78–81), and clinical trials have shown a reduction in AGE-hemoglobin independent of HbA_{1c} lowering (36).

Placebo-controlled clinical trials have been conducted with aminoguanidine in types 1 and 2 diabetes examining renal outcomes (76, 82). Reductions in proteinuria and a decrease in progression of retinopathy were observed, although the study did not demonstrate a statistically significant beneficial effect on the progression of overt nephropathy. Further clinical evaluation of this agent has been limited as a result of concerns over long-term toxicity, with some patients developing myeloperoxidase and

TABLE 1. Interventions targeting the AGE pathway

Action	Compound/therapeutic agent (references)	Effect in animal studies	Human/clinical trials (phase)	Safety concerns/adverse effects
Inhibition of AGE formation	Aminoguanidine (76–79)	↓ Neuropathy ↓ Nephropathy ↓ Retinopathy	↓ Nephropathy ↓ Retinopathy (III)	Glomerulonephritis ↓ Vit B6 ↓ iNOS
	ALT-946 (81, 82)	↓ Nephropathy ↓ Renal AGE		
	Pyridoxamine (83–85)	↓ Nephropathy ↓ Retinopathy ↓ Cholesterol ↓ Weight		
	OPB-9195 (86–88)	↓ Nephropathy ↓ Stenosis after vascular injury ↓ BP		↓ Vit B6
	LR-90 (89, 90)	↓ Nephropathy ↓ Oxidative stress ↓ ECM fibrosis		Weight gain
Putative cross-link breakers	PTB (91, 92)	↓ AGE		
	Alagebrium (ALT-711) (40, 64, 93–95)	↓ Nephropathy	↓ Arterial stiffness	
		↓ BP	↓ Pulse pressure ↑ Diastolic heart function (III)	
RAGE blockade	Anti-RAGE $F(ab')_2$ and sRAGE (65, 96)	↓ Nephropathy ↓ Atherogenesis ↓ Neuropathy		
AGE clearance	Lysozyme (97)	↓ Nephropathy ↓ AGE ↓ Atherosclerosis		
Reducing exogenous exposure	Low-AGE diet (6, 109)		↓ AGE ↓ CRP	

BP, Blood pressure; CRP, C-reactive protein; iNOS, inducible nitric oxide synthase; Vit B6, vitamin B6.

antineutrophil antibodies (83), and indeed in a small number of subjects, glomerulonephritis (76).

Pyridoxamine is a derivative of vitamin B6. It prevents the degradation of protein-Amadori intermediates to protein-AGE products. In murine models it has reduced hyperlipidemia and prevented AGE formation (84, 85). Thomas *et al.* (86) have shown that pyridoxamine antagonizes angiotensin II-induced elevation in serum and renal AGEs, prevents renal hypertrophy, and decreases salt retention in experimental models. Pyridoxamine also prevents diabetes-induced retinal vascular lesions. Some preliminary clinical trials with this agent have been performed (87).

OPB-9195 is a thiazolidine derivative. This compound has not been tested in humans, nor is there any evidence for planned clinical trials. OPB-9195 has been shown to prevent the progression of glomerular sclerosis and reduced urinary albumin excretion (88) in association with decreases in renal TGF-β and VEGF expression (89).

Methylene bis 4,4′-(-2 chlorophenylureido phenoxyisobutyric acid) (LR-90) has been investigated in a number of animal studies by Figarola *et al.* (90). LR-90 inhibited albuminuria and reduced serum creatinine concentration and circulating AGE levels in diabetic rats without any effect on glycemic control. LR-90 prevented glomerulosclerosis and collagen deposition in association with reduced glomerular AGE accumulation. The effect of LR-90 is also currently being tested on macrovascular complications in a range of animal studies. Interestingly, LR-90 has recently inhibited S100b-induced expression of RAGE and other proinflammatory genes in human monocytes (91), suggesting that this agent has novel antiinflammatory properties with the potential for additional protective effects against diabetic vascular complications. There are plans, not yet fully disclosed, for this agent to be evaluated further in the clinical context.

Putative cross-link breakers

The actions of the prototype putative cross-link breaker N-phenacylthiazolium bromide (PTB) were first reported over 10 yr ago. In *in vitro* experiments, PTB was shown to cleave cross-links. Subsequently, it was shown in diabetic rat models to decrease tissue AGE accumulation (92) but was not shown to decrease proteinuria (93).

Subsequently, a more stable derivative of PTB was generated, known as ALT-711 or alagebrium (3-phenyacyl-4,5-dimethylthiazolium chloride). In a series of studies in various models of diabetic complications, alagebrium was shown to confer end-organ benefits. With respect to diabetic renal disease, alagebrium was shown to attenuate the development of albuminuria in diabetic rats in association with modest effects on blood pressure and a reduction in renal TGF-β and collagen deposition. This renoprotective effect of alagebrium has been reproduced by several groups (46, 94).

In further studies, diabetic rats treated for 4 months with alagebrium showed reduced cardiac collagen III expression, increased collagen solubility, and reduced RAGE and AGE-R3 gene expression in association with reduced relative left ventricular weight and decreased cardiac B-natriuretic peptide mRNA levels, an indirect marker of cardiac function (95). In another study in diabetic apoE−/− mice, alagebrium attenuated atherosclerosis by over 30% (66), an effect similar to that seen with the inhibitor of AGE formation aminoguanidine. Although it was initially postulated that alagebrium was acting *in vivo* to cleave preformed AGE cross-links, there is an increasing body of data to suggest alternative and/or additional modes of action for this drug (96).

A few clinical trials have been conducted with this cross-link breaker, although these studies have not been restricted to subjects with diabetes. The Patients with Impaired Ejection Fraction and Diastolic Dysfunction: Efficacy and Safety of Alagebrium trial showed a beneficial effect in ejection fraction in heart failure (http://www.alteon.com/). Another randomized placebo-controlled trial in hypertensive subjects showed a reduction in pulse pressure and arterial stiffness (97). It is anticipated that in the near future, clinical trials focusing on the potential renoprotective effects of this agent will commence.

Another therapeutic approach that is being considered involves sRAGE or sRAGE that blocks AGEs from binding to RAGE. It remains as yet to be elucidated fully as to whether sRAGE acts as an antagonist inhibiting RAGE dependent signaling pathways, or acts to bind to various RAGE ligands such as AGEs, thus preventing these putative proinflammatory molecules from acting on other receptors such as scavenger receptors to promote end-organ injury (98). Studies with RAGE knockout mice that do not express sRAGE or full-length RAGE suggest that the key mechanism of action of sRAGE is via inhibition of RAGE dependent phenomena. It is anticipated that in the next few years, either sRAGE or possibly a nonpeptide RAGE antagonist will be examined clinically, although it is possible that nondiabetic diseases may be the focus of the RAGE clinical development program.

Other agents and interventions

Poly(ADP ribose) polymerase (PARP) inhibits glyceraldehyde-3-phosphate dehydrogenase, resulting in increased AGE formation. PARP inhibitors have been used in animal studies, resulting in improved neuropathy (99), endothelial and diastolic function (100, 101).

Benfotiamine, a lipid-soluble thiamine (vitamin B1) derivative, prevents activation of three major pathways of hyperglycemic damage (hexosamine pathway, intracellular AGE formation, and the diacylglycerol-protein kinase C pathway) by increasing the activity of transketolase, the rate-limiting enzyme of the nonoxidative branch of the pentose phosphate pathway (102). In animal studies, high-dose thiamine and benfotiamine therapy increased transketolase expression in renal glomeruli, and inhibited the development of microalbuminuria and diabetes-induced hyperfiltration (103). Benfotiamine has improved nerve conduction velocity in the peroneal nerve (104) in diabetic patients, and a short 3-wk clinical study showed alleviation of painful neuropathy (105), but long-term human data are still lacking. Recent studies also show that benfotiamine prevents macrovascular and microvascular endothelial dysfunction and oxidative stress after a high-AGE meal (106).

ACE inhibitors as well as angiotensin II antagonists appear to decrease the formation of AGEs, as assessed in a series of *in vitro*

studies as well as in animal models of diabetes (98, 107, 108). Furthermore, ACE inhibitors, based on *in vitro*, preclinical, and clinical studies, appear to promote sRAGE expression, thus providing an additional mechanism to inhibit AGE induced organ injury (98).

A low-AGE diet administered for 6 wk in a clinical trial resulted in lower serum AGE levels and inflammatory markers such as C-reactive protein (6). Furthermore, in an apoE knockout mouse model, a low-AGE diet reduced neointimal formation after arterial injury and inhibited atheroma formation as assessed by plaque accumulation at the aortic root (109).

Inhibition of absorption of dietary AGEs may be a novel approach to reduce the deleterious effects of AGEs. AST-120 is an oral adsorbent that attenuates the progression of chronic renal failure by removing uremic toxins. It also binds to CML and has decreased serum levels of AGEs in nondiabetic subjects with chronic renal failure (110).

Oral hypoglycemic agents like metformin and pioglitazone decrease the formation of AGEs by minimizing hyperglycemia, but have also been shown *in vitro* to prevent formation of AGEs and AGE cross-linking (111) independent of their effects on glucose *per se*.

Conclusions

The biochemical process of advanced glycation appears to be enhanced in the diabetic milieu as a result of not only hyperglycemia but also other stimuli such as oxidative stress and lipids. A heterogenous group of chemical moieties is generated that appears to induce directly and indirectly, via activation of key intracellular signaling pathways and generation of proinflammatory and prosclerotic cytokines, various pathological processes ultimately enhancing the development and progression of diabetic vascular complications. A range of pharmacological strategies, predominately being examined in preclinical contexts, appears to show great promise in reducing AGE induced injury by interfering with either the accumulation of AGE ligands or interrupting the AGE-RAGE interaction. It is anticipated that over the next few years, findings from clinical studies will assist clinicians in determining the relevance of targeting advanced glycation as an approach to reducing diabetic complications.

Acknowledgments

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Disclosure Information: None applicable.

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