Negative Consequences of Glycation

Michael Brownlee

The Diabetes Control and Complications Trial (DCCT) established unequivocally that the effects of inadequate insulin action (as monitored by the level of hyperglycemia) are associated with the incidence and progression of diabetic retinopathy, nephropathy, and neuropathy. How does hyperglycemia induce the functional and morphologic changes that characterize diabetic complications? Increasing evidence points to a major role for sugar-derived advanced glycation end products (AGEs), which form inside and outside cells as a function of glucose concentration. Recent work in this area supports a central role for reactive oxygen species (ROS) in both the formation of AGEs, and in AGE-induced pathologic alterations in gene expression. Inhibition of ROS may also be centrally important in the action of drugs that prevent complications in diabetic animal models.
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INCREASES IN ADVANCED glycation end product (AGE) accumulation precede and are accompanied by histological evidence of diabetic microvascular damage in the retina, the kidney, and the peripheral nerve.[1-5] AGEs may arise by several mechanisms. Formation of many AGEs involves the participation of reactive oxygen species (ROS). AGEs can be produced by autoxidation of the so-called Amadori product, a 1-amino-1-deoxyketose produced by the reaction of reducing sugars with protein amino groups.[6] Recently, it has been shown that dicarbonyl AGE intermediates may also form from metal-catalyzed autoxidation of sugars, with glyoxal and arabinose as intermediates. Nonoxidative pathways also exist, the best studied of which involve generation of the reactive dicarbonyl methylglyoxal from triose phosphates formed during glycolysis and the reactive dicarbonyl 3-deoxyglucosone.[7-20] In vivo, the Amadori adduct appears to be the more significant precursor of AGEs,[21] while in vitro it appears that approximately 50% of the AGE carboxymethyllysine originates from Amadori product oxidation, and 50% by other pathways.[22] In cultured endothelial cells, however, increased methylglyoxal production accounts for all of the increase in AGE formation.[23] Glucose has the slowest rate of glycosylation product formation of any naturally occurring sugar. Thus, the rate of AGE formation by such intracellular sugars as fructose, glucose-6-phosphate, and glyceraldehyde-3-phosphate is considerably faster than the rate for glucose.[10] For this reason, the rate of intracellular AGE formation is much more rapid than the rate of AGE formation in the extracellular compartment. The reactive dicarbonyl intermediates formed from Amadori products and from sugars react with protein amino groups to form a variety of AGEs. Increased levels of both 3-deoxyglucosone and methyglyoxal have been reported in diabetes.[18,24,25] A 2-oxoaldehyde reductase has been isolated and cloned which reduces 3-deoxyglucosone to 3-deoxyfructose. This enzyme appears to be identical to aldehyde reductase.[26] Glyoxylase I specifically converts methylglyoxal to D-lactate via the intermediate S-D-lactoylglutathione.[27] The nature and efficiency of such enzymes could be an important determinant of the amount of AGEs that form at any given level of blood glucose in both diabetic and nondiabetic patients. Inherited differences in the ability to enzymatically detoxify AGE-intermediates such as 3-deoxyglucosone and/or methylglyoxal may be one important genetic factor responsible for determining the impact of a given level of glycemia on diabetic complications.

There are 3 general mechanisms by which AGE formation may cause pathological changes. First, rapid intracellular AGE formation by glucose, fructose, and more highly reactive metabolic pathway-derived intermediates can directly alter protein function in cells that do not require insulin for glucose transport, such as microvascular endothelial cells and neurons. Second, extracellular AGEs alter matrix-matrix, matrix-cell, and cell-cell interactions. Third, AGE interactions with cellular receptors alter the level of gene expression for a variety of molecules involved in the genesis of vascular, and perhaps also neural pathology, by generation of reactive oxygen species and activation of the pleiotropic transcription factor NFκB.

AGES INCREASE MUCH MORE RAPIDLY INSIDE CELLS THAN OUTSIDE, ALTERING INTRACELLULAR PROTEIN FUNCTION

AGEs have been thought to form only on long-lived extracellular macromolecules, because the rate of AGE formation from glucose is so slow that more rapidly turned over intracellular proteins would not exist long enough to accumulate them. Recently, however, it has been shown that AGEs do, in fact, form on proteins in vivo. After only 1 week, AGE content increases 13.8-fold in endothelial cells cultured in high glucose-containing media.[28] This extremely rapid rate of AGE formation most likely reflects hyperglycemia-induced increases in intracellular glycolytic intermediates, which are much more reactive than glucose. Recently, we have shown[23] that hyperglycemia-induced increases in endothelial cell macromolecular endocytosis can be completely prevented by inhibition of methylglyoxal-derived intracellular AGEs. These data support the hypothesis that AGE modification of intracellular proteins can alter vascular cell function.

AGES INTERFERE WITH NORMAL MATRIX-MATRIX, MATRIX-CELL, AND CELL-CELL INTERACTIONS

AGE formation alters the functional properties of several important matrix molecules. Recently, Monnier[10] showed that the formation of these AGE crosslinks on extracellular matrix

From the Departments of Medicine and Pathology, Albert Einstein College of Medicine, Bronx, NY.

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Address reprint requests to Michael Brownlee, MD, Departments of Medicine and Pathology, Albert Einstein College of Medicine, 1300 Morris Park Ave, Bronx, NY 10461.

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require the participation of ROS.[29] Collagen was the first matrix protein used to show that glucose-derived AGEs form covalent, intermolecular bonds.[30,31] On type I collagen, this crosslinking induces an expansion of the molecular packing.[32] Soluble plasma proteins such as LDL and immunoglobulin (Ig) G are also covalently crosslinked by AGEs on collagen.[33-35] The lumenal narrowing that characterizes diabetic vessels may arise, in part, from accumulation of subendothelial AGE-linked plasma proteins. AGE formation on type IV collagen from basement membrane inhibits lateral association of these molecules into a normal network-like structure by interfering with binding of the noncollagenous NC1 domain to the helix-rich domain.[36] In vitro AGE formation on intact glomerular basement membrane increases its permeability.[37]

AGE formation on extracellular matrix not only interferes with matrix-matrix interactions, it also interferes with matrix-cell interactions. For example, AGE-modification of type IV collagen's cell-binding domains decreases endothelial cell adhesion.[38] AGE-modification of either basement membrane components or whole retinal basement membrane causes reduced proliferation of retinal pericytes and increased proliferation of retinal endothelial cells; the same changes observed in diabetic patients.[39] These AGE-induced abnormalities in extracellular matrix function alter the structure and function of intact vessels. AGEs decrease elasticity in large vessels from diabetic rats, even after vascular tone is abolished, and increase fluid filtration across the carotid artery.[40] Defects in the vasodilatory response to nitric oxide correlate with the level of accumulated AGEs in diabetic animals, because of dose-dependent quenching by AGEs.

AGE-RECEPTORS MEDIATE PATHOLOGICAL CHANGES IN GENE EXPRESSION

Specific receptors for AGEs were first identified on monocytes and macrophages. These receptors, designated p60 and p90, have been shown to be identical to OST-48 and 80K-H, respectively.[41] AGE-protein binding to these receptors stimulates macrophage production of interleukin-1, insulin-like growth factor I, tumor necrosis factor-α, and granulocyte-macrophage colony-simulating factor at levels that have been shown to increase glomerular synthesis of type IV collagen and to stimulate proliferation of both arterial smooth muscle cells and macrophages.[42-44]

Vascular endothelial cells also express AGE-specific receptors. A 35-kd and a 46-kd AGE-binding protein have been purified to homogeneity from endothelial cells.[45-47] The N-terminal sequence of the 35-kd protein was identical to lactoferrin, while the 46-kd protein was novel. A full-length 1.5-kb cDNA for the 46-kd protein was cloned and sequenced. This novel AGE-binding protein, designated receptor for AGEs (RAGE), appears to be a member of the Ig superfamily, with 3 disulfide-bonded Ig homology units. Other putative AGE receptors include galectin-3[41] and the scavenger receptor type II. In endothelial cells, AGE-binding to its receptor induces changes in gene expression that include alterations in thrombomodulin, tissue factor, and vascular cell adhesion molecule-1.[48-50] These changes induce procoagulatory changes in the endothelial surface, and increase the adhesion of inflammatory cells to the vessel wall. In addition, endothelial AGE-receptor binding appears to mediate in part the hyperpermeability induced by diabetes.[51]

The RAGE receptor appears to mediate signal transduction through the generation of oxygen free radicals. Reactive oxygen species are generated by AGE binding to endothelial cells. These reactive oxygen species activate the free radical-sensitive transcription factor NF-kappaB, a pleiotropic regulator of many "response-to-injury" genes. This signal transduction cascade can be blocked by antibodies to either of the AGE-receptor components and by antibodies to AGEs themselves.[52] The antioxidant alpha lipoic acid blocks the AGE receptor-induced production of oxygen radicals and activation of NFkappaB in cultured endothelial cells,[53] which may explain its beneficial effect in the treatment of diabetic peripheral sensory neuropathy.[54,55] Intracellular AGE formation may also affect DNA function directly. AGEs that form on prokaryotic DNA in vitro can cause mutations and DNA transposition in bacteria and mammalian cells.[56-59] Incubation of nucleotides with Amadori products or methyglyoxal yields N2-1-(1-carboxyethyl)guanine as a major AGE product.[60]

PHARMACOLOGICAL INHIBITION OF AGE FORMATION

Pharmacological agents that specifically inhibit AGE formation have made it possible to investigate the role of AGEs in the development of diabetic complications in animal models. The hydrazine compound aminoguanidine was the first AGE inhibitor discovered,[30] and it has been by far the most extensively studied. Aminoguanidine reacts mainly with non–protein-bound dicarbonyl intermediates such as 3-deoxyglucosone[61] and methylglyoxal.[62] Because a variety of antioxidants have been shown to inhibit intracellular AGE formation[63] we recently evaluated the ability of therapeutic levels of aminoguanidine to function as a scavenger of ROS.[64] Aminoguanidine effectively scavenged hydroxyl radicals in vitro, and prevented diabetes-induced elevation of vitreous lipid peroxidation in vivo. The effects of aminoguanidine on diabetic pathology have been investigated in the retina, the kidney, the nerve, and the artery. In the rat retina, diabetes causes a 19-fold increase in the number of acellular capillaries. Aminoguanidine treatment of diabetics prevented excess AGE accumulation, and reduced the number of acellular capillaries by 80%. Aminoguanidine treatment had a similar effect on the number of diabetic eyes positive for microaneurysms. Diabetes-induced pericyte dropout was also markedly reduced by aminoguanidine treatment.[65] Aminoguanidine treatment also inhibited the development of accelerated diabetic retinopathy in the spontaneous hypertensive rat model, suggesting that hypertension-induced deposition of AGEs in the retinal vasculature plays an important role in the acceleration of diabetic retinopathy by hypertension.[66] Secondary intervention studies have shown that aminoguanidine treatment is as effective as islet transplantation in retarding the progression of established disease in the rat model, but no reversal is observed.[67] Agents clearly need to be developed that can disrupt already formed AGEs, as well as prevent the formation of new AGEs.

Similar results have been obtained in animal models of diabetic kidney disease.[68-70] Diabetes increased AGEs in the

renal glomerulus, and aminoguanidine treatment prevented this diabetes-induced increase. Untreated diabetic animals developed albuminuria that averaged 30 mg/24 h by 32 weeks. This was more than a 10-fold increase above control levels. In aminoguanidine-treated diabetics, the level of albumin excretion was reduced by nearly 90%.[68] In hypertensive diabetic rats, aminoguanidine treatment also prevented albuminuria without affecting blood pressure.[70] Untreated diabetic animals also developed the characteristic structural feature of human diabetic nephropathy, increased fractional mesangial volume. When diabetic animals were treated with aminoguanidine, this increase in fractional mesangial volume was completely prevented.

In the peripheral nerve of diabetic rats, both motor nerve and sensory nerve conduction velocity are decreased after 8 weeks of diabetes.[71] Nerve action-potential amplitude is decreased by 37%, and peripheral nerve blood flow by 57% after 24 weeks of diabetes.[72] Aminoguanidine treatment prevents each of these abnormalities of diabetic peripheral nerve function.[71,72]

Inhibition of AGE formation by aminoguanidine treatment also ameliorates the effects of diabetes on large arteries. In animal models, aminoguanidine treatment increased elasticity as measured by static compliance, aortic input impedance, and left ventricular power output. Abnormal increases in fluid filtration across the carotid wall were also significantly reduced.[40]

The place of aminoguanidine and other inhibitors of AGE formation and/or intracellular oxidant stress for the treatment of diabetic complications must ultimately be defined by multicentered, randomized, double-blinded clinical studies.

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