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The Critical Role of Methylglyoxal and Glyoxalase 1 in Diabetic Nephropathy

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COMMENTARY

The discovery of increased formation of methylglyoxal (MG) by cell metabolism in high glucose concentration in vitro suggested possible relevance to diabetes and diabetes complications (1,2). MG is the precursor of quantitatively important advanced glycation end products (AGEs) of protein and DNA- and MG-derived AGEs increase in experimental and clinical diabetes (3,4). Increased MG and its metabolism by glyoxalase 1 (Glo1) was linked to clinical microvascular complications (nephropathy, retinopathy, and neuropathy) (5). Current clinical treatment decreasing MG and MG-derived AGEs, such as insulin lispro (6,7), has some clinical benefit in diabetic nephropathy (8), although the decrease in MG-derived AGE exposure is minor—~17% (7). Greater benefits may be achieved with specific and effective anti-MG targeted therapy. An outstanding research problem is to gain unequivocal evidence that MG glycation is a key mediator of vascular complications and, if possible, provide some pointers as to how MG glycation could be effectively countered. In this issue, the study by Giacco et al. (9) provides key evidence by a functional genomic approach manipulating expression of Glo1 to increase or decrease endogenous MG glycation. The outcomes show that development of experimental diabetic nephropathy is driven by increased levels of MG glycation and increasing renal expression of Glo1 prevents this. Recent research has shown Glo1 expression may be increased by small molecule inducers (10). Taken together, these findings suggest that prevention and treatment of diabetic nephropathy and possibly other complications of diabetes may be improved by development of Glo1 inducers.

MG is an endogenous α-oxoaldehyde or dicarbonyl metabolite formed mainly from the degradation of triose phosphates—a minor spontaneous "leak" from the metabolic flux through anaerobic glycolysis (Fig. 1). Other usually minor contributions to MG formation are from the oxidation of acetone—increased in diabetic ketoacidosis—catabolism of threonine, and nonenzymatic degradation of glucose and proteins glycated by glucose (11). MG is a highly potent glycating agent with specific reactivity ~20,000-fold higher than that of glucose. This is tolerable in vivo because efficient detoxification of MG by Glo1 maintains the concentration of MG in plasma approximately 50,000-fold lower than that of glucose. In diabetes, however, MG concentrations and MG-derived AGEs increase in plasma and at sites of complications development (4,12,13). MG is formed mainly inside cells but a minor fraction leaks out and so glycation of both cellular and extracellular proteins by MG increases (4,14,15).

MG is unlike glucose in its glycation reaction with proteins. Glucose reacts with proteins on lysine side chain or N-terminal amino groups to initially form fructosamine adducts—such as glycated hemoglobin A_{1c}. MG is rather an arginine-directed glycating agent mainly forming the hydroimidazolone adduct MG-H1 (16) (Fig. 1). As a consequence, the functional impairment of protein is more likely by MG glycation because arginine residues are more common in functional domains of protein than lysine residues (19.5 vs. 12.5%) (17). Moreover, arginine residues within functional domains are hot spots for MG modification. For example, arginine residues within the RGD and GFOGER motifs of integrin-binding domains of collagen IV lining the walls of blood vessels are hot spots for MG glycation and modification triggers detachment of endothelial cells (15). MG glycation has the potential to seek out and damage sensitive sites in tissues and blood vessels. Although the steady-state levels of MG-modified proteins is low, usually 1–5%, their physiological effects are amplified by a "gatekeeper" role in dysfunction, such as MG-modified mitochondrial

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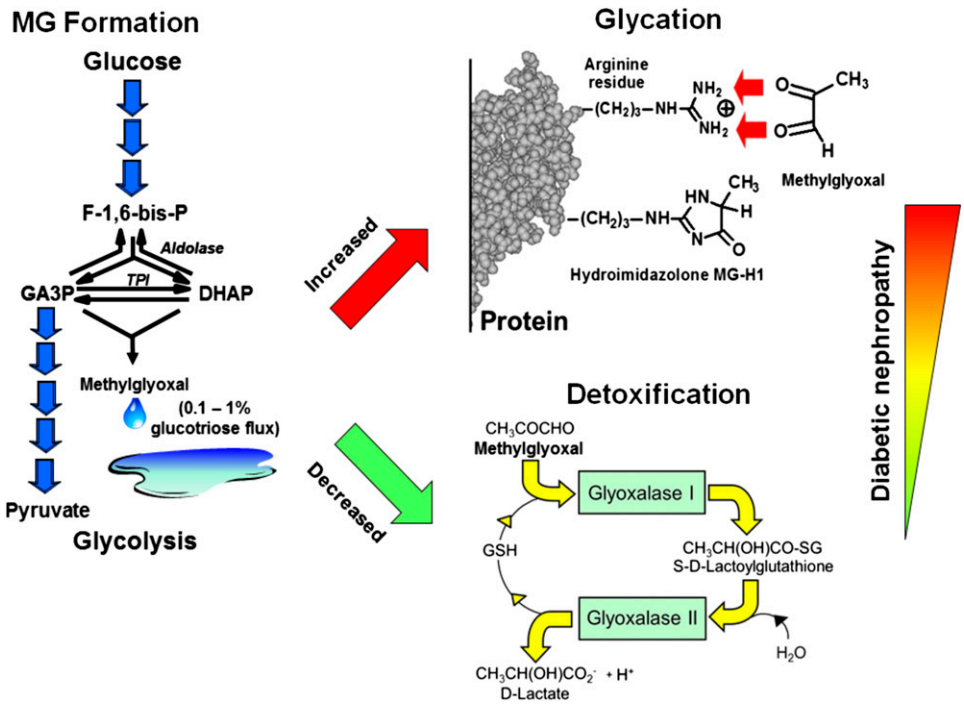

Figure 1—MG formation, protein glycation, and detoxification by the glyoxalase system in diabetic nephropathy. DHAP, dihydroxyacetone phosphate; F-1,6-bis-P, fructose 1,6-bisphosphate; GA3P, glyceraldehyde-3-phosphate; TPI, triose phosphate isomerase.

proteins leaking reactive oxygen species causing oxidative stress, cell detachment from MG-modified extracellular matrix, and atherogenic transformation of MG modification of LDL (17).

The first line of defense against MG glycation is the glyoxalase system (Fig. 1). This is a glutathione-dependent enzymatic pathway in the cytosol of all cells. Glo1 catalyses the first step and hence is a key regulator of cellular and extracellular levels of MG and MG-derived AGEs. It is estimated that an adult human produces ~3 mmol MG per day but only ~0.3% of this forms glycation adducts—the remaining 99.7% is metabolized, mostly by Glo1 (16). In diabetic patients, the flux of formation of MG is increased twofold to fourfold, depending on glycemic control, and the formation of MG-derived AGEs is increased similarly. MG-derived AGEs may be increased disproportionately higher because of downregulation of Glo1 and increased degradation of Glo1 at some sites (18,19).

Giacco et al. (9) produced mice expressing Glo1 small interfering RNA that knocked down Glo1 expression by 45–65%. These mice showed increased MG-H1 residues in the proteins of renal glomeruli and tubules, with concomitant development of albuminuria and mesangial expansion in the nondiabetic state. They also produced a line of Glo1 transgenic mice with human Glo1 expression under the preproendothelin-1 promoter, yielding increased Glo1 expression in the vascular endothelium, glomerular mesangial cells, and tubular epithelium. Glo1 transgenic mice prevented increased renal MG-H1 content in streptozotocin-induced diabetes with concomitant prevention of albuminuria and mesangial expansion.

Weaknesses of the study are absence of supporting estimates of MG content of the kidney and corroboration of immunohistochemical detection of MG-H1 with quantitative mass spectrometric measurement, as used in other mouse disease models (20). These may emerge in future studies. An unexpected finding was no significant further increase of MG-H1 content in the kidneys of Glo1 knockdown mice with streptozotocin-induced diabetes. This may be due to increased turnover of MG-H1 modified proteins, preventing further elevation of the steady-state protein content of MG-H1. MG-H1–modified proteins are targeted for cellular proteolysis, decreasing the half-life of the protein substrate. If there is no compensatory increase in protein expression, the renal content of proteins susceptible to MG glycation will be decreased. If there is compensatory gene expression, protein targets of MG glycation may have a transcriptional signature in diabetic nephropathy. Proteomic and transcriptional signatures of increased MG glycation in diabetic nephropathy and other vascular complications are areas for future research.

Giacco et al. (9) provide encouragement for the development of Glo1 inducers for the prevention of

diabetic nephropathy and to explore the use of MG-H1–modified proteins and peptides as biomarkers of early-stage diabetic renal disease.

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

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