Food Sci. Biotechnol. Vol. 17, No. 6, pp. 1131 ~ 1138 (2008)

RESEARCH REVIEW

Food Science and Biotechnology

© The Korean Society of Food Science and Technology

Advanced Glycation Endproduct-induced Diabetic Complications

Hyun-Sun Lee[1], Chung-Oui Hong, and Kwang-Won Lee*

Division of Food Bioscience and Technology, College of Life Science and Biotechnology, Korea University, Seoul 136-701, Korea
[1]Institute of Life Science and Natural Resource, Korea University, Seoul 136-701, Korea

Abstract Diabetic complications are a leading cause of blindness, renal failure, and nerve damage. Additionally, diabetes-accelerated atherosclerosis leads to increased risk of myocardial infarction, stroke, and limb amputation. At the present time, 4 main molecular mechanisms have been implicated in hyperglyceamia-mediated vascular damage. In particular, advanced glycation endproducts (AGE), which are formed by complex, heterogeneous, sugar-derived protein modifications, have been implicated as a major pathogenic process for diabetic complications. Recently, AGE inhibitors such as aminoguanidin, ALT-946, and pyridoxamine have been reported. Such an integrating paradigm provides a new conceptual framework for future research on diabetes complications and on discovering drugs to prevent the progression of AGE-induced maladies.

Keywords: advanced glycation endproduct, diabetes mellitus, diabetic complication, inhibitor, reactive oxygen species (ROS)

Introduction

Diabetes mellitus (DM) defines the condition that occurs when the body can no longer utilize glucose normally. Three-thousand years ago, the ancient Egyptians described clinical features that were similar to DM. Today, DM afflicts nearly 194 million people worldwide, and this figure is expected to increase to almost 333 million by the year 2025; a new 800,000 cases are diagnosed every year in the USA (1). DM consists of absent or markedly diminished insulin secretion and/or ineffective insulin action, and is a syndrome with interrelated metabolic, vascular, and neuropathic components (2). In humans, diabetic complications occur within both types of diabetes (type 1 and type 2), and have an important role in the increased morbidity and mortality suffered by such individuals (3).

The nonenzymatic browning, or glycation, reaction was originally investigated by the French biochemist Louis Camille Maillard in 1912, where he observed yellow-brown colors when reducing-sugars were heated with amino acids (4). This reaction is widely used in the food industry to control food texture; however, it is now acknowledged as also being involved in the pathogenesis of various diseases, particularly DM and neurodegenerative diseases such as Alzheimer's disease (5,6). The glycation reaction involves a series of nonenzymatic reactions between the carbonyl groups on reducing sugars and the amino groups on proteins, nucleic acids, or phospholipids; a Schiff base is formed followed by Amadori rearrangement and further nonoxidative and/or oxidative modification, which leads to advanced glycation endproduct formation (7). Hemoglobin A1c (HbAlc) is the direct combination of glucose and adult hemoglobin (HbA), and was first characterized as a glycoprotein by Bookchin and Gallop in 1968 (8). In the 1990s, the American Diabetes Association began making HbA1c-based treatment recommendations, following the publication of data from the Diabetes Control and Complications Trial (DCCT). Within both research and clinical settings, HbA1c has become the gold standard for therapeutic management of DM (8). The edible plants such as the leaves of *Stelechocarpus cauliflorus* (9) and puerariafuran from the roots of *Pueraria lobata* (10) for reducing advanced glycation endproducts (AGEs) had reported. These basic discoveries of potential inhibitors of AGEs can offer a therapeutic approach for the prevention of diabetic or other pathogenic complications.

The following article reviews some of the important roles AGEs have in the initiation and progression of eyesight-threatening disorders such as diabetic retinopathy, glaucoma, cataract formation, and age-related macular degeneration (AMD). It also considers pharmacological strategies for preventing or neutralizing the effects of AGEs, as well as recent developments in potential therapies for AGE-induced diseases.

Mechanisms of Diabetic Complications

In both animal models and patients with diabetes, effective glycaemic control reduces the incidence of diabetic complications; thus chronic hyperglycaemia contributes to the pathogenesis of long-term complications (11,12). It is not clearly understood how hyperglycaemia contributes to tissue damage and functioning, but progress to date suggests that diabetic complications are linked to the glucose-induced derangement of several biochemical pathways. As shown in Fig. 1, the 4 main hypotheses are as follows: 1) increased polyol pathway flux, 2) activation of protein kinase C (PKC) isoforms, 3) increased hexosamine pathway flux, and 4) increased AGE formation (13).

A. Polyol pathway

Aldose reductase is the first and the rate-limiting enzyme of the polyol pathway, which converts monosaccharides

*Corresponding author:
Tel: +82-2-3290-3027; Fax: +82-2-925-1970
E-mail: kwangwon@korea.ac.kr
Received September 28, 2007; Revised April 22, 2008;
Accepted April 22, 2008

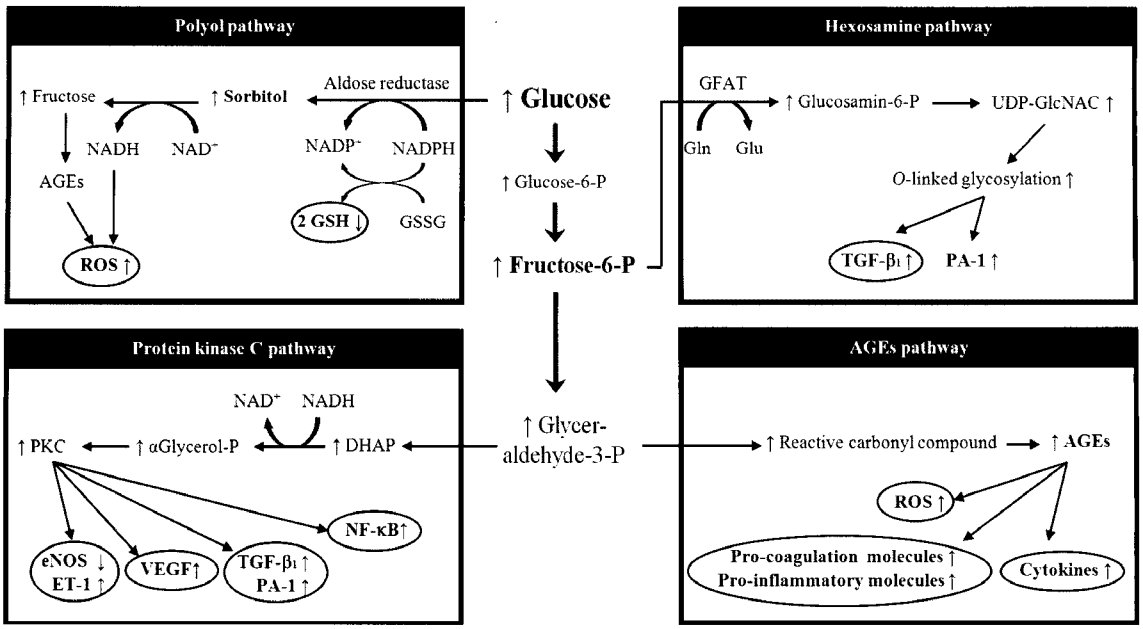

Fig. 1. Potential mechanism by which hyperglycaemia-induced mitochondrial superoxide overproduction activates 4 pathways of hyperglycaemic damage.

(e.g., glucose) to polyols or sugar alcohols (e.g., sorbitol). This enzyme is widely distributed throughout the body, including within the tissues that are susceptible to chronic diabetes complications (e.g., retina, lens, cornea, glomeruli, Schwann cells of the peripheral nerves, and endothelium) (14). Aldose reductase has a low affinity for glucose, and at physiological glucose concentrations it is preferentially channeled into the glycolytic or pentose-phosphate pathway under the action of hexokinase, which has a much higher affinity for glucose. Under hyperglycaemic conditions, and in tissues where insulin does not regulate glucose entry, intracellular glucose levels rise and glucose is increasingly diverted through the polyol pathway for conversion into sorbitol, with nicotinamide adenine dinucleotide phosphate hydrogenase (NADPH) concomitantly reduced to $NADP^+$; the converted sorbitol is then oxidized to fructose with NAD^+ reduced to NADH (13,15). The NADPH that is consumed by the reduction of glucose to sorbitol is required for the regeneration of reduced glutathione (GSH) (13). Increased levels of NADH inhibit the activity of glyceraldehydes-3-phosphate dehydrogenase (GAPDH), thereby continuously increasing concentrations of triose phosphate and methylglyoxal, which are precursors of AGEs (16). Increased polyol pathway flux is associated with various other cellular and metabolic abnormalities. These include the depletion of myo-inositol; the reduced activity of Na^+/K^+-ATPase; abnormalities in PKC dependent protein phosphorylation; reductions in intracellular reduced glutathione concentrations; and alterations of the redox state, which may impair the activity of many cellular enzymes (17).

B. PKC activation

In the PKC pathway, intracellular hyperglyaemia increases the synthesis of diacylglycerol (DAG), which is a critical activating cofactor for the classic isoforms of PKC (18,19). When PKC is activated by hyperglycemia, it has a variety of effects on gene expression. For example, vasodilator-producing endothelial nitric oxide (NO) synthase (eNOS) is decreased, while the vasoconstrictor endothelin-1 (ET-1) is increased; transforming growth factor-β1 (TGF-β1) and plasminogen activator inhibitor-1 (PA-1) are also increased (20). Furthermore, the abnormal activation of PKC has been implicated in the following: blood-flow abnormalities due to decreased NO and increased ET-1, increased vascular permeability-induced expression of vascular endothelial growth factor (VEGF), and finally, vascular occlusion by matrix protein accumulation-induced TGF-β1. In addition to affecting PKC activation, the activation of the protein nuclear factor kappa B (NF-κB), which acts as a master switch to turn on inflammation response, is also affected (18).

C. Increased hexosamine pathway

When cellular glucose levels are high, the majority is metabolized via glycolysis - converting first to glucose-6 phosphate and then to fructose-6 phosphate, and on through the rest of the glycolytic pathway (21). Some fructose-6 phosphate, however, is diverted into a signaling pathway where the enzyme GFAT (glutamine: fructose-6 phosphate amidotransferase) converts it to glucosamine-6 phosphate, and subsequently, uridine diphosphate *N*-acetyl glucosamine (UDP-GlcNAC). *N*-Acetyl glucosamine phosporylates and modifies the serine and threonine residues of transcription factors, often resulting in pathologic changes in gene expression (22). For example, increased transcription factor Sp1 modification results in increased transforming growth factor-SP1 and PA-1 expressions, both of which aggravate blood vessels in diabetes (22).

D. Advanced AGEs

Louis-Camille Maillard was the first to describe the process of glycation, also known as the Maillard reaction (23). Glycation is now recognized as the non-enzymatic modification of proteins, nucleotides, and lipids by saccharide derivatives, and is a complex cascade of

Table 1. Consequences of AGE formation and deposition

AGEs in atherosclerosis
- Accumulation in the vascular matrix → narrowing & occlusion
- Vascular endothelial dysfunction (e.g., inactivating NO) → procoagulant state, vasoconstriction, hypertension
- Glycoxidation of LDL → slow degradation of LDL, lipid peroxidation, oxidative stress
- Trapping of plasma proteins → initiation of complement activation, oxidation
- Monocyte chemotaxis/activation → cytokine & growth factor release vascular tissue proliferation
- Increased endothelial cell permeability → vascular leakage
AGEs in renal disease
- Matrix expansion, vascular leakage, basement membrane thickening → glomerular hypertrophy & glomerular sclerosis
- Glomerular sclerosis → albuminurea
- Delayed clearance of AGE-peptide → uremic complications
AGEs in diabetic neuropathy
- Accumulation in vasa nervosum → wall thickening & occlusion, ischemia
- Vascular endothelial dysfunction → occlusion, ischemia
- Glycation of myelin → myelin damage
- Glycation of growth factors [nerve growth factor (NGF), fibroblast growth factor (FGF)] → loss of function
- Accumulation on macrophages → macrophage activity → myelin & vascular degeneration
AGEs in diabetic retinopathy
- Increased endothelial cell permeability → vascular leakage & retinal damage
- Vessel wall thickening and coagulation → occlusion, ischemia
- Induction of autocrine vascular endothelial growth factor (VEGF) synthesis → angiogenesis, neovascularization

reactions yielding a heterogeneous class of compounds collectively termed advanced glycation endproducts, or AGEs. AGEs found within diabetic retinal vessels and renal glomeruli have been implicated in the pathogenesis of diabetes, renal failure, and aging (7). And *in vitro*-prepared AGE protein modifications were shown to be toxic, immunogenic, and capable of triggering cellular injuries (23).

AGEs and Diabetic Complications

Increased tissue and plasma concentrations of AGEs have been detected in various disease states, including DM, chronic renal failure, atherosclerosis, arterial hypertension, and Alzheimer's disease (Table 1) (5,6). Increased AGE levels were also found in patients with primary rheumatoid arthritis and osteoporosis (24,25). Yet details of the pathophysiological implications of AGES are just recently becoming better understood.

As shown in Fig. 2, diabetes may promote inflammation via the formation of AGEs that interact with endothelial receptors. For example, adhesion molecules and chemotactic factors mediate the entry of particular types of leukocytes into the arterial wall (26). The first step in adhesion, the 'rolling' of monocytes along the endothelial surface, is mediated by selectins, which bind to carbohydrate ligands

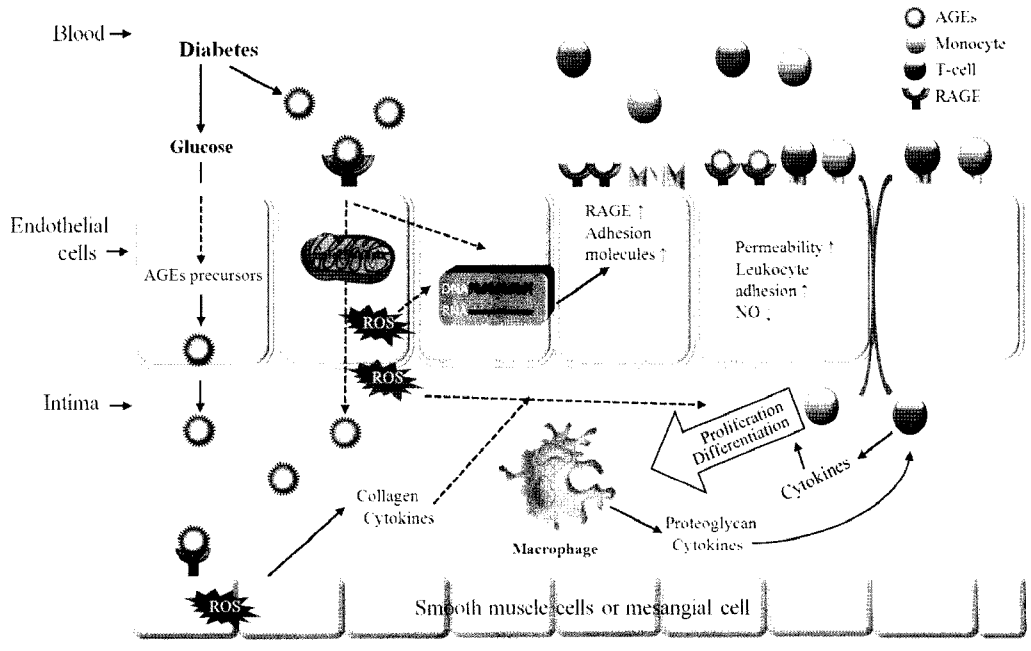

Fig. 2. Mechanisms leading to the inflammation induced by AGEs that interact with endothelial receptors.

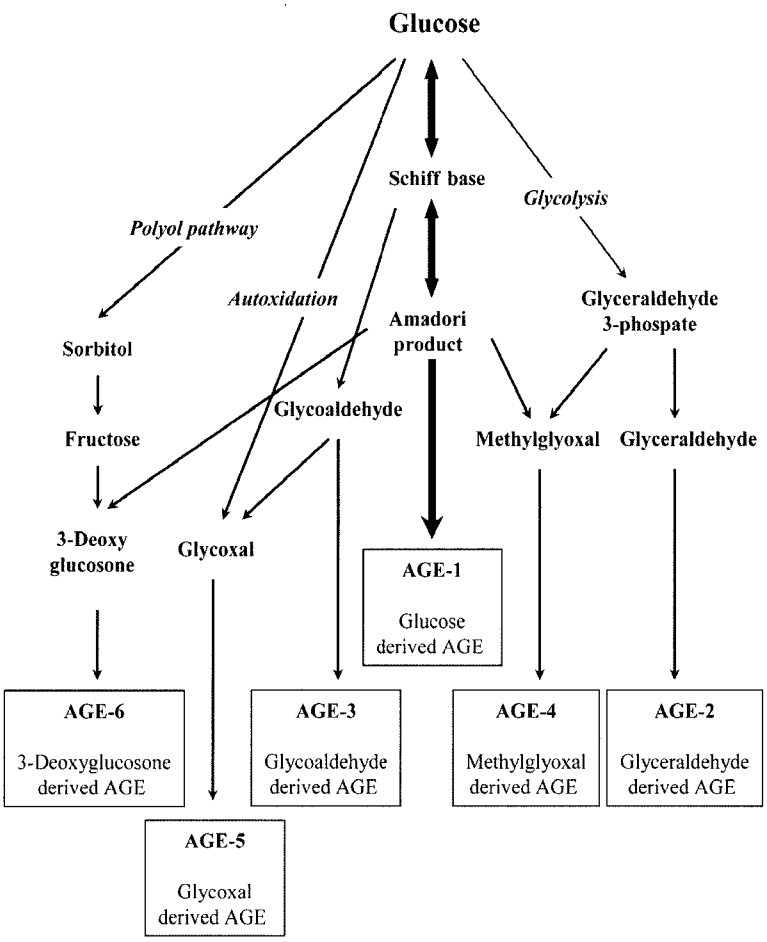

Fig. 3. Alternative routes for *in vivo* AGE formation. Adopted from Sato *et al.* (28).

on leukocytes. Studies of mice deficient in P- and E-selectins, or the intercellular adhesion molecule-1 (ICAM-1), have revealed the roles of these adhesion molecules in atherosclerosis (26). Integrin VLA-4, found on monocytes and T cells, mediates the firm adhesion of these cells to the endothelium; it also interacts with vascular cell adhesion molecule-1 (VCAM-1) on the endothelium and the CS-1 splice variant of fibronectin (26).

Formation of Various AGEs *in vivo* and Related Toxicity

During normal aging, AGE formation and accumulation occur in various tissues; in DM, both occur at accelerated rates. Previous research has shown that AGEs arise *in vivo* not only from glucose, but also from reducing sugars and dicarbonyl compounds (27).

In a recent report, Sato *et al.* (28) described how glyceraldehyde- and glycolaldehyde-derived AGEs (glycer-AGEs and glycol-AGEs), but not glucose-derived AGEs (glu-AGEs), contribute to neuronal cell toxicity in diabetes, suggesting that various endogenous AGEs have different biological activities among tissues and cells. Recent studies have suggested that AGEs arise not only from sugars, but also from carbonyl compounds derived from the autoxidation of sugars as well as from other metabolic pathways (26,29). They also describe the contributions of glucose, α-hydroxyaldehydes (glyceraldehyde and glycolaldehyde), and dicarbonyl compounds [methylglyoxal (MGO), glyoxal (GO), and 3-deoxyglucosone] to protein glycation, along with their developed anti-AGE antibodies that specifically recognize 6 distinct classes of AGE structure: glucose-derived AGEs (AGE-1), glyceraldehyde-derived AGEs (AGE-2), glycolaldehyde-derived AGEs (AGE-3), methylglyoxal-derived AGEs (AGE-4), glyoxal-derived AGEs (AGE-5), and 3-deoxyglucosone-derived AGEs (AGE-6); but not carboxymethl lycine (CML). Furthermore, these studies have shown that AGE-2 and AGE-3, but not AGE-1 or CML, contribute to neuronal cell toxicity in diabetic patients; it was also emphasized that both types have high toxicity (Fig. 3). Takeuchi *et al.* (29) reported that in type 2 diabetic patients, serum non-CML AGE levels were significantly correlated with mean fasting blood glucose levels over the previous 2 months and with HbA1c over the past 1 month; however, in 66 patients, CML-AGE levels were not correlated with clinical parameters. Some researchers have proposed that both CML and non-CML AGEs are present in the blood, and that non-CML AGEs, rather than CML AGEs, should be evaluated more closely when investigating the pathophysiology of AGE-related diseases (28,29).

AGE Formation During Diabetic Conditions

The advanced glycation process is enhanced under hyperglycemia and/or in conditions where the protein and

Table 2. Drugs specifically developed as anti-AGEs

Compound	Researched data	Ref.
AGE inhibitors		
Aminoguanidine (AMG) : guanidine structure	Trapping of reactive dicarbonyl intermediates	50
	Inhibitor of nitric oxide synthase	65
	Disadvantage: rapid renal clearance and toxic	66
OPB-9195 : hydrazine derivative	Inhibits pentosidine generation and traps dicarbonyl intermediates of AGEs	67
	Chelating activity	51
	Disadvantage: induces vitamin B_6 deficiency	68
LR compounds : aromatic compound	LR-90, LR-9, and LR-74	52
	Interact with several reactive dicarbonyl species and chelating activity	52
	Inhibited lipid peroxidation	69
Pyridoxamine : vitamin	Natural form of vitamin B_6	56
	Interferes with post-Amadori oxidative reactions by binding catalytic redox metal ions	60
	Traps reactive low molecular weight carbonyl compounds	59,70
	Phase II trials are ongoing	61
Benfotiamine : vitamin	Lipophilic derivative of vitamin B_1	71
	Shunting of triose glycolytic intermediates towards the reductive pentose pathway	71
	Prevented hexosamine and PKC activation	72
AGE breakers		
PTB	*N*-Phenacyl thiazolinium bromide Cleave AGE-protein cross-links	73
ALT-711	*N*-Phenacyl-4,5-dimethylthiazolium chloride Cleave AGE-protein cross-links and inhibition of AGE formation	62
	Phase II trials are ongoing	67,68
Receptors of AGE (RAGE) blockers		
Soluble RAGE	Effective results in db/db mice	69
Anti-RAGE antibodies	Effective results in type I and type II animal models	70,71

lipid turnover period is prolonged (28-32). For example, AGEs were identified in the mesenteric vessels of STZ-treated diabetic rats within 3 weeks (32), and in their skeletal muscle arteries within 4-6 weeks (33).

It was postulated that AGEs contribute to the development of diabetes-associated vascular diseases (34). AGEs that form on the matrix component of vessel walls can cause structural damage by decreasing elasticity and increasing thickness, rigidity, and narrowing of the vessel lumen. AGEs increase collagen cross-linking, leading to the arterial stiffness that is commonly observed in normal ageing, and which occurs at an accelerated rate in diabetes (35).

By forming cross links and recognizing the receptor for AGE (RAGE) on cellular surfaces, AGEs change the endothelial cell properties that are relevant to vascular disease pathogenesis (36). Much evidence suggests that one consequence of AGE-RAGE interaction is reactive oxygen species (ROS) generation, at least in part via the activation of NADPH (37,38). Studies have indicated that a key target of cellular ROS is the activation of the transcription nuclear factor NF-κB. Indeed, AGE-RAGE interaction activates NF-κB, a critical factor transducing a variety of inflammatory and pro- or anti-apoptotic signals in cells, depending on the time course, site, and chronicity of the stimulus.

Superoxide anions, which can inactivate eNOS (39), are generated by AGE and RAGE interactions on vascular matrix proteins (40). In addition, an *in vitro* study demonstrated that ET-1 could potentiate oxidized low-density lipoprotein (LDL)-induced superoxide anion formation (41). Lastly, AGE–RAGE interactions increase the formation of ROS, which participate in vascular endothelial cell dysfunction (42).

ROS and Diabetes

Under diabetic conditions, free radical levels are altered, and the function of the antioxidant defense system is compromised. There is existing evidence that an acute increase in plasma glucose may enhance free radical production via the autoxidation of glucose (43), and by the advanced glycation process that attenuates antioxidant enzymes activity (44). The advanced glycation process can also intracellularly activate the polyol pathway, which produces an imbalance in the NADH/NAD^+ ratio and favors the production of free radicals (45).

In rats, total superoxide dismutase (SOD) (Cu-Zn-SOD and Mn-SOD) activity increased in the heart, aorta, and blood from the 2^{nd} week after diabetes onset, and continued

up to the 4th week, after which it tended to decline (46).

Glucose exposure causes intracellular sorbitol and fructose levels to increase, leading to a reduction in NADPH cell stores (13); this could inhibit NO synthase and glutathione reductase activities. Decreased levels of NO can lead to vasoconstriction and tissue injury, while a reduction in glutathione increases the susceptibility of endothelial cells to damage by hydrogen peroxide (47).

A previous study reported that ROS increased LDL oxidation, AGE formation, and platelet and monocyte activation (48). Hydroxyl radicals have been implicated in diabetes-induced endothelial dysfunction (43) and are capable of oxidizing lipids, damaging cell membranes, and oxidizing thiol groups (49).

Pharmacological Inhibition of AGEs

A variety of pharmacological compounds and strategies have been studied *in vitro,* as well as *in vivo,* for their potential in preventing the formation and local accumulation of AGEs. Until recently, the mechanisms of action for all AGE inhibitors were poorly understood. In general, they are assumed to function in the trapping of reactive dicarbonyl species (50), in antioxidant activity by transition metal chelation (51,52), and in other activities, including free radical scavenging, as AGE breakers, AGE receptor blocking (53-55), AGE receptor signal blocking, glycemia reduction by anti-diabetic therapy (56), Amadori reaction inhibition, and finally, the shunting of trioses-P towards the pentose-P pathway via transketolase activation (57-59). Practically, inhibitors can be distinguished in drugs specifically developed as AGE inhibitors or AGE breakers, as RAGE and receptor signaling blockers, and as other therapeutic compounds possessing AGE inhibitor activity, including dietary antioxidants. As shown in Table 2, these drugs can be divided into different classes according to their mechanisms of action.

Although animal models confirmed the beneficial effects of aminoguanidin (AMG) against diabetic complications, AMG did not achieve successful clinical trials (50). This may be attributed to its rapid renal clearance, its moderate *in vivo* dicarbonyl scavenging effects at pharmacological concentrations, and its toxicity. In type 1 diabetic patients, pyridoxamine (PM) has shown a favorable safety profile (60), and phase II trials are ongoing to evaluate the efficacy of PM in inhibiting the progression of proteinuria and hyperlipidemia in diabetic patients with early stage kidney disease (61). Phase II clinical trials of ALT-711 were initiated in 1998 (62). In patients with diastolic heart failure, ALT-711 treatment (410 mg daily) resulted in decreased left ventricular mass and improved left ventricular diastolic filling and quality of life (63,64).

Conclusion

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia and alterations in fat and protein metabolism, and by the occurrence of a specific set of long-term microvascular and neurologic complications. Strict glycemic control is the first therapy in preventing the progression of diabetic complications. Yet, despite glycemic control, patients appear to be more or less susceptible to the diabetic complication-induced formation of substances known as AGEs, which accumulate over time. AGEs are found in the vessels of the eyes, kidneys, and extremities. They are also found in other major blood vessels and lead to plaque formation and atherosclerosis, contributing to heart disease. The long-term therapeutic significance of the most recently developed AGE inhibitors or breakers remains to be demonstrated. Despite the encouraging results obtained from *in vitro* and *in vivo* studies, most clinical trials on AGE inhibitors and breakers have been more or less unsatisfactory, in part because of their side effects. But sooner rather than later, the ability to pharmacologically inhibit AGEs might prove to be clinically useful in preventing not only diabetic complications, but also other diseases induced by AGEs.

Acknowledgments

This work was supported by a grant (#106019-03-2-SB010) from the Agricultural R&D Promotion Center (ARPC) under the Ministry for Food, Agriculture, Forestry and Fisheries, Republic of Korea.

References

1. Evans E, Patry R. Management of gestational diabetes mellitus and pharmacists' role in patient education. Am. J. Health-Syst. Ph. 61: 1460-1465 (2004)
2. Argoff CE, Cole BE, Fishbain DA, Irving GA. Diabetic peripheral neuropathic pain: Clinical and quality-of-life issues. Mayo Clin. Proc. 81: 3-11 (2006)
3. Sarti C, Gallagher J. The metabolic syndrome: Prevalence, CHD risk, and treatment. J. Diabetes Complicat. 20: 121-132 (2006)
4. Somoza V. Five years of research on health risks and benefits of Maillard reaction products: An update. Mol. Nutr. Food Res. 49: 663-672 (2005)
5. Bierhaus A, Hofmann MA, Ziegler R, Nawroth PP. AGEs and their interactions with AGE receptors in vascular disease and diabetes mellitus: AGE concept. Cardiovasc. Res. 37: 586-600 (1998)
6. Vlassara H, Stricker LJ, Teichberg S, Fuh H, Li YM, Steffes M. Advanced glycation end-products induce glomerular sclerosis and albuminuria in normal rats. P. Natl. Acad. Sci. USA 91: 11704-11708 (1994)
7. Yamagishi S, Nakamura K, Matsui T. Advanced glycation end products (AGEs) and their receptor (RAGE) system in diabetic retinopathy. Curr. Drug. Discov. Technol. 3: 83-88 (2006)
8. Weissman AJ. Intensive diabetes treatment and cardiovascular disease. New Engl. J. Med. 354: 1751-1752 (2006)
9. Wirasathien L, Pengsuparp T, Suttisri R, Ueda H, Moriyasu M, Kawanishi K. Inhibitors of aldose reductase and advanced glycation end-products formation from the leaves of *Stelechocarpus cauliflorus* R.E. Fr. Phytomedicine 14: 546-550 (2007)
10. Jang DS, Kim JM, Lee YM, Kim YS, Kim JH, Kim JS. Puerariafuran, a new inhibitor of advanced glycation end products (AGEs) isolated from the roots of *Pueraria lobata.* Chem. Pharm. Bull. 54: 1315-1317 (2006)
11. Reichard P, Nilsson BY, Rosenqvist U. The effect of long-term intensified insulin treatment on the development of microvascular complications of diabetes mellitus. New Engl. J. Med. 329: 304-309 (1993)
12. Service FJ, Rizza RA, Daube JR, O'Brian PC, Dyck PJ. Near normoglycaemia improved nerve conduction and vibration in diabetic neuropathy. Diabetologia 28: 722-727 (1985)
13. Brownlee M. Biochemistry and molecular cell biology of diabetic complications. Nature 414: 813-820 (2001)
14. Misawa S, Kuwabara S, Kanai K, Tamura N, Nakata M, Sawai S, Yagui K, Hattori T. Aldose reductase inhibition alters nodal Na^+ currents and nerve conduction in human diabetics. Neurology 66:

1545-1549 (2006)
15. Pacher P, Szabo C. Role of poly (ADP-ribose) polymerase-1 activation in the pathogenesis of diabetic complications: Endothelial dysfunction, as a common underlying theme. Antioxid. Redox. Sign. 7: 1568-1580 (2004)
16. Williamson JR, Chang K, Frangos M, HasanKS, Ido Y, Kawamura T, Nyengaard JR, Enden M, Kilo C, Tilton RG. Hyperglycemic pseudohypoxia and diabetic complications. Diabetes 42: 801-813 (1993)
17. Ii S, Ohta M, Kudo E, Yamaoka T, Tachikawa T. Redox state-dependent and sorbitol accumulation-independent diabetic albuminuria in mice with transgene-derived human aldose reductase and sorbitol dehydrogenase deficiency. Diabetologia 47: 541-544 (2004)
18. Abebe W, MacLeod KM. Protein kinase C-mediated contractile responses of arteries from diabetic rats. Brit. J. Pharmacol. 101: 465-471 (1990)
19. Legan E. Effects of streptozotocin-induced hyperglycemia on agonist-stimulated phosphoinositol turnover in rat aorta. Life Sci. 45: 371-378 (1989)
20. Palm F. Intrarenal oxygen in diabetes and a possible link to diabetic nephropathy. Clin. Exp. Pharmacol. P. 33: 997-1001 (2006)
21. Kolm-Litty V, Sauer U, Nerlich A, Lehmann R, Schleicher ED. High glucose-induced transforming growth factor beta1 production is mediated by the hexosamine pathway in porcine glomerular mesangial cells. J. Clin. Invest. 101: 160-169 (1998)
22. Xu Y, He Z, King GL. Introduction of hyperglycemia and dyslipidemia in the pathogenesis of diabetic vascular complications. Curr. Diab. Rep. 5: 91-97 (2005)
23. Takeuchi M, Kikuchi S, Sasaki N, Suzuki T, Watai T, Iwaki Bucala R, Yamagishi S. Involvement of advanced glycation end-products (AGEs) in Alzheimer's disease. Curr. Alzheimer Res. 1: 39-46 (2004)
24. Hein G, Wiegand R, Lehmann G, Stein G, Franke S. Advanced glycation end-products pentosidine and *N*-carboxymethyllysine are elevated in serum of patients with osteoporosis. Rheumatology 42: 1242-1246 (2003)
25. Suzuki D, Miyata T, Saotome N, Horie K, Inagi R. Immunohistochemical evidence for an increased oxidative stress and carbonyl modification of proteins in diabetic glomerular lesion. J. Am. Soc. Nephrol. 10: 822-832 (1999)
26. Lusis AJ. Atherosclerosis. Nature 407: 233-241 (2000)
27. Reddy S, Bichler J, Wells-Knecht KJ, Thorpe SR, Baynes JW. Ne-(carboxymethyl) lysine is a dominant advanced glycation end product (AGE) antigen in tissue proteins. Biochemistry 34: 10872-10878 (1995)
28. Sato T, Shimogaito N, Wu X, Kikuchi S, Yamagishi S, Takeuchi M. Toxic advanced glycation end products (TAGE) theory in Alzheimer's disease. Am. J. Alzheimers Dis. Other Demen. 21: 197-208 (2006)
29. Takeuchi M, Makita Z, Yanagisawa K, Kameda Y, Koike T. Detection of noncarboxymethyllysine and carboxymethyllysine advanced glycation end products (AGE) in serum of diabetic patients. Mol. Med. 5: 393-405 (1999)
30. Zeng J, Dunlop RA, Rodgers KJ, Davies MJ. Evidence for inactivation of cysteine proteases by reactive carbonyls via glycation of active site thiols. Biochem. J. 398: 197-206 (2006)
31. Nawroth PP, Bierhaus A, Vogel GE, Hofmann MA, Zumbach M, Wahl P, Ziegler R. Non-enzymaticglycation and oxidative stress in chronic illness and diabetes mellitus. Med. Klin. 94: 29-38 (1999)
32. Rumble JR, Cooper ME, Soulis-Liparota T, Cox A, Wu L. Vascular hypertrophy in experimental diabetes: Role of advanced glycation end-products. J. Clin. Invest. 99: 1016-1027 (1997)
33. Hill MA, Ege EA. Active and passive mechanical properties of isolated arterioles from STZ-induced diabetic rats: Effect of aminoguanidine treatment. Diabetes 43: 1450-1456 (1994)
34. Goldin A, Joshua AB, Schmidt AM, Creager MA. Advanced glycation end products sparking the development of diabetic vascular injury. Circulation 114: 597-605 (2006)
35. Odetti P, Aragno I, Rolandi R, Garibaldi S, Valentini S. Scanning force microscopy reveals structural alterations in diabetic rat collagen fibrils: Role of protein glycation. Diabetes Metab. 16: 74-81 (2000)
36. Yan SD, Schmidt AM, Anderson MG, Zhang J, Brett J. Enhanced cellular oxidant stress by the interaction of advanced glycation end-products with their receptors/binding proteins. J. Biol. Chem. 269: 9889-9897 (1994)
37. Chappey O, Dosquet C, Wautier MP, Wautier JL. Advanced glycation end-products, oxidant stress, and vascular lesions. Eur. J. Clin. Invest. 27: 97-108 (1997)
38. Schmidt AM, Hori O, Brett J, Yan SD, Wautier JL, Stern D. Cellular receptors for advanced glycation end-products: Implication for induction of oxidant stress and cellular dysfunction in the pathogenesis of vascular lesions. Arterioscler. Thromb. 14: 1521-1527 (1994)
39. Galle J, Schneider R, Winner B, Lehmann-Boden C, Schinzel R. Glyco-oxidized LDL impair endothelial function more potently than oxidized LDL: Role of enhanced oxidative stress. Atherosclerosis 138: 65-77 (1998)
40. Donato R. RAGE: A single receptor for several ligands and different cellular responses: The case of certain S100 proteins. Curr. Mol. Med. 7: 711-724 (2007)
41. Galle J, Lenhmann-Bodem C, Hubner U, Heinloth A, Wanner C. CyA and OxLDL cause endothelial dysfunction in isolated arteries through endothelinmediated stimulation of O(2)(-) formation. Nephrol. Dial. Transpl. 15: 339-346 (2000)
42. Schleicher E, Friess U. Oxidative stress, AGE, and atherosclerosis. Kidney Int. Suppl. 106: 17-26 (2007)
43. Pieper GM, Dondlinger LA. Plasma and vascular tissue arginine are decreased in diabetes: Acute arginine supplementation restores endotheliumdependent relaxation by augmenting cGMP. J. Pharmacol. Exp. Ther. 283: 684-691 (1997)
44. Matsuzawa-Nagata N, Takamura T, Ando H, Nakamura S, Kurita S, Misu H, Ota T, Yokoyama M, Honda M, Miyamoto K, Kaneko S. Increased oxidative stress precedes the onset of high-fat diet-induced insulin resistance and obesity. Metabolism 57: 1071-1077 (2008)
45. Forbes JM, Coughlan MT, Cooper ME. Oxidative stress as a major culprit in kidney disease in diabetes. Diabetes 57: 1446-1454 (2008)
46. Kakkar R, Mantha SV, Kalra J, Prasad K. Time course study of oxidative stress in aorta and heart of diabetic rats. Clin. Sci. 91: 441-448 (1996)
47. Wang YK, Hong YJ, Huang ZQ. Protective effects of silybin on human umbilical vein endothelial cell injury induced by H_2O_2 *in vitro*. Vasc. Pharmacol. 43: 198-206 (2005)
48. Ha H, Lee HB. Reactive oxygen species amplify glucose signalling in renal cells cultured under high glucose and in diabetic kidney. Nephrology 10: 7-10 (2005)
49. Munzel T, Heitzer T, Harrison DG. The physiology and pathophysiology of the nitric oxide/superoxide anions. Herz 22: 158-172 (1997)
50. Thornalley PJ, Yurek-George A, Argirov AK. Kinetics and mechanism of the reaction of aminoguanidine with alpha-oxaldehydes glyoxal, methylglyoxal, and 3-deoxyglucosone under physiological conditions. Biochem. Pharmacol. 60: 55-65 (2000)
51. Price DL, Rhett PM, Thorpe SR, Baynes JW, Chelating activity of advanced glycation end-product inhibitors. J. Biol. Chem. 276: 48967-48972 (2001)
52. Peyroux J, Sternberg M. Advanced glycation endproducts (AGEs): Pharmacological inhibition in diabetes. Pathol. Biol. 54: 405-419 (2006)
53. Goova MT, Li J, Kislinger T, Qu W, Lu Y, Bucciarelli LG. Blockade of receptor for advanced glycation end-products restores effective wound healing in diabetic mice. Am. J. Pathol. 159: 513-525 (2001)
54. Flyvberg A, Denner L, Schrijvers B, Tilton RG, Mogensen TH, Paludan SR. Long-term renal effects of a neutralizing RAGE antibody in obese type 2 diabetic mice. Diabetes 53: 166-172 (2004)
55. Jensen IJ, Denner L, Schrijers BF, Tilton RG, Rash R, Flyvgerg A. Renal effects of a neutralizing RAGE-antibody in long-term streptozotocin-diabetic mice. J. Endocrinol. 188: 493-501 (2006)
56. Booth AA, Khalifah RG, Todd P, Hudson BG. *In vitro* kinetics studies of formation of antigenic advanced glycation end products (AGEs). J. Biol. Chem. 272: 5387-5430 (1997)
57. Bolton WK, Cattran DC, Williams ME, Adler SG, Appel GB for the

ACTIONI Investigator Group. Randomized trial of an inhibitor of formation of advanced glycation end products in diabetic nephropathy. Am. J. Nephrol. 24: 32-40 (2004)
58. Bakris GL, Bank AJ, Kass DA, Neutel JM, Preston RA, Oparil S. Advanced glycation end-product cross-link breakers: A novel approach to cardiovascular pathologies related to the aging process. Am. J. Hypertens.17: 23-30 (2004)
59. Onorato JM, Jenkins AJ, Thorpe SR, Baynes JW. Pyrodoxamine, an inhibitor of advanced glycation reactions, also inhibits advanced lipoxidation reactions. J. Biol. Chem. 275: 21177-21184 (2000)
60. Voziyan PA, Khalifah RG, Thibaudeau C, Yildiz A, Jacob J, Seriani S. Modification of proteins *in vitro* by physiological levels of glucose. J. Biol. Chem. 278: 46616-46624 (2003)
61. Voziyan PA, Hudson BG. Pyridoxamine: The many virtues of a maillard reaction inhibitor. Ann. NY Acad. Sci. 1043: 807-816 (2005)
62. Yang S, Litchfield JE, Baynes JW. AGE-breakers cleave model compounds but not break Maillard crosslinks in skin and tail collagen from diabetic rats. Arch. Biochem. Biophys. 412: 42-46 (2003)
63. Vasan S, Foiles P, Founds H. Therapeutic potential odd breakers of advanced glycation end product-protein cross links. Arch. Biochem. Biophys. 419: 89-96 (2003)
64. Little WC, Zile MR, Kitzman DW, Hundley WG, O'Brien TX, Degroof RC. The effect of alagebrium chloride (ALT 711), a novel glucose crosslink breaker, in treatment of elderly patients with diastolic heart failure. J. Card. Fail. 11: 191-195 (2005)
65. Chen XM, Kitts DD. Determining conditions for nitric oxide synthesis in Caco-2 cells using Taguchi and factorial experimental designs. Anal. Biochem. 381: 185-192 (2008)
66. Thornalley PJ. Use of aminoguanidine (Pimagedine) to prevent the formation of advanced glycation endproducts. Arch. Biochem. Biophys. 419: 31-40 (2003)
67. Miyata T, Ueda Y, Yamada Y, Izuhara Y, Wada T, Jadoui M. Accumulation of carbonyls accelerates the formation of pentosidine, advanced glycation end product: Carbonyl stress in uremia. J. Am. Soc. Nephrol. 9: 2349-2356 (1998)
68. Miyata T, Yamamoto M, Izuhara Y. From molecular footprints of disease to new therapeutic interventions in diabetic nephropathy. Ann. NY Acad. Sci. 1043: 740-749 (2005)
69. Figarola JL, Scott S, Loera S, Xi B, Synold T, Weiss I. Prevention of early renal disease, dyslipidemia, and lipid peroxidation in STZ-diabetic rats by LR-9 and LR-74 novel AGE inhibitors. Diabetes Metab. Res. 21: 533-544 (2005)
70. Voziyan PA, Metz TO, Baynes JW, HudsonBG. A post-Amadori inhibitor pyridoxamine also inhibits chemical modification of proteins by scavenging carbonyl intermediates of carbohydrate and lipid degradation. J. Biol. Chem. 277: 3397-3403 (2002)
71. Babei-jadidi R, Karachalian N, Ahmed N, Battab S, Thornalley PJ. Prevention of incipient diabetic nephropathy by high-dose thiamine and benfotiamine. Diabetes 52: 2110-2120 (2003)
72. Hammes HP, Du X, Edelstein D, Taguchi T, Matsumara T, Ju Q. Benfotiamine blocks three major pathways of hyperglycemic damage and prevents experimental diabetic retinopathy. Nat. Med. 9: 294-299 (2003)
73. Vasan S, Zhang X, Karpurniotu A, Bernhagen J, Teichberg S. An agent cleaving glucose-derived protein crosslinks *in vitro* and *in vivo*. Nature 382: 211-212 (1996)