Advanced glycation end products in the pathogenesis of chronic kidney disease

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Advanced glycation end products (AGEs) are stable posttranslational modifications of proteins formed by the spontaneous reaction with glucose and related metabolites. Important AGEs quantitatively are methylglyoxal (MG)-derived hydroimidazolone MG-H1, N_ε-carboxymethyl-lysine (CML), and glucosepane. They contribute to the development of chronic kidney disease (CKD). Cellular proteolysis of AGE-modified proteins forms AGE free adducts, glycated amino acids, which are cleared by the kidneys and excreted in urine. Dietary AGEs mainly supplement the endogenous flux of AGE free adduct formation. AGE free adducts accumulate markedly in plasma with decline in glomerular filtration rate. A key precursor of AGEs is the dicarbonyl metabolite MG, which is metabolized by glyoxalase 1 (Glo1) of the cytoplasmic glyoxalase system. Proteins susceptible to MG modification are collectively called the dicarbonyl proteome. Abnormal increase of MG dicarbonyl stress is a characteristic of CKD, driven by down-regulation of renal Glo1, increasing flux of MG-H1 formation. Protein inactivation and dysfunction linked to the dicarbonyl proteome contributes to CKD development. The receptor for AGEs, RAGE, is important in development of CKD, but its interaction with AGEs *in vivo* remains enigmatic; other ligands and ternary complexation may be influential. Prevention of diabetic kidney disease (DKD) by overexpression of Glo1 in transgenic animal models has stimulated the development of small-molecule inducers of Glo1 expression, Glo1 inducers, to prevent AGE formation. *trans*-Resveratrol–hesperetin combination therapy is a Glo1 inducer. In clinical trial it demonstrated a profound improvement in insulin resistance and vascular inflammation. It may find future therapeutic application for treatment of DKD.

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Advanced glycation end products

Advanced glycation end products (AGEs) are a group of compounds formed by the nonenzymatic reaction of reducing sugars and related metabolites with proteins and amino acids. The process is called glycation or the Maillard reaction. Major precursors of AGEs *in vivo* are the early-stage glycation adduct N_ε-fructosyl-lysine (FL) and dicarbonyl metabolites methylglyoxal (MG), glyoxal, and 3-deoxyglucosone (3-DG).[1] Major AGEs quantitatively are MG-derived hydroimidazolone (MG-H1) and FL-derived N_ε-carboxymethyl-lysine (CML) and the cross-link glucosepane (Figure 1). AGEs are formed as glycated amino acid residues of proteins, which are conventionally called AGE residues of proteins, although they are often called protein-bound AGEs in renal research. AGE-modified proteins are degraded to related glycated amino acids, called AGE free adducts. AGEs are also formed from glucose osmolyte, MG, and other glucose degradation product dicarbonyls absorbed from thermally processed dialysis fluids in renal replacement therapy. AGEs may also be absorbed from glycated proteins in food, mainly as AGE free adducts. AGEs may be quantified robustly by stable isotopic dilution analysis liquid chromatography and tandem mass spectrometry (LC-MS/MS).[1] AGEs represent relatively long-lived and potentially damaging posttranslational modification of proteins. They are mostly damaging through modification of functional domains of proteins, producing protein inactivation or dysfunction.

Herein we review evidence of protein-derived AGEs. There are also AGEs formed by MG and glyoxal modification of nucleotides and basic phospholipids, phosphatidylethanolamine and phosphatidylserine.[2,3] These have been little-studied in chronic kidney disease (CKD),[4] and so the coverage below focuses on protein-derived AGEs.

The formation of AGEs is suppressed by enzymatic metabolism of the precursor glycating agents or glycation adduct FL. MG and glyoxal are metabolized mainly by the cytoplasmic glyoxalase system. The glyoxalase system consists of 2 enzymes, glyoxalase 1 (Glo1) and glyoxalase 2, and a catalytic amount of reduced glutathione (GSH). Glo1 catalyzes the GSH-dependent metabolism of MG to S-D-lactoylglutathione, and glyoxalase 2 catalyzes the hydrolysis of S-D-lactoylglutathione to D-lactate, reforming GSH consumed in the Glo1 catalyzed step[5] (Figure 2). 3-DG and likely also 3,4-dideoxyglucosone-3-ene (3,4-DGE) found in thermally processed peritoneal dialysis (PD) fluids with glucose osmolyte are metabolized by aldoketo reductases

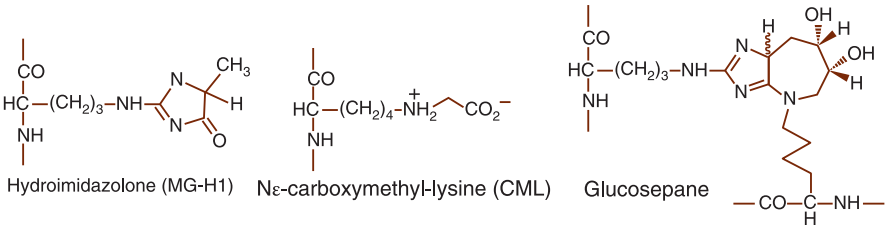

Figure 1 | Major advanced glycation end products in chronic kidney disease.

(AKRs) and aldehyde dehydrogenases. The kidney has a very high activity of aldose reductase, approximately 2% total protein, in the inner medulla that primarily reduces glucose to sorbitol to counter high extracellular osmotic pressure.[6] Steady-state levels of FL residues are suppressed by enzymatic metabolism by fructosamine-3-phosphokinase, resulting in deglycation of precursor lysine residues.[1]

Dicarbonyl stress

Dicarbonyl stress is the abnormal accumulation of MG and related dicarbonyl compounds, leading to increased AGE formation and related cell and tissue dysfunction. Dicarbonyl stress is a driver of CKD development, as evidenced by development of nephropathy in Glo1-deficient mice.[7] Dicarbonyl stress may also be consequence of renal failure, as evidenced by dicarbonyl stress with loss of clearance in bilateral nephrectomized rats.[8] Dicarbonyl stress occurs in patients with CKD,[5] including accumulation of MG without increase in D-lactate in nondiabetic subjects.[9] D-Lactate is a marker of flux of formation of MG.[10] This suggests that the driver of dicarbonyl stress in CKD is down-regulation of Glo1 rather than increased formation of MG in nondiabetic subjects. Decreased urinary excretion of MG is not a major contributing factor because little MG is excreted, although this may be more important for 3-DG.[10] Down-regulation of Glo1 in the kidney is a common feature of experimental diabetic nephropathy and diabetic kidney disease (DKD). It may be driven by decreased Glo1 expression in response to hypoxia-inducible factor-1α and inflammatory signaling conflicting with transcription factor Nrf2, and by increased proteolysis. Overexpression of Glo1 prevented renal senescence[11] and development of diabetic nephropathy,[7,12] the latter even when only in endothelial and tubular epithelial cells.[7] This suggests that reversing renal down-regulation of Glo1 may provide a new route to therapy.[10]

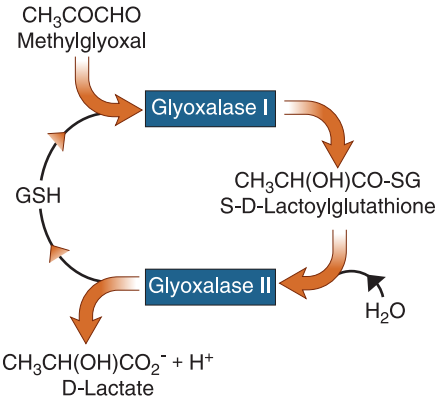

Figure 2 | The glyoxalase system. Schematic of the glyoxalase metabolic pathway.[10]

The flux of MG formation in a healthy adult human subject is approximately 3 mmol MG per 24 hours, and >99% is normally metabolized enzymatically. MG concentration of PD fluids, 2 to 7 μM, is therefore not a major increment to MG exposure. For 3-DG, the flux of formation is approximately 0.13 mmol 3-DG per 24 hours, and approximately 90% is normally metabolized enzymatically with approximately 10% excreted. PD fluids containing 100 to 400 μM 3-DG increase exposure to 3-DG markedly, although 3-DG has approximately 200-fold lower reactivity than MG in protein glycation.[5,10]

Accumulation of AGEs in renal failure: the profound increase of AGE free adducts

AGE free adducts are the major form by which AGEs are eliminated from the body. Decreased clearance in CKD markedly influences the plasma concentrations of AGE free adducts.[8,13] The increase of AGE free adducts in clinical renal failure, studied in patients receiving hemodialysis (HD) and PD renal replacement therapy was 4- to 40-fold, whereas the increase in AGE residues of plasma protein was 2- to 5-fold.[13] Drivers of AGE free adduct accumulation are increased flux of formation of AGEs and decreased clearance. The flux of formation of AGEs is indicated by the total excretion of AGE free adducts in dialysate and urine. In PD patients, flux of AGE formation was increased markedly with respect to healthy controls: 9-fold for MG-H1 and 2-fold for CML, pentosidine, and 3-DG-derived hydroimidazolones 3DG-H. The flux of excretion of MG-H1 free adduct in PD patients was 4- to 713-fold higher than of other AGEs, indicating MG-H1 is a dominant AGE in renal failure.[13]

AGE residue contents of plasma protein have been studied as biomarkers of mortality risk in renal failure with contrary outcomes or low marginal increased relative risk.[14,15] Plasma protein AGEs, such as MG-H1 and 3DG-H, may be increased by dicarbonyl stress;[13] CML and glucosepane may be increased by elevated FL residue precursor and/or decreased FL metabolism;[16–18] and pentosidine residue content may be increased by elevated pentosephosphate pathway activity providing increased level of the pentose precursor.[19] AGE residue content