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Dicarbonyl Stress and Atherosclerosis: Is It All RAGE?

Diabetes 2014;63:3587–3589 | DOI: 10.2337/db14-0953

Atherosclerotic vascular disease is a major cause of cardiovascular (CV) morbidity and mortality and a significant driver of health care costs in patients with diabetes. Systemic abnormalities, including dyslipidemia, insulin resistance, hyperinsulinemia, hyperglycemia, oxidative stress, accentuated renin-angiotensin system, and tissue inflammation, have been proposed to play a role in mediating accelerated atherosclerosis in diabetes (1). Hyperglycemia not only predicts CV disease risk in individuals without known CV disease or diabetes (2), but atherosclerosis is also quite prevalent in prediabetes (3). Although difficult to achieve, near-normal glycemic control is not typically associated with further reduction in macrovascular events in patients with type 2 diabetes (4). Consequently, elucidating mechanisms by which hyperglycemia affects atherosclerosis is necessary to identify novel therapeutic targets.

Increased mitochondrial reactive oxygen species (ROS) has been identified as a common upstream event that mediates the atherogenic effects of hyperglycemia (5). ROS increases the formation of advanced glycation end products (AGEs), augments the expression of the receptor for AGEs (RAGE) and its ligands, activates protein kinase C (PKC) isoforms (primarily PKCβ isoform), and accentuates flux through the hexosamine and polyol pathways (5). Among these, the ligand-RAGE axis appears to play a major role in vascular dysfunction in diabetes. AGEs, S100/calgranulins, and high-mobility group box 1 are principal endogenous ligands for RAGE (6). Persistent activation of RAGE promotes atherosclerosis by activating a diverse array of intracellular signaling pathways that stimulate expression of cytokines, cellular adhesion molecules, growth factors, ROS, and vascular matrix metalloproteinases (6). Diabetes-induced atherosclerosis and vascular dysfunction are attenuated in RAGE-null mice confirming the pivotal role played by the ligand-RAGE axis (7,8).

AGEs are formed by rearrangement of Amadori products or as a result of reactions between dicarbonyls and amino acid residues (lysine, arginine, and cysteine) (9). Methylglyoxal (MG) is a highly reactive α-dicarbonyl and a major precursor of cellular and circulating AGEs (9). MG is primarily derived from triose phosphate intermediates formed during glycolysis (9). In humans, plasma/tissue MG concentrations are ~1–5 μmol/L (10). More than 99% of cellular MG is metabolized by glyoxalase 1 (GLO1), GLO2, and reduced glutathione to D-lactate (11). Glycation of arginine residues by MG results in the formation of hydroimidazolones (MG-H1, MG-H2, and MG-H3) and that of lysine leads to N^{ϵ}-(carboxymethyl) lysine (CML) and N^{ϵ}-(carboxyethyl) lysine (CEL) (12). MG-H1 is the most prevalent MG-derived AGE with plasma concentration of ~17 μmol/L in healthy individuals (12). Unlike CML/CELs, MG-Hs have a higher binding affinity to RAGE (K_D, ~40 nmol/L vs. 100 μmol/L) (13). Hyperglycemia and diabetes are associated with increased formation and decreased metabolism of MG (14,15). Thus, MG-Hs are capable of sustained activation of RAGE in the vasculature and circulating inflammatory cells (Figure 1).

Overexpression of GLO1 reduces dicarbonyl stress and attenuates diabetes-induced endothelial dysfunction, impaired neovascularization, and nephropathy (16–19). Similarly, knockdown of GLO1 in rodents is associated with increased MG-H1 levels in the kidney and, even in the face of normoglycemia, is associated with mesangial sclerosis and proteinuria, features typical of diabetic nephropathy (19). These studies suggest that dicarbonyl stress triggers renal pathology directly and independent of glycemia. Whether dicarbonyl stress similarly modulates atherosclerosis is unknown. In this issue of *Diabetes*, Tikellis et al. (20) examined the atherogenic effects of dicarbonyl and the potential role of RAGE in that process. To that end, they demonstrate that dicarbonyl stress due

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See accompanying article, p. 3915.

to exogenous MG supplementation or chemical inhibition of GLO1 in normoglycemic apolipoprotein E knockout ($apoE^{-/-}$) mice is associated with increased atherosclerosis. Furthermore, they show that the atherogenic effects of dicarbonyl stress due to excess MG exposure are significantly abrogated in RAGE-deficient $apoE^{-/-}$ mice. These results suggest that excess MG initiates and augments atherosclerosis predominantly through RAGE activation and independent of glycemia.

Endothelial cells exposed to MG and aortas of MG-treated mice demonstrated increased functional adhesiveness and expression of genes associated with leukocyte adhesion and vascular inflammation (intercellular adhesion molecule-1 [ICAM-1], vascular cell adhesion molecule-1 [VCAM-1]) in wild-type mice. However, endothelial cells and aortas of RAGE/$apoE^{-/-}$ mice subjected to dicarbonyl stress had a significantly reduced leukocyte adhesion but a higher gene expression of VCAM-1, ICAM-1, and tetherin. Cellular mechanisms mediating these differential effects of RAGE activation and/or non-RAGE–mediated downstream pathways need further elucidation. Similarly, cell permeable superoxide dismutase mimetic prevented GLO1 inhibitor–induced expression of MCP-1, ICAM-1, and VCAM-1 in endothelium of RAGE knockout but not in wild-type mice. It is likely that MG activates distinct ROS-generating pathways in RAGE replete versus deficient endothelium.

BBGC, a chemical inhibitor of GLO1, was used to induce dicarbonyl stress in $apoE^{-/-}$ mice. However, a recent study examined if GLO1 overexpression decreased atherosclerosis in streptozotocin-treated $apoE^{-/-}$ mice, and if GLO1 knockdown can promote atherogenesis in nondiabetic $apoE^{-/-}$ mice (21). In contrast to the current report by Tikellis et al., despite a decrease in GLO1 activity (by ~75%) and increased aortic MG-H1 content, *Glo1* and *apoE* double knockout mice did not show increased atherosclerosis compared with $apoE^{-/-}$ mice. Furthermore, GLO1 overexpression prevented renal dysfunction but not atherosclerosis in streptozotocin-treated $apoE^{-/-}$ mice. Thus, chemical inhibition and gene ablation of GLO1 appear to have differential effects on atherosclerosis. As opposed to global inhibition of GLO1 with BBGC, reduced expression of GLO1 may be variable and tissue- or cell-specific (circulating inflammatory cells vs. resident vascular cells), which may explain the discrepant results. Nonetheless, future studies are needed to reconcile these seemingly contrasting results.

Glycation of LDL by MG promotes arterial atherogenicity by increasing small, dense LDL levels and modifying apoB100; both changes favor increased LDL retention in the vascular wall (22). In mouse models of atherosclerosis ($apoE^{-/-}$), plasma cholesterol is transported in lipoprotein remnants, as opposed to LDL in humans

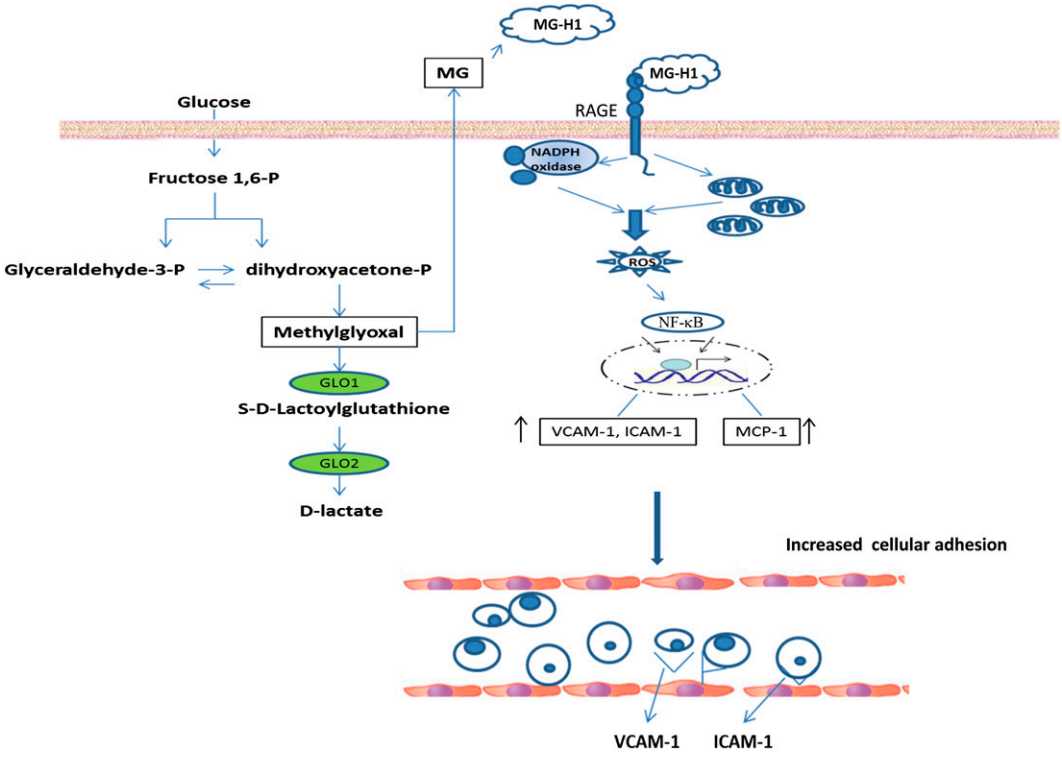

Figure 1—Dicarbonyl stress and RAGE signaling. Increased flux through the glycolytic pathway increases MG formation. Reduced GLO1 activity in this setting decreases the degradation of MG, resulting in excess formation of MG-H1. MG-H1 activates RAGE. RAGE activation leads to increased ROS production that subsequently activates the transcription factor, nuclear factor-κB (NF-κB), to stimulate the expression of adhesion molecules and chemokines. P, phosphorylation.

(23). Thus, the magnitude of the effects of dicarbonyl stress on lipids may be different and species-dependent. In addition, mice do not exhibit coronary atherosclerosis or unstable plaques. Rupture-prone arterial plaques in humans are characterized by lower GLO1 expression and increased levels of MG-H1, inflammatory cytokines, and cellular apoptosis markers (24). Higher plasma levels of AGEs (CML, CEL, and pentosidine) increase the risk of incident CV events in individuals without prior CV events (25). Although the causal relationship cannot be ascertained, findings from these human studies suggest that AGEs may play an important role in atherosclerosis/plaque rupture and serve as a potential biomarker that helps improve CV risk reclassification.

In conclusion, the findings by Tikellis et al. (20) are novel and highlight the glucose-independent effects of dicarbonyl stress on atherosclerosis that are primarily mediated through RAGE activation. Whether GLO1 can be therapeutically targeted to reduce dicarbonyl stress and prevent atherosclerosis remains to be determined.

Funding. This work was supported by the Intramural Research Program of National Institute of Diabetes and Digestive and Kidney Diseases and the Extramural Research Program, National Heart, Lung, and Blood Institute, National Institutes of Health.

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

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