Getting Better Without AGE
New Insights Into the Diabetic Heart

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A century ago, Maillard first observed that incubation of glucose with amino acids led to the formation of a yellow-brown pigment due to nonenzymatic glycosylation.1 This unstable compound known as a Schiff base can undergo rearrangement over several days to form the more stable Amadori-type product.2 One well-known example is hemoglobin A1C, the adduct of glucose with the N-terminal valine amino group of the β-chain of hemoglobin.3 This process of nonenzymatic protein glycation differs from enzyme-dependent α-glycosylation, the latter being a reversible process whereby proteins are modified at specific residues to effect signal transduction much like phosphorylation.4,5 Glycated proteins, in contrast, can further evolve over time, undergoing complex rearrangements to yield crosslinked proteins known as advanced glycation end products (AGEs)2,6 (Figure). Importantly, and in contrast to Amadori product precursors, AGEs are virtually irreversible once formed. Long-lived structural proteins such as collagen are particularly vulnerable to AGE crosslinks by nature of their slow turnover rate.

AGE crosslinking alters protein biochemistry by reducing enzymatic activity,6 altering biophysical properties,9,10 and changing protein interactions with other enzymes. In the case of collagen, AGE links form throughout the molecule, contrasting to the more limited terminal positions for normal crosslinking, and this increases its tensile stiffness. In addition, AGE crosslinks render collagen less digestible by metalloproteinases,11,12 which in turn favors its chronic accumulation in tissue. This can be particularly relevant to disorders in which collagen synthesis is stimulated by inflammatory cytokines, neurohormones, or mechanical stress. AGE formation does not require diabetes, although the existence of elevated sugar increases the likelihood of early less stable glycation products forming, and so is an important contributor to crosslink formation. However, studies have also shown that AGE crosslinks develop simply over time, ie, aging itself.5 These senescent changes play an important role in disorders such as diverse as Alzheimer’s disease13 and arterial and myocardial stiffening that contributes prominently to cardiovascular risks in the elderly.7

AGE also plays an important role in cell signaling, working by interaction with specific receptors including RAGE, AGE-R2, and AGE-R3. RAGE signaling has been linked to the activation of p21(ras), NF-κB, oxidant radical formation, proinflammatory cytokines and growth factors (eg, interleukin-6, tumor necrosis factor-α, and tissue growth factor β1), and adhesion molecule expression.14,15 Such signaling is implicated in diabetic glomerular nephropathy and with endothelial dysfunction and diabetic vasculopathy. In contrast, AGE-R3 (Galectin-3) may play a protective role, as mice lacking galectin-3 display accelerated glomerulopathy.16 AGE has been implicated in reducing endothelial cell function17 and triggering premature senescence in part due to abnormalities in nitric oxide physiology and the generation of oxidant species such as peroxynitrite.18 AGE has further been linked to upregulation of connective tissue growth factor (CTGF),19 also known as insulin-like growth factor–binding protein-related protein. CTGF is a potent inducer of extracellular matrix synthesis and angiogenesis, and it is often increased in tissues of models of diabetes.20

There are a variety of approaches that can block the pathophysiologic effects of AGE. First, AGE and AGE crosslink formation can be inhibited by compounds such as aminoguanidine (AG), pyridoxamine, or OPB-9195 [(±)-2-iso-propylidenehydrazono-4-oxo-thiazolidin-5-ylacetanilide].21 These compounds are designed to trap carbonyl intermediates to prevent modification of nucleophilic residues in proteins, and are all potent inhibitors of AGE formation and ultimate AGE crosslinking. Studies using AG were among the first to demonstrate a role of AGE crosslinks in vascular stiffening and reduced cardiac diastolic compliance in various experimental models involving diabetes mellitus or aging. Such investigations reported that AG treatment enhanced cardiac diastolic compliance,22 blunted age-dependent decline in arterial distensibility,23 and inhibited CTGF expression and CTGF-dependent matrix accumulation in diabetic nephropathy.24 In addition to preventing AGE formation, these agents have been reported to chelate metal ions at concentrations that are physiologically relevant, so that part of their effects may be as antioxidants.25

A second method for inhibiting AGE effects is to target RAGE by infusing a soluble extramembrane portion of the receptor (sRAGE) to act as a false ligand or using RAGE antibodies. sRAGE administration has been demonstrated to improve wound healing in diabetic mice26 and to suppress early acceleration of atherosclerosis as well as stabilize established atherosclerosis in diabetic apolipoprotein E–null mice.27,28 Newer small molecular alternatives to RAGE inhibition are presently under development.

Another approach to inhibiting AGE pathophysiology is the use of crosslink breaker compounds. These agents contain a thiazolium structure that can break α-carbonyl compounds.

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by cleaving the carbon-carbon bond between carbonyls. Among this class, 4,5-dimethyl-3-phenacylthiozolium chloride (ALT-711) has been the most widely studied. Incubation of AGE crosslinked collagen with ALT-711 in vitro restores MMP digestibility of the collagen, a key often used marker of link-breaker effect. In aged dogs, ALT-711 was shown to enhance cardiac diastolic compliance in association with an improvement in net cardiac output due to greater net filling and stroke volume.\(^{29}\) In nonhuman primates, ALT-711 improved arterial distensibility as reflected by the pulse wave velocity.\(^{30}\) Effects required several weeks to fully develop and were sustained for several weeks after drug cessation. Most recently, ALT-711 has been tested in older human subjects with basal elevation of arterial pulse pressure and reduced arterial compliance.\(^{31}\) The drug improved arterial compliance and lowered pulse pressure compared with placebo control, and importantly, these changes occurred without a disproportionate effect on mean arterial pressure. While such studies have highlighted the physiological potency and potential clinical efficacy of this class of drug, there has been very little assessment to date regarding the impact of ALT-711 on AGE-dependent signaling or restoring structural molecular physiology.

In the investigation by Candido and colleagues,\(^{32}\) reported in this issue of *Circulation Research*, important new insights regarding the impact of breaking AGE crosslinks on the diabetic heart are revealed. Using a standard rat model of diabetes induced by streptozotocin, the investigators treated animals for 4 months of ALT-711 versus placebo. They report enhanced collagen solubility, reduced collagen III, reduced expression of RAGE protein and AGE-R3 mRNA, and reduced CTGF expression in ALT-711-treated rats compared with diabetic controls. Some of these changes, for example, the decline in CTGF and RAGE expression, were observed in nondiabetic control rats treated with ALT-711 as well, suggesting a mechanism that may not require excessive accumulation AGE crosslinks. Other changes were observed only in the diabetic animals.

One important limitation of the study was the absence of functional analysis to couple with the structural and molecular investigation. The authors did report a significant decline in brain natriuretic peptide and reduced left ventricular mass with ALT-711 treatment, and this may well have reflected a fall in filling stresses associated with improved diastolic distensibility. However, changes in systolic function as well as alternative signaling mechanisms may have played a role, and this important question remains to be answered. Prior studies have generally shown an association between increased collagen type I and/or III ratio with diastolic chamber stiffening.\(^{33}\) Thus, the physiological consequence of the ALT-711–induced decline in type III collagen is unclear.

Furthermore, whether changes in cardiac properties were the direct result of ALT-711 or due in part to reduced systemic unloading remains unclear. ALT-711 treatment did not diminish arterial systolic pressure in the present study. However, similar findings have been previously reported in dog and nonhuman primates,\(^{29,30}\) despite a significant decline in systemic vascular resistance, a result of increased stroke volume and thus cardiac output. In contrast, the recent human trial of ALT-711 did not reveal significant effects on vascular resistance, although diabetic subjects were not specifically targeted in this study.\(^{31}\) The results for TGF\(\beta1\) and \(\beta\)ig-h3, the markers of TGF\(\beta1\) activity, were somewhat surprising in that AGE and AGE-RAGE interaction have been shown to increase TGF\(\beta1\) expression in kidney and vasculature in various diabetic model studies.\(^{34}\) This may reflect specific features of the present model and study time points or differences between cardiac and other end-organ AGE-RAGE and AGE crosslink signaling. Finally, while enhanced collagen solubility and amount might be anticipated by a breaker compound, the mechanisms for alterations in AGE receptor or CTGF expression are less clear. As noted, similar declines were observed in normal hearts, suggesting a mechanism that may not entail AGE links.

ALT-711 is presently in advanced phase II clinical trials that aim to establish dose ranges, test its efficacy for the treatment of systolic hypertension with and without ventricular hypertrophy, and determine its utility to improve patients with cardiac failure symptoms despite preserved ejection fraction. The latter is perhaps most directly relevant to the study of Candido et al.,\(^{32}\) in that reduced diastolic compliance is thought to play a role in this disorder. The results of these
trials are anticipated shortly and should help clarify the potential that this and future similar agents may play in the treatment of disorders featuring AGE-dependent stiffening and altered signaling. In the meantime, the work of Candido and colleagues and other recent studies supports the contention that unlike fine wine, hearts improve without “AGE.” Ongoing efforts to block the impact of AGE and AGE crosslinks may soon offer a novel and important avenue to enhance cardiovascular health.

References


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