AGE, RAGE, and ROS in Diabetic Nephropathy

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Summary: Diabetic nephropathy is a major cause of morbidity and mortality in diabetic patients. Two key mechanisms implicated in the development of diabetic nephropathy include advanced glycation and oxidative stress. Advanced glycation is the irreversible attachment of reducing sugars onto amino groups of proteins to form advanced glycation end products (AGEs). AGE modification of proteins may lead to alterations in normal function by inducing cross-linking of extracellular matrices. Intracellular formation of AGEs also can cause generalized cellular dysfunction. Furthermore, AGEs can mediate their effects via specific receptors, such as the receptor for AGE (RAGE), activating diverse signal transduction cascades and downstream pathways, including generation of reactive oxygen species (ROS). Oxidative stress occurs as a result of the imbalance between ROS production and antioxidant defenses. Sources of ROS include the mitochondria, auto-oxidation of glucose, and enzymatic pathways including nicotinamide adenine dinucleotide phosphate reduced (NAD[P]H) oxidase. Beyond the current treatments to treat diabetic complications such as the optimization of blood pressure and glycemic control, it is predicted that new therapies designed to target AGEs, including AGE formation inhibitors and cross-link breakers, as well as targeting ROS using novel highly specific antioxidants, will become part of the treatment regimen for diabetic renal disease.

Semin Nephrol 27:130-143

Keywords: *Diabetes mellitus, diabetic nephropathy/complications, advanced glycation end-products, receptors, oxidative stress*

Diabetes mellitus is one of the biggest epidemics affecting human health in the 21st century. The alarming increase, particularly of type 2 diabetes, is attributed largely to globalization, along with vast changes in human behavior, lifestyle, and increased lifespan.[1] The complications associated with diabetes are a major cause of morbidity and mortality, with diabetic nephropathy being one of the major chronic microvascular complications in both type 1 and 2 diabetic patients. Many factors influence the development of diabetic nephropathy and other complications including genetic, hemodynamic, environmental, and metabolic factors (Fig. 1). The major contributing factor is persistent hyperglycemia with the Diabetes Control and Complications Trial[2] and the United Kingdom Prospective Diabetes Study[3] showing that intensive control of hyperglycemia can reduce the occurrence and progression of diabetic microvascular complications including nephropathy. The mechanisms whereby hyperglycemia leads to diabetic complications continue to be under active investigation. This review focuses on advanced glycation and oxidative stress, 2 key players in the pathogenesis of diabetic nephropathy.

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Supported in part by the Juvenile Diabetes Research Foundation, National Health and Medical Research Council of Australia, Diabetes Australia, and the National Institutes of Health. J.M.F. is a Juvenile Diabetes Research Foundation career development awardee; A.L.Y.T. is supported by a Kidney Health Australia Medical Research Funding Award.

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0270-9295/07/$ - see front matter

 doi:10.1016/j.semnephrol.2007.01.006

ADVANCED GLYCATION END PRODUCTS

Advanced glycation end products (AGEs) are bound covalently, reducing sugar modifications of proteins and lipoproteins.[4] AGEs accumulate as a result of natural aging in a time-dependent man-

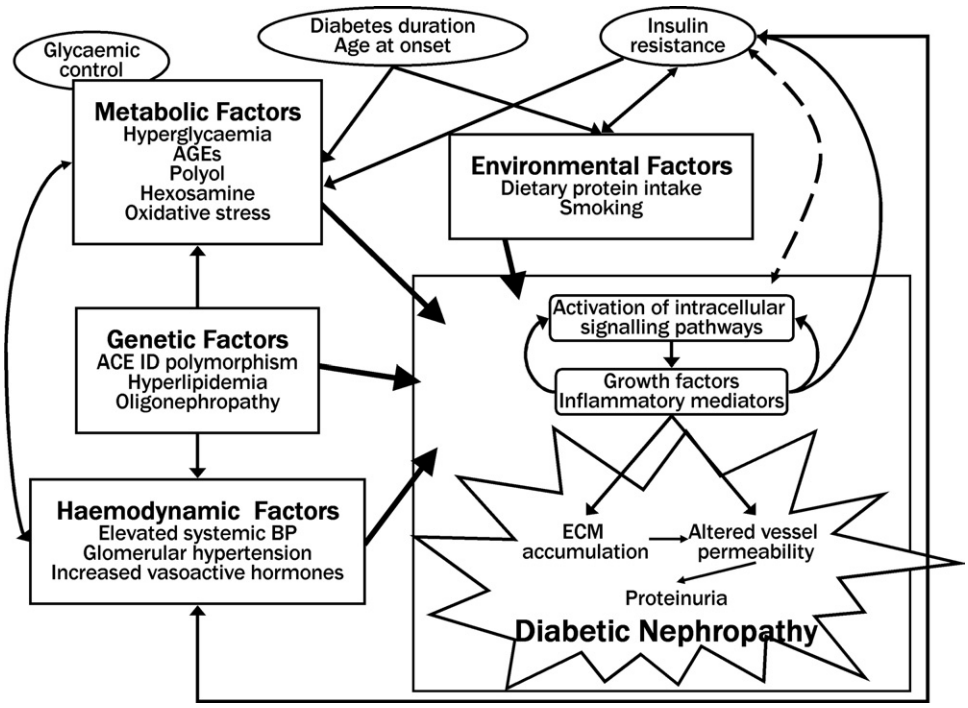

Figure 1. Potential interactions between metabolic, hemodynamic, genetic, and environmental factors in the pathogenesis of diabetic nephropathy. ACE, angiotensin-converting enzyme; BP, blood pressure.

ner, as supported in experimental models and in human beings. It is thought that the physiologic role of advanced glycation is to identify senescent proteins for degradation. During aging, AGE formation may result from reduced AGE defenses, long-term exposure of proteins to reducing sugars such as glucose, increased insulin resistance, and/or deteriorating renal function. In diabetes, AGE formation is enhanced by persistent hyperglycemia and oxidative stress, leading to more extensive modification of long-lived proteins such as skin collagen, although short-lived proteins also become targets for advanced glycation. Exogenous AGEs may be absorbed into the circulation from reactions between sugars and proteins in foods or from curing of tobacco. Indeed, AGE content is high in cooked and processed foods, especially those rich in proteins, fat, and sugar.[5] It is considered that dietary AGEs are similar to endogenous AGEs with regard to their prooxidant, proinflammatory, and signaling properties.[6] Thus, the levels of circulating AGE levels in diabetic patients may be a reflection of both endogenously formed and exogenously ingested AGEs.

Importantly, the kidney is a target for AGE-mediated damage and also the main contributor to increasing circulating AGE concentrations via a decrease in renal function, by the clearance of AGEs.[7]

GENESIS AND STRUCTURE OF AGES

Advanced glycation (advanced glucosylation or glycosylation) is the nonenzymatic process whereby the carbonyl (aldehyde or ketone) of reducing sugars such as glucose react nonenzymatically with lysine and N-terminal amino groups in a variety of proteins, lipoproteins, and nucleic acids, leading to the formation of early glycation products via the Maillard reaction.[4] These go through further rearrangements leading to the formation of various reactive intermediate products including α-dicarbonyls or oxoaldehydes. Examples of α-dicarbonyls include 3-deoxyglucosone, glyoxal, and methylglyoxal. α-dicarbonyls react with amino groups of intracellular and extracellular proteins to form AGEs, a heterogeneous class of stable and irreversible covalent adducts. Many AGEs such as pentosidine have intrinsic florescence and hence tissue and plasma fluorescence may be used as markers of AGE accumulation. Other AGEs such as carboxymeth-

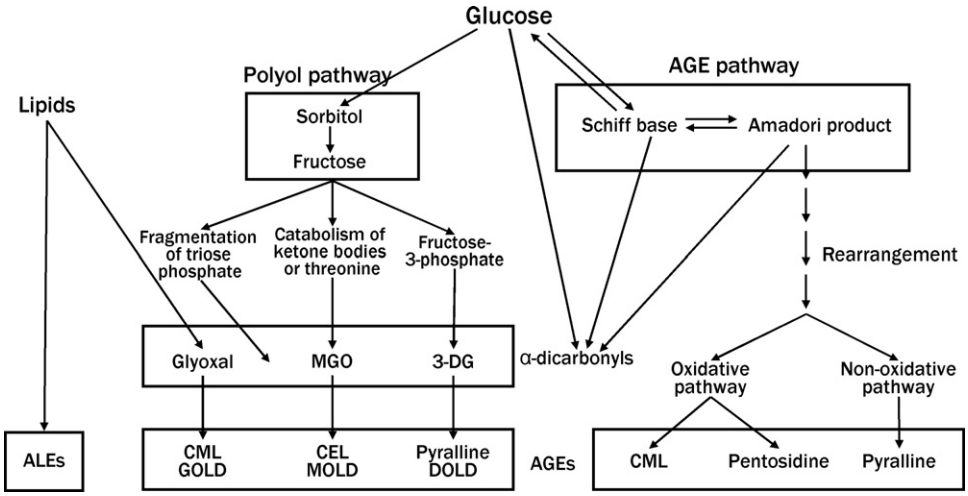

Figure 2. Possible pathways for AGE formation. Intermediate lipid metabolism, the polyol pathway, and advanced glycation leads to formation of α-dicarbonyl intermediates, AGEs, and advanced lipoxidation end-products (ALEs). This can occur through oxidative or nonoxidative pathways. MGO, methylglyoxal; 3-DG, 3-deoxyglucosone; CEL, carboxyethyllysine; GOLD, glyoxal lysine dimer; MOLD, methylglyoxal lysine dimer; DOLD, deoxyglucosone lysine dimer.

yllysine (CML) are not fluorescent. In vivo, the Maillard reaction is slow under homeostatic conditions because glucose, the predominant sugar used for fuel, is the least reactive of the biological sugars to the Maillard reaction.[4]

Glycation may be accompanied by oxidation and induction of intracellular metabolic pathways such as the polyol pathway, to form glycoxidation products including CML, carboxyethyllysine, and pentosidine. AGEs also can be generated through nonoxidative pathways, for example, pyralline, a methylglyoxal lysine dimer derived from methylglyoxal during nonoxidative anaerobic glycolysis, or deoxyglucosone lysine dimer derived from 3-deoxyglucosone released during Amadori rearrangements (Fig. 2).[4]

The effects of AGEs can be classified as receptor-independent or -dependent (Fig. 3). First, AGEs modify long-lived structural components of the basement membrane or extracellular matrix (ECM). This may occur by increasing the expression of protein components such as type IV collagen in the kidney or via abnormal interactions of AGEs with other matrix compo-

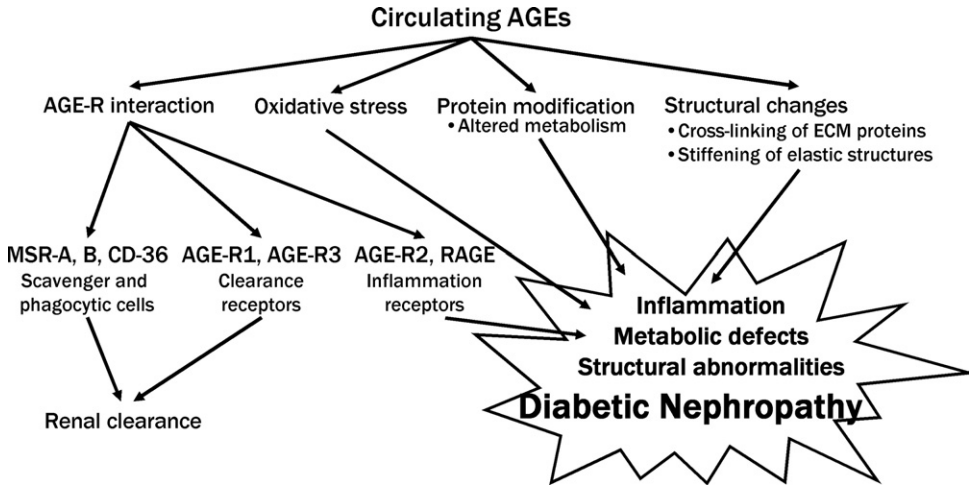

Figure 3. AGEs and their receptor-independent or -dependent effects leading to renal clearance or inflammation, metabolic defects, and structural abnormalities. TCA, tricarboxylic acid cycle; MSR-A, B, macrophage scavenger receptor types A, B.

nents and cellular matrix receptors. In addition, covalent intermolecular and intramolecular cross-links can form between glycated ECM proteins leading to structural alterations including changes in packing density, surface charge, membrane permeability, resistance to proteolytic digestion, and thermal stability. AGEs disrupt normal cell-matrix contact or prevent physiologic cellular growth and intercellular contact, thus preventing maintenance of tissue integrity and normal function.[4]

The formation of AGEs is not exclusive to glycation of extracellular proteins. AGEs and α-dicarbonyl intermediates also can form from intracellular components and this can occur after only days of hyperglycemia or via increases in reducing sugars owing to altered metabolism.[8]

RECEPTOR-MEDIATED EFFECTS OF AGES

AGEs also mediate their effects via receptors (Fig. 3) including the receptor for AGE (RAGE), macrophage scavenger receptor types I and II (types A and B1/CD36), oligosaccharyl transferase-48 (AGE-R1), 80K-H phosphoprotein (AGE-R2), galectin-3 (AGE-R3), CD-36,[4] and the recently identified ezrin, radixin, and moesin proteins.[9] Other multiligand receptors including megalin also bind AGEs. AGE receptors are expressed on various cell types such as monocytes, macrophages, endothelial cells, mesangial cells, podocytes, tubular epithelial cells, astrocytes, microglia, and smooth muscle cells.[10] Many AGE-receptors have multiple ligands and can be activated by non-AGE moieties, as well as a range of structurally distinct AGEs. It remains unknown which AGEs have the greatest affinity and activating potential for AGE receptors. Ultimately, the total empiric binding capacity rather than the specific AGE moiety that is binding may be more important.

RAGE

Although RAGE first was described as a receptor for AGEs, it later was discovered to be a multiligand receptor that recognizes a pattern or common motif. Its primary function is in the innate immune response in which it plays a major role in host pathogen defense. Other ligands of RAGE other than AGEs include amphoterin, amyloid β-peptide, S100/calgranulins, and Mac-1.[11]

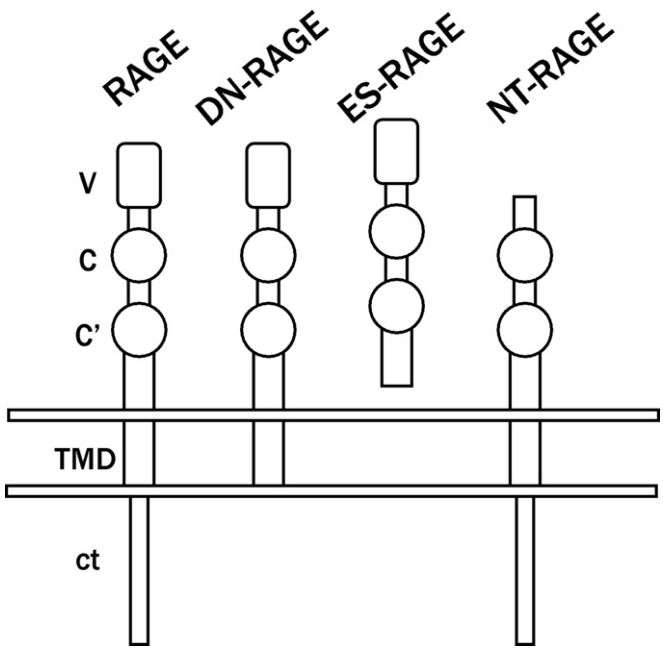

Figure 4. RAGE, dominant-negative (DN)-RAGE, and the major splice variants of RAGE - endogenous secretory (ES)-RAGE and N-truncated (NT)-RAGE. RAGE has 3 extracellular domains: 1 terminal V-type domain and 2 C-type domains (V-C-C'), a transmembrane (TMD) domain, and a cytosolic tail (ct).

RAGE is a multiligand member of the immunoglobulin superfamily with 394 amino acids, a single hydrophobic transmembrane domain (19 amino acids), and a highly charged COOH-terminal cytosolic tail (43 amino acids) that mediate intracellular signaling pathways. Extracellularly, RAGE has a terminal V-type ligand binding domain and 2 C-type domains (V-C-C') (Fig. 4).[11]

In addition to full-length RAGE, there are other messenger RNA (mRNA) splice variants for RAGE that encode truncated proteins with various biological properties (Fig. 4).[12] Endogenous secretory or soluble RAGE (sRAGE) lacks the COOH terminal and transmembrane domains. It is secreted in a paracrine way and can bind extracellular ligands independently of direct cell contact. Excess sRAGE may competitively bind RAGE ligands, preventing their interaction with the cell surface RAGE receptor and hence preventing cellular signaling. Hence, the balance between synthesis of sRAGE and full-length RAGE may be a

key determinant of AGE-induced pathology. Low (picomolar) levels of sRAGE have been found in the plasma of animals and human beings, produced by native expression of the truncated form of RAGE. This suggests that therapeutically, exogenous administration of sRAGE of the same species may not trigger an immunologic response. Indeed, infusion of sRAGE of the same species into animals such as mice is not immunogenic, even up to 6 months.[13] The potential therapeutic value of sRAGE has been observed both in experimental diabetic atherosclerosis[14] and nephropathy.[15] Other RAGE isoforms include the secreted RAGE isoform that lacks the transmembrane domain only, and N-truncated RAGE, which lacks the terminal V-type domain. N-truncated RAGE is anchored in the cell membrane but does not bind ligands and its role remains unknown.

In the human kidney, RAGE protein is found in tubular epithelial cells,[16] mesangial cells,[17] podocytes,[15,18] and within vascular and neural compartments. In diabetes, RAGE expression is increased at sites of macrovascular and microvascular injury. This is supported by AGE and RAGE colocalization in susceptible organs in diabetes.[19]

RAGE binding by AGEs or other ligands activates diverse signal transduction cascades including $p21^{ras}$, p38, p44/p42 (erk1/2, extracellular signal-related kinase), and stress-activated protein kinase/c-Jun N-terminal kinase mitogen-activated protein (MAP) kinases, the Janus kinase/signal transducers and activators of transcription pathway, and protein kinase C (PKC) pathway. Signal transduction leads to downstream consequences including generation of reactive oxygen species (ROS) and activation of transcription factors such as nuclear factor kappa B (NF-κB).[11] One important consequence of NF-κB translocation is the up-regulation of RAGE itself because the promoter region of RAGE contains functional binding elements for NF-κB.[20] AGE-RAGE induction of NF-κB or other pathways contributes to the release of proinflammatory cytokines, and the expression of adhesion molecules and growth factors that are implicated in the pathogenesis of diabetic complications. These include transforming growth factor-β1 (TGF-β1), vascular endothelial growth factor, connective tissue growth factor, interleukin-1β and -6, insulin-like growth factor-1, platelet-derived growth factor, tumor necrosis factor (TNF)-α, and vascular cell adhesion molecule (VCAM)-1.[11]

AGE, RAGE, AND DIABETIC NEPHROPATHY: ANIMAL STUDIES

Animal studies support the role of AGEs and RAGE in the pathogenesis of diabetic nephropathy. First, diabetic animals have significant increases in renal AGEs assayed by a range of techniques.[21] Second, pathologic changes in the diabetic kidney are reduced with AGE formation inhibitors such as aminoguanidine,[22] ALT-946,[22] OPB-9195,[23] EXO-226,[24] and A717,[25] or other approaches to reduce AGE accumulation such as the cross-link breaker ALT-711 (alagebrium-chloride; Alteon Inc., Ramsey, NY).[26] These renal pathologic changes also can be diminished by treating the diabetic animals with soluble RAGE[15] or a RAGE-specific neutralizing antibody.[27] Third, genetic manipulation of RAGE expression influences the renal phenotype in the setting of diabetes. For example, diabetic transgenic mice that overexpress human RAGE have more advanced renal disease when compared with diabetic wild-type mice. These changes included increases in albuminuria and serum creatinine levels, mesangial expansion, and advanced glomerulosclerosis.[28] Consistent with these findings, RAGE knockout mice made diabetic by using streptozotocin have less renal injury in comparison with diabetic wild-type mice. In particular, these RAGE knockout mice do not have significant mesangial expansion or glomerular basement membrane thickening.[15] Finally, normal rats or mice administered with AGE-albumin develop renal changes reminiscent of those seen in diabetic nephropathy including increased renal AGE content and glomerular volume, glomerular basement membrane thickening, mesangial matrix expansion, NF-κB activation, and increased collagen IV and TGF-β mRNA expression.[29] These changes are reduced with administration of the AGE inhibitor aminoguanidine[29] or a RAGE-specific neutralizing antibody.[30]

AGE, RAGE AND DIABETIC NEPHROPATHY: HUMAN DIABETES

Clinical studies in both type 1 and 2 diabetes strongly implicate AGEs in the development of diabetic complications. Type 1 diabetic patients that advance from normal renal function to subsequent microalbuminuria, clinical nephropathy, and hemodialysis have significantly increased serum levels of fluorescent non-CML AGEs, but not CML or pentosidine.[31] Other investigators also have shown that CML in type 1 diabetic patients correlates with the presence and severity of nephropathy and retinopathy.[32,33] A lower mean glycated hemoglobin value in type 1 diabetic patients with intensified insulin treatment, as observed in the Diabetes Control and Complications Trial, also is associated with less carotid intima-media thickening, and this has been postulated to be linked to less AGE accumulation.[34] It should be noted, however, that associations between AGE accumulation and the development of diabetic complications remained significant even after adjustment for the glycated hemoglobin level.[33]

Similarly, in type 2 diabetic patients there also are significant increases in serum AGE concentrations, including increased CML–human serum protein[35] and hydroimidazolone levels.[36] CML–human serum protein levels were higher in those patients with retinopathy or microalbuminuria.[35] In addition, increases in circulating AGE peptides correlated with the severity of renal impairment in diabetic subjects.[37] The severity of diabetic nephropathy in human beings correlated to the extent of AGE formation in glomerular and tubulointerstitial compartments.[18] Furthermore, these patients had increased podocyte RAGE expression.[18]

OTHER AGE RECEPTORS

It has been proposed that AGE-R1, -R2, and -R3 interact closely in the AGE receptor complex, a molecular aggregate on cell surfaces involved in AGE catabolism.[38] AGE-R1 or P60 is a 48-kd, type I integral membrane protein originally discovered in the lumen of the endoplasmic reticulum and was thought to act as a stabilizing component of the oligosaccharyltransferase system. Later, it was identified on cell surfaces where it bound AGEs significantly. It has been suggested that AGE-R1 may have a protective effect against AGE-induced injury. In diabetic kidney disease, AGE-R1 expression is suppressed in both human beings[39] and nonobese diabetic mice.[40] Moreover, in mesangial cells, up-regulation of AGE-R1 enhances AGE removal and down-regulates RAGE and downstream signaling pathways such as NF-κB activity and MAP kinase phosphorylation, whereas down-regulation of AGE-R1 increases AGE-induced MAP kinase activation.[10] Furthermore, mice transgenic for AGE-R1 are protected against the development of diabetic nephropathy.[41]

AGE-R2 or P90 is an 80- to 90-kd protein, found to be identical to an 80- to 87-kd AGE-inducible tyrosine-phosphorylated protein. It initially was thought to act as a substrate for kinase C, but later was found to be involved in the intracellular signaling of various receptors, including the fibroblast growth factor receptor. The P90 protein is located in the plasma membrane and can bind to other adaptor molecules such as Shc. Because P90 is phosphorylated when exposed to AGEs, it was suggested that it has a role in the early stages of AGE signaling, and hence was termed *AGE-R2*.

AGE-R3 or galectin-3 is a 32-kd protein that binds to carbohydrates, laminin, and immunoglobulin E and is associated with several cellular functions including activation, inflammation, tumor growth activity, and apoptosis. Galectin-3 binds to AGE ligands with high affinity and increases in surface expression of AGE-R3 leads to an increase in AGE-ligand binding and endocytosis by macrophages. It is proposed that AGE-R3 is involved in the regulation of AGE turnover and hence maintenance of tissue integrity,[42] a compensatory event to combat increasing circulating and tissue AGE concentrations in diabetes. In diabetic rats, increases in glomerular AGE-R3 mRNA and protein expression were observed 2 months after induction of diabetes and continued to increase as compared with undetectable levels in nondiabetic rats until 12 months of age.[42] The importance of AGE-R3 in AGE turnover is best shown in deficient mice that have accelerated diabetic glomerulopathy, increased proteinuria, and mesangial expansion.[43]

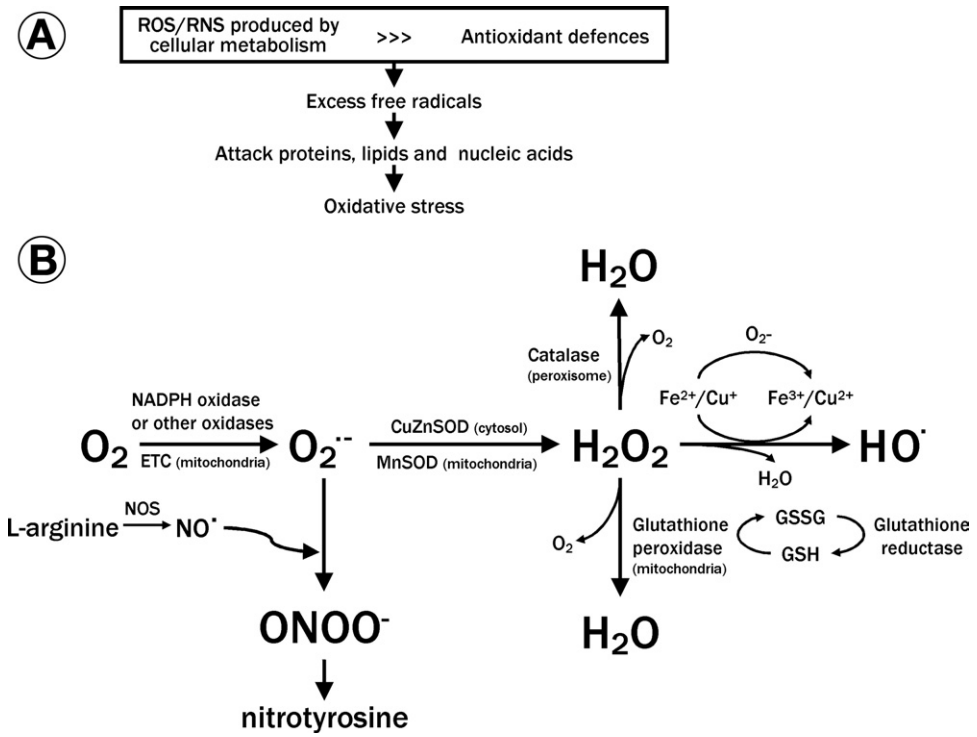

Figure 5. Oxidative stress and the generation of reactive species. (A) Oxidative stress results when highly reactive molecules including ROS and RNS are not sufficiently removed by antioxidant defences. (B) Generation of reactive species may occur when oxygen is converted to $O_2^{\cdot -}$, which then is dismutated to H_2O_2 by SOD. H_2O_2 may be converted to H_2O by catalase or glutathione peroxidase, or to $HO^{\cdot}$ by reaction with copper (Cu) or iron (Fe). In addition, $O_2^{\cdot -}$ also can react rapidly with $NO^{\cdot}$ to form $ONOO^-$.

OXIDATIVE STRESS

Oxidative Stress and Antioxidant Defense

Oxidative stress is defined as the excess formation or insufficient removal by antioxidant defenses of highly reactive molecules including ROS and reactive nitrogen species (RNS) (Fig. 5A). Examples of ROS include free radicals such as superoxide ($O_2^{\cdot -}$), hydroxyl ($HO^{\cdot}$), peroxyl ($O_2^{\cdot}$), hydroperoxyl ($HO_2^{\cdot}$), and nonradical species such as hydrogen peroxide (H_2O_2) and hydrochlorous acid (HOCl). Examples of RNS include free radicals such as nitric oxide ($NO^{\cdot}$) and nitrogen dioxide ($NO_2^{\cdot}$), and nonradicals such as peroxynitrite ($ONOO^-$), alkyl peroxynitrates (RONOO), and nitrous oxide (HNO_2). The major free radical implicated in diabetic complications is $O_2^{\cdot -}$, which can be produced by various sources including the mitochondrial electron transport chain (ETC) during normal oxidative phosphorylation, by nicotinamide adenine dinucleotide phosphate reduced (NAD[P]H) oxidase, xanthine oxidase, cyclooxygenase, lipoxygenase, cytochrome P-450, and nitric oxide synthase in certain contexts (Fig. 5B).[44]

In normal conditions, $O_2^{\cdot -}$ is eliminated rapidly by antioxidant defense mechanisms. $O_2^{\cdot -}$ can dismutate spontaneously to form H_2O_2. Alternatively, superoxide dismutase (SOD) can catalyze the dismutation of $O_2^{\cdot -}$ to H_2O_2. SOD has 3 major isoforms: cytosolic CuZnSOD (SOD1), mitochondrial MnSOD (SOD2), and extracellular SOD (SOD3). H_2O_2 is converted to H_2O and O_2 via catalase in lysosomes or glutathione peroxidase (GPx) in the mitochondria and cytosol. In the presence of transition metals such as iron and copper, H_2O_2 can be converted to the highly reactive $HO^{\cdot}$ radical via the Fenton reaction. Excess $O_2^{\cdot -}$ also can react with $NO^{\cdot}$ to form $ONOO^-$ (Fig. 5B).[44]

Common effects of the various ROS described earlier such as $O_2^{\cdot -}$, H_2O_2, $HO^{\cdot}$, and $ONOO^-$ include oxidation of important macromolecules including lipids, DNA, proteins, and carbohydrates. ROS can induce peroxidation of

membrane lipids that may alter membrane structure and fluidity and hence function. This may result in the production of toxic lipid peroxides. DNA damage may result in the modification of transcription factors, thus modulating the expression of a range of proteins including cytokines and enzymes involved in glucose respiration. Oxidants also can increase signaling molecules such as p38 or c-Jun N-terminal kinase MAP kinases.[44]

Oxidative Stress in Diabetes

Nonenzymatic sources

It is widely recognized that oxidative stress is a key component in the development of diabetic complications. Nonenzymatic sources of oxidative stress induced by diabetes include glucose auto-oxidation, advanced glycation, the polyol pathway, and the mitochondrial ETC.[45] It has been suggested that the primary initiating event in the development of diabetic complications is $O_2^{\cdot -}$ formation by mitochondria.[46] One theory suggests that hyperglycemia induces changes in the mitochondrial voltage gradient by increasing electron donors of the ETC or via uncoupling protein-1.[46] Another hypothesis is that hyperglycemia may inhibit F_0F_1-adenosine triphosphate (ATP) synthase, slowing electron transfer and ATP synthesis, leading to an excess of electrons that would combine with molecular O_2 to form $O_2^{\cdot -}$. Indeed, diabetic rats have mitochondrial enlargement in renal proximal tubules associated with disturbed ATP metabolism.[47] Alternatively, production of excess O_2 also may result when NAD^+ cannot be regenerated during electron transfer and NADH oxidase is activated, generating $O_2^{\cdot -}$ as a byproduct. Moreover, mitochondrial swelling induced by permeability transition pore opening in isolated rat liver mitochondria inhibits the activity of ETC complex I.[48] Furthermore, diabetic rats have altered mitochondrial permeability transition evident in kidney[49] mitochondria. In addition to induction of mitochondrial permeability transition, oxidative damage also can affect mitochondrial function by altering oxidative phosphorylation, calcium homeostasis, and protein turnover.[50]

Enzymatic sources

It has been recognized that there are a number of enzymatic sources of ROS, however, these are not discussed within this section but have been comprehensively reviewed previously.[45,51] NAD(P)H oxidase is a membranous enzyme consisting of 5 subunits: 2 membrane-associated subunits, p22phox and gp91phox, and 3 major cytosolic subunits, p47phox, p40phox, and p67phox. Gp91phox has other homologues including nox-1 and nox-4.[52] NAD(P)H oxidase is a major source of cellular $O_2^{\cdot -}$ and is an important source of vascular $O_2^{\cdot -}$ in both nondiabetic and diabetic patients. Diabetic rats also show significantly increased NAD(P)H oxidase activity and subunit expression within the kidney.[53-55] In support of this, prevention of NAD(P)H oxidase assembly by initiation of membranous translocation of p47phox and p67phox from the cytosol using ruboxistaurin reduces ROS generation in glomeruli of diabetic rats.[53] Cultured endothelial cells exposed to high glucose levels activate NAD(P)H oxidase via PKC, leading to ROS generation.[56] AGE treatment of human endothelial cells also leads to oxidative stress that is attenuated with the NAD(P)H oxidase inhibitor diphenyliodonium.[57]

Changes in the antioxidants enzymes GPx, catalase, CuZnSOD, and MnSOD also may contribute to oxidative stress in diabetes. In diabetic rats, GPx activity is decreased in the liver, brain, kidney, and heart, whereas catalase activity is increased in heart and kidney, but not liver and brain. CuZnSOD activity in diabetes is decreased in heart, but not liver, brain, or kidney.[58] Interestingly, overexpression of MnSOD in diabetic mice attenuates diabetic renal injury.[59] Furthermore, MnSOD overexpression in cultured glomerular mesangial cells prevents the increase in hyperglycemia-induced cellular $O_2^{\cdot -}$ and collagen synthesis.[59]

INTERPLAY BETWEEN AGES AND ROS

This review has focused on advanced glycation accumulation and oxidative stress as mechanisms involved in the pathogenesis of diabetic complications, particularly nephropathy. However, there is increasing evidence to suggest that there

is interplay between these and other pathways responsible for diabetic complications.[46] For example, oxidative stress may facilitate both the formation of intracellular AGEs and cross-linking in diabetes.[60] Human diabetic glomerular lesions show colocalization of oxidative stress and AGE-modified proteins.[61] Moreover, in spontaneously diabetic rats, good glycemic control prevents the increase in both glycation and oxidation end products in collagen.[62] Indeed, studies using a range of antioxidants have been successful in reducing AGE formation. For example, the use of antioxidants such as butylated hydroxytoluene and probucol leads to decreased renal AGE concentrations in diabetic rats.[21]

Although these studies show that ROS can trigger AGE generation, there also is evidence to suggest that the converse occurs with AGE formation triggering ROS production. For example, AGEs induce decreases in the activities of antioxidant enzymes such as SOD and catalase,[63] decreases glutathione stores, or can directly stimulate ROS production.[64] In addition, biological effects of AGEs may be modulated by changes in oxidative stress. Antioxidant treatment of cultured cells prevents the AGE-induced activation of NF-κB, TGF-β1, and cell death.[65] Furthermore, depletion of the intracellular antioxidant glutathione in cultured rat mesangial cells decreases the AGE concentrations required to activate downstream signaling pathways including NF-κB and PKC-β1.[65] In addition, antioxidant administration to mice infused with AGE albumin prevents the increase in endothelial cell oxidant stress as measured by thiobarbituric acid reactive substance generation and NF-κB translocation.[30] Indeed, it has been suggested that glycation of antioxidative enzymes also may enhance ROS production and cellular oxidative damage.[66]

POTENTIAL INTERVENTIONS FOR DIABETIC COMPLICATIONS

Targeting AGEs

Dietary reduction of AGEs

Potential interventions for diabetic complications are summarized in Fig. 6. The first approach to consider is a dietary reduction in exogenously derived AGEs. Patients with diabetic nephropathy have been reported to have decreased renal excretion of exogenously derived AGEs and diabetic patients on a high AGE diet may have an increased risk of renal and vascular injury.[67] Thus, decreasing the AGE content in the diet may be an important adjunct therapy in the treatment of diabetic nephropathy.

Low dietary AGE intake in animal models, including those with diabetes, is associated not only with decreased atherosclerosis,[68] but also with decreased nephropathy.[69] Diabetic patients on a low-AGE diet have decreased serum AGE levels and a reduction in the inflammatory mediators TNF-α and VCAM-1.[70] Furthermore, a reduction in dietary AGE intake by nondiabetic, chronic renal failure patients with increased serum AGE levels leads not only to a decrease in serum AGE levels, but also reduced TNF-α, VCAM-1, and vascular endothelial growth factor levels.[71] Moreover, long-term dialysis patients have significant correlations between dietary AGE intake and serum AGE levels that appear to be independent of dietary constituents such as fat, protein, and carbohydrate.[72]

AGE formation inhibitors

One of the earliest strategies used to reduce AGE accumulation was the use of AGE formation inhibitors. These agents act in a variety of ways including trapping of reactive carbonyl and dicarbonyl compounds, chelation of transition metal ions, and direct inhibition of the conversion of Amadori intermediates to AGEs.[73] A number of AGE formation inhibitors have been described including aminoguanidine, ALT-946, pyridoxamine, and OPB-9195.[74] The first agent to be investigated extensively was aminoguanidine, a nucleophilic hydrazine compound that inhibits in vitro and in vivo formation of AGEs via binding to early glycation and glycoxidation products, dicarbonyl intermediates, and aldehyde products.[75] Aminoguanidine is a nonspecific inhibitor because it also inhibits inducible nitric oxide synthase and diamine oxidase.[76] Aminoguanidine has been shown to slow the development of diabetic complications including nephropathy.[22] Diabetic rats treated with aminoguanidine and other AGE formation inhibitors including OPB-9195, ALT-946, and pyridoxamine have shown reduced renal AGE accumulation, less mesangial expansion, and

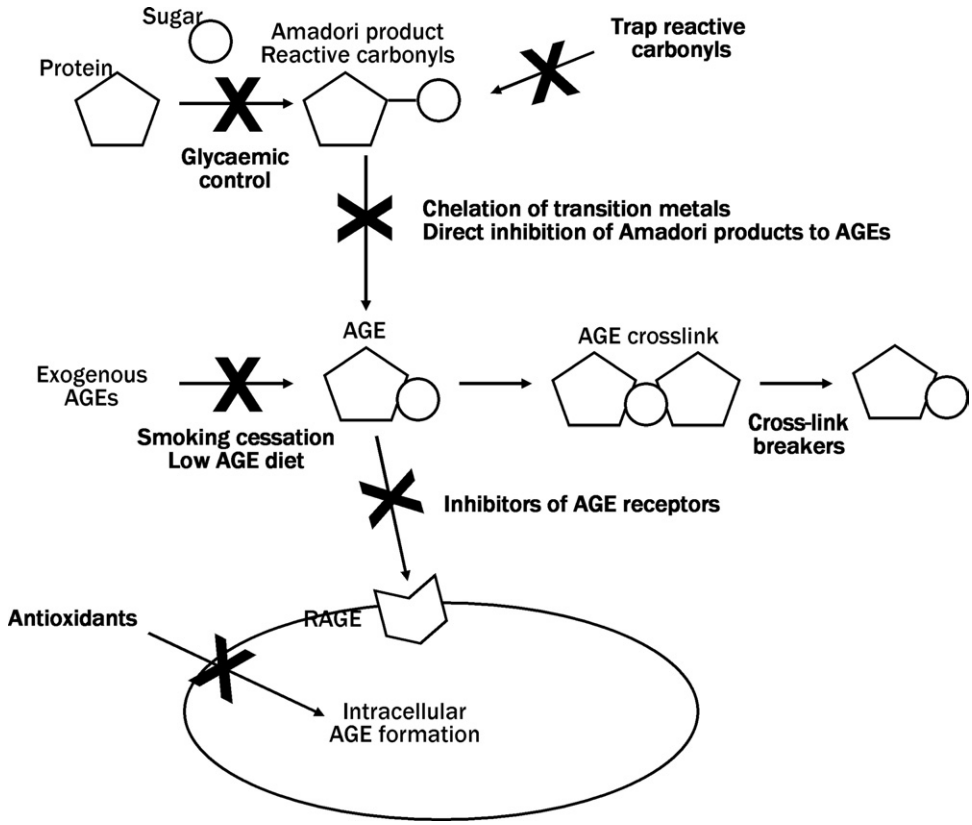

Figure 6. Therapeutic intervention to reduce AGE-induced damage.

slower progression of glomerulosclerosis and albuminuria.[22,77] Furthermore, the renoprotective effects of these agents appear to be related to the duration of the treatment.[78] In human clinical studies, type 1 diabetic patients with nephropathy treated with pimagedine (aminoguanidine hydrochloride) were shown to have slower decreases in glomerular filtration rate, but overall there was no significant beneficial effect on the progression of overt nephropathy.[79] Unfortunately, aminoguanidine interferes with several important regulatory systems[76] and toxic side effects were observed with use of this agent in clinical trials. Thus, it has been discontinued for further clinical development.[80] Interestingly, one of the current clinical therapies for diabetic nephropathy, angiotensin-converting enzyme inhibitors, have been identified as potent inhibitors of AGE formation[81] and it is postulated that at least some of the nonhemodynamic renoprotection conferred by angiotensin-converting enzyme inhibitors may involve effects on AGE accumulation.

AGE cross-link breakers

AGE cross-link breakers are compounds that reduce AGE accumulation by cleavage of preformed AGE-mediated cross-links.[4] Examples of AGE cross-link breakers include N-phenacylthiazolium bromide (PTB) and alagebrium chloride, 4,5-Dimethyl-3-(2-oxo2-phenylethyl)-thiazolium chloride (ALT-711).[26,82] Indeed, ALT-711 has been reported to attenuate renal injury in experimental diabetes[26] and is deemed safe in human clinical trials in other nondiabetic diseases (www.alteon.org). Clinical trials are now warranted to confirm that renoprotective effects of ALT-711 (alagebrium chloride; Alteon Inc.) also are seen in human beings.

Inhibitors of AGE binding

Inhibitors of AGE receptor ligand binding include soluble RAGE and RAGE-specific neutralizing antibodies, which have been used in both in vivo and in vitro studies to block the biological effects of RAGE. Indeed, diabetic mice treated with sRAGE have less albuminuria and glomerulosclе-

rosis.[15] RAGE-specific neutralizing antibodies administered to diabetic mice prevent diabetes-induced renal changes including mesangial expansion and albuminuria.[27] RAGE is considered an attractive target for developing new treatments for diabetic complications with an active program currently in development to target this receptor specifically (http://www.lifesciencesworld.com/news/view/8312).

TARGETING ROS

A large number of experimental studies have been performed using a range of antioxidants to assess their potential actions as renoprotective agents. This has included the use of vitamins C and E and α-lipoic acid. Rat mesangial cells treated with vitamin E and a ROS scavenger nitecapone have less AGE-dependent NF-κB activation and normalization of PKC activity.[65] Diabetic rats treated with the ROS scavenger nitecapone normalized urinary sodium excretion and oxidative stress parameters, prevented hyperfiltration, focal glomerulosclerosis, and albuminuria, and inhibited activation of glomerular PKC activity.[83] The potential beneficial effects of antioxidant therapy in human beings remain controversial. Type 2 diabetic patients administered vitamin C have improved endothelial dysfunction in their forearm resistance vessels.[84] Type 1 diabetic patients with high-dose vitamin E supplementation have normalized baseline retinal blood flow and creatinine clearance, suggesting a role in improving retinal hemodynamics and renal function in diabetic patients.[85] In the Cambridge Heart Antioxidant Study, vitamin E administration to patients with coronary atherosclerosis decreased the primary trial end point of cardiovascular death and myocardial infarction.[86] However, in the Heart Outcomes Prevention Evaluation trial, administration of vitamin E to older patients with an increased risk of cardiovascular events including a significant proportion with diabetes, there was no significant effect on primary and secondary cardiovascular outcomes.[87] Idebenone, a short analogue of CoQ10 that acts as a potent free radical scavenger, protects Friedriech ataxia patients from iron/ROS injury in the heart muscle and reduces cardiac hypertrophy in these patients.[88] It remains to be determined if such a strategy, potentially targeting mitochondrial ROS generation, may be useful in patients with diabetic nephropathy.

It remains unexplained as to why no clear-cut beneficial effects of antioxidants that have undergone trials to date have been observed. A number of explanations have been raised including low absorption rates and the likelihood that conventional antioxidants such as various vitamins may not effectively reduce $O_2^{\cdot -}$ levels and indeed may be prooxidants in certain contexts. It has been suggested that more effective reductions in $O_2^{\cdot -}$ concentrations may be seen with a catalytic antioxidant, such as a SOD/catalase mimetic, that would continuously scavenge ROS. Indeed, the MnSOD mimetic, MnTBAP, prevented hyperglycemia-induced ROS injury in endothelial cells.[89] The role of such an approach in diabetic complications remains to be elucidated.

CONCLUSIONS

There is strong evidence to support the pathogenicity of excess AGE accumulation and ROS generation in diabetic nephropathy. It is essential to further understand these pathways and other metabolic and hemodynamic factors that may interact to contribute to the pathogenesis of diabetic complications. Such knowledge would aid in designing therapeutic interventions to add to the current treatment regimens that focus on blood glucose and blood pressure control. Combination therapies targeting multiple pathways are likely to be more successful than targeting single pathways alone. A variety of therapies including AGE formation inhibitors, AGE cross-link breakers, sRAGE, and free-radical scavengers ultimately may be proven to be the appropriate adjunct therapies to optimize the prevention of diabetes-associated renal injury.

A range of issues still need to be resolved in the field of advanced glycation. In particular, it is critical to further understand the relative importance of RAGE and the other AGE receptors that have been identified recently. With respect to oxidative stress in diabetic complications, further elucidation of the relative importance of the various sources of ROS generation is required. Furthermore, current pharmacologic strategies to target ROS generation and its biological effects do not appear to be particularly

potent and are relatively nonselective. Finally, it is critical that we understand in more detail how AGE and ROS interact, not only with each other, but also with other relevant pathways in diabetic complications such as the renin-angiotensin system. This would allow us to design therapies that target these pathways appropriately and in the long term provide better treatment strategies for diabetic patients at risk of or with established renal disease.

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