Role of Advanced Glycation End Products in Diabetic Nephropathy

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Abstract. Nonenzymatic reactions between sugars and the free amino groups on proteins, lipids, and nucleic acids result in molecular dysfunction through the formation of advanced glycation end products (AGE). AGE have a wide range of chemical, cellular, and tissue effects through changes in charge, solubility, and conformation that characterize molecular senescence. AGE also interact with specific receptors and binding proteins to influence the expression of growth factors and cytokines, including TGF-β1 and CTGF, thereby regulating the growth and proliferation of the various renal cell types. It seems that many of the pathogenic changes that occur in diabetic nephropathy may be induced by AGE. Drugs that either inhibit the formation of AGE or break AGE-induced cross-links have been shown to be renoprotective in experimental models of diabetic nephropathy. AGE are able to stimulate directly the production of extracellular matrix and inhibit its degradation. AGE modification of matrix proteins is also able to disrupt matrix–matrix and matrix–cell interactions, contributing to their profibrotic action. In addition, AGE significantly interact with the renin-angiotensin system. Recent studies have suggested that angiotensin-converting enzyme inhibitors are able to reduce the accumulation of AGE in diabetes, possibly via the inhibition of oxidative stress. This interaction may be a particularly important pathway for the development of AGE-induced damage, as it also can be attenuated by antioxidant therapy. In addition to being a consequence of oxidative stress, it is now clear that AGE can promote the generation of reactive oxygen species. It is likely that therapies that inhibit the formation of AGE will form an important part of future therapy in patients with diabetes, acting synergistically with conventional approaches to prevent diabetic renal injury.

The growing epidemic of diabetes will ultimately affect more people than any other disease in the Western world. More than 150 million people currently have diabetes, and twice that number is at high risk of developing diabetes in the next 5 to 10 yr (1). Despite the clear and present danger of diabetes, knowledge of the mechanisms by which accumulation of sugars contributes to blindness, cardiovascular disease, and kidney failure remains limited (2). Among the irreversible changes that occur as a result of hyperglycemia is the formation of advanced glycation end products (AGE) through a reaction between sugars and the free amino groups on proteins, lipids, and nucleic acids. These chemically heterogeneous compounds are known to have a wide range of chemical, cellular, and tissue effects implicated in the development and progression of diabetic pathology (Figure 1). In particular is the role of AGE in diabetic nephropathy, in which their importance is demonstrated in studies using inhibitors of advanced glycation to prevent albuminuria without influencing glycemic control (3).

In addition, direct in vivo exposure to AGE is able to generate lesions similar to those seen in diabetic nephropathy (4). This article discusses some of the mechanisms by which AGE may influence renal structure and function.

AGE and Extracellular Matrix Proteins
Diabetic nephropathy is characterized by the accumulation of extracellular matrix (ECM) protein in the glomerular mesangium and tubulointerstitium. In its simplest terms, this can be explained as an imbalance between the synthesis and degradation of ECM components, leading to the pathologic accumulation of collagens, fibronectins, and laminins. AGE are able to influence this balance in a variety ways.

Because of their slow turnover, ECM proteins are especially susceptible to AGE modification, resulting in alterations of both structure and function. For example, the formation of inter- and intramolecular cross-links after the glycation of collagen leads to structural alterations, including changes in packing density (5) and surface charge, manifested by increased stiffness, reduced thermal stability, and resistance to proteolytic digestion (6–8). The reduction in collagen pepsin solubility (as a result of the increased number of acid-stable cross-links in diabetic collagen) is reflected in a marked increase in acid-insoluble collagen in diabetic tissue (9). Cleavage of AGE-induced cross-links by agents such as N-phenacylthiazolium bromide and ALT-711 restores collagen solubility (10,11) associated with a reduction in matrix accumulation within the kidney (11).
Cell–matrix interactions may also be disrupted by matrix glycation, contributing to changes in cellular adhesion (12), altered cell growth, and loss of the epithelial phenotype. In addition, heterotypic interactions between matrix proteins are disturbed by AGE modifications. The affinity of laminin and fibronectin for type IV collagen and heparan sulfate proteoglycan is decreased after AGE modification (13,14). Glycation also inhibits the homotypic interactions required for polymeric self-assembly of type IV collagen (7) and laminin (14). These changes may be particularly apparent in the glomerular basement membrane, where the induction of chemical cross-links between amines leads to an increase in protein permeability (15). Cross-link breakers, however, are able to prevent the development of albuminuria in experimental diabetes (11).

ECM composition may also be substantially altered by AGE. The expression of extracellular proteins such as fibronectin and types I and IV collagen is increased by AGE in a dose- and time-dependent manner, in the presence (16) or absence of hyperglycemia (17–19). For example, the glomerular expression of type IV collagen and laminin is increased after the direct injection of AGE into mice (18). This has been considered to be a direct effect via AGE-specific receptors involving activation of the JAK/STAT signal transduction pathway (19), leading to the induction of profibrotic cytokines and growth factors, including TGF-β1, PDGF-B, and CTGF (2,20). CTGF (also known as IGF-binding protein-related protein-2) is a potent profibrotic agent and is increased in diabetic nephropathy (21). Inhibitors of advanced glycation such as aminoguanidine can prevent increased expression of CTGF in diabetes, associated with a reduction in tissue AGE levels and the prevention of mesangial expansion (22). In addition, we recently demonstrated that soluble AGE including carboxymethyllysine-containing proteins are able to induce the expression of CTGF and fibronectin production in cultured human mesangial cells (22). Similar changes also have been reported in human dermal fibroblasts, where the AGE-induced upregulation of CTGF is mediated through the receptor for AGE (RAGE) (23).

Excessive ECM production is also compounded by the increased numbers of interstitial fibroblasts, myofibroblasts, and infiltrating macrophages in diabetic nephropathy. Although some of these cells migrate to the interstitium as a result of chemokines released in response to injury, the transdifferentiation of tubular epithelial cells into a mesenchymal phenotype (tubulointerstitial-mesenchymal transdifferentiation [TMT]) has also been implicated in the accelerated fibrogenesis seen in diabetic nephropathy (24). TMT is regulated by several growth factors and cytokines, including TGF-β1, fibroblast growth factor, IL-1, and EGF (24). In addition, we have reported that AGE may induce TMT, potentially contributing to their profibrotic action (20). The mechanism of this action is the subject of ongoing research, but it seems to be receptor mediated, as blockade of the RAGE receptor prevents TMT, suggesting a crucial role for the AGE/RAGE interaction in cell differentiation. This process seems to be dependent on the activation of TGF-β1, as TMT in response to AGE can be blocked by a neutralizing antibody to TGF-β1 (Figure 2) (20). This downstream signaling pathway may be further regulated by intracellular mediators of the Smad family, in particular Smad2 or Smad3, which are phosphorylated by the type I TGF-β receptor (25). Recent reports also suggest that basement membrane composition and integrity is important for the maintenance of epithelial phenotype. Zisberg et al. (26) described how type I collagen (known to be upregulated by AGE and diabetes) promotes TMT. In addition, inhibition of assembly of type IV collagen NC1 hexamers (as occurs with collagen glycation (7)) facilitates EMT in vitro, possibly though the upregulation of TGF-β1 in tubular epithelial cells that follows the disruption of basement membrane architecture (26).

At the same time that matrix synthesis is augmented by AGE, the expression and activity of degradative matrix metalloproteinases are also reduced. Not only are AGE-modified proteins more resistant to enzymatic digestion, but also experimental diabetes is associated with a reduction in the matrix-degradative capacity of the kidney (27). This effect is replicated in vitro, with a 45% reduction in the matrix-degrading activity of matrix metalloproteinases secreted by mesangial cells after growth on glycated matrix (28). In both instances, glycation results not only in a decrease in the expression and activity of matrix metalloproteinases secreted by mesangial cells but also in the increased expression and activity of tissue metalloproteinase inhibitors.
Interactions between AGEs and the Renin-Angiotensin System

Overactivity of the intrarenal renin-angiotensin system (RAS) has been strongly implicated in the pathogenesis of diabetic nephropathy, although the source of this activation is not yet established (29). AGE significantly interact with the RAS as demonstrated by the reversal in AGE-induced collagen production by captopril in vitro, possibly by attenuating RAGE expression and JAK2/STAT activity (19). We have now demonstrated that in vivo angiotensin-converting enzyme (ACE) inhibition attenuates the formation and accumulation of AGE in experimental diabetes (30). This may represent a direct effect, as simultaneous incubation of ACE inhibitors with glucose and protein prevents the in vitro formation of AGE, possibly though metal chelation (31). In addition, ACE inhibitors reduce the formation of reactive oxygen species (ROS) associated with diabetic nephropathy and therein reduce the formation of AGE through glycoxidation (2). Treatment with ramipril seems to be similar in efficacy to aminoguanidine in reducing glomerular and tubular protein oxidation as assessed by renal nitrotyrosine levels (Figure 3). The source of oxidative stress in the diabetic kidney remains controversial. In vitro studies suggest a possible role for mitochondrial oxidation (2). In addition, the expression and activity of NAD(P)H oxidase...
may represent an important vascular and renal source of oxidative stress in conditions such as diabetes (32,33). Our own studies have demonstrated an increased expression of the membrane-bound nox-4 subunit in the diabetic kidney (30). However, this does not seem to be modified at the gene level by either ACE inhibition or with the AGE inhibitor aminoguanidine (30). By contrast, increased expression of cytosolic p47phox has been reported after 2 wk of streptozocin-induced diabetes, and this NAD(P)H subunit could be attenuated by either quinapril or candesartan (34). However, a link between oxidative stress and the production of AGE is difficult to establish in vivo. Indeed, AGE may generate ROS through stimulation of membrane-bound NAD(P)H oxidase via the RAGE receptor (33). In addition, AGE directly augment the formation of ROS through catalytic sites in their molecular structure (35).

### AGE, Transcription Factors, and Growth Factors

AGE result in the expression and activation of a number of transcription factors implicated in the development of diabetic nephropathy, including nuclear factor κB (NF-κB) and protein kinase C (PKC). This effect may be both direct (through AGE receptors) and indirect, via generation of free oxygen radicals leading to the production of cytokines, adhesion molecules, and chemokines (Figure 1). These pathways may be synergistic, as depletion of intracellular antioxidants reduces the AGE concentration needed for mesangial cell activation of NF-κB (36). In addition, the promoter region of the RAGE receptor gene contains NF-κB binding sites (37), potentially producing a self-perpetuating pathway.

AGE contribute to the release of proinflammatory cytokines and expression of growth factors and adhesion molecules implicated in the pathogenesis of the complications of diabetes. These include VEGF, CTGF, TGF-β1, IGF-I, PDGF, TNF-α, IL-1β, and IL-6 (16,20,38). In particular, the induction of TGF-β1 seems to be the key intermediate step for many of the AGE-mediated effects on cell growth and matrix homeostasis as noted earlier (20). In addition, inhibitors of advanced glycation reduce the overproduction of TGF-β1 in diabetic animals, independent of glycemic status (16). The transcriptional upregulation of TGF-β1 in diabetes seems to be mediated via PKC-dependent pathways for which AGE are a potent stimulus. Moreover, renoprotective treatment with inhibitors of AGE has been shown to attenuate renal PKC overexpression in diabetic rats (38). Some studies have also suggested an important role for oxidative stress in the AGE-induced TGF-β1 transcription. This is illustrated by the finding that antioxidants are able to prevent the upregulation of TGF-β1 after exposure to AGE (36). However, the failure of antioxidant therapy to prevent end-organ injury in the diabetic cohort of the Heart Outcomes Prevention Evaluation (HOPE) study casts some doubt on the pathologic significance of oxidative stress in progressive diabetic renal injury (39,40).

### The Future of AGE Inhibition

AGE accumulation in the glomerular and tubulointerstitial compartments and structural alterations of ECM proteins correlate with the severity of diabetic nephropathy. There is now strong evidence that AGE are directly pathogenic. Interventions to reduce renal AGE accumulation seem to be renoprotective in the context of diabetes. However, advanced glycation is only one pathway by which renal injury may be induced in diabetes. It seems likely that an interaction of metabolic and hemodynamic factors compound the deleterious effects of the diabetic milieu and reduce the threshold for injury via common mechanisms (40). Therapies that target multiple pathways may indeed be more successful than those that target one alone. It remains to be determined whether a combination of hemodynamic and metabolic pathways is more effective than any individual therapy in preventing diabetes-associated renal injury.

### References

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