α-Oxoaldehyde metabolism and diabetic complications

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Abstract

The factors responsible for variable susceptibility to diabetic nephropathy are not clear. According to the non-enzymatic glycation hypothesis, diabetes-related tissue damage occurs due to a complex mixture of toxic products, including α-oxoaldehydes, which are inherently toxic as well as serving as presursors for advanced glycation end-products. Protective mechanisms exist to control this unavoidable glycation, and these are determined by genetic or environmental factors that can regulate the concentrations of the reactive sugars or end-products. In diabetes these protective mechanisms become more important, since glycation stress increases, and less efficient defence systems against this stress could lead to diabetic complications. Some of these enzymatic control mechanisms, including those that regulate α-oxoaldehydes, have been identified. We have observed significant increases in production of the α-oxoaldehydes methylglyoxal and 3-deoxyglucosone in three human populations with biopsy-proven progression of nephropathy. The increase in methylglyoxal could be secondary to defects in downstream glycolytic enzymes (such as glyceraldehyde-3-phosphate dehydrogenase) that regulate its production, or in detoxification mechanisms such as glyoxalase. Other mechanisms, however, appear to be responsible for the observed increase in 3-deoxyglucosone levels. We present results of our studies on the mechanisms responsible for variable production of α-oxoaldehydes by measuring the activity and characteristics of these enzymes in cells from complication-prone and -resistant diabetic patients. New therapeutic interventions designed to control these endogenous mechanisms could potentially enhance protection against excessive glycation and prevent or reverse complications of long-term diabetes.

Introduction

It is well recognized that individuals with diabetes vary considerably in their propensity to develop complications. In attempting to understand these observations, a genetic basis for susceptibility (or resistance) to the development of complications has been proposed. This suggests that factors in addition to glycaemic control may be important in an individual’s predisposition to the development of complications. Support for this hypothesis is quite strong, including familial clustering of complications in siblings with Type I diabetes [1], and in Pima Indians with Type II diabetes [2,3]. Diabetic nephropathy is more likely to occur in siblings (85%) if nephropathy is present in a proband compared with those with no evidence of nephropathy (15%) [4]. In the Diabetes Control and Complications Trial (DCCT), diabetic first-degree relatives of those with progression of nephropathy had higher rates of renal disease [1], associated with either Type I or II diabetes. In another study of diabetic siblings, remarkably similar histological patterns of nephropathy were observed on renal biopsy [5], irrespective of age or duration of diabetes.

The non-enzymatic glycation hypothesis of diabetic complications, which over the past decade has gained increasing acceptance as one of the significant mechanisms contributing to diabetic complications, proposes that chemically reactive sugars and the resulting AGEs (advanced glycation end-products) lead to the tissue damage seen in diabetes [6–9]. These sugars consist of a large variety of carbohydrates [9–12], including glucose and the α-oxoaldehydes 3DG (3-deoxyglucosone) and MG (methylglyoxal) (in the text we will refer to these compounds as MG or 3DG, rather than the more generic term α-oxoaldehydes). Both MG and 3DG are particularly reactive, forming adducts with amino groups of proteins, nucleic acid and phospholipids up to 10 000 times more readily than does glucose.

Although the chemical reactions with these sugars are primarily non-enzymatic, their production and detoxification is ultimately controlled by upstream and downstream enzymatic mechanisms. This suggests that genetic and environmental factors that influence these mechanisms

Key words: 3-deoxyglucosone, diabetic complications, glycation, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), methylglyoxal, α-oxoaldehyde.

Abbreviations used: AGE, advanced glycation end-product, 3DG, 3-deoxyglucosone, GAPDH, glyceraldehyde-3-phosphate dehydrogenase, GBM, glomerular basement membrane, MG, methylglyoxal

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could regulate tissue glycation and potentially account for some of the variable complication rates observed when different individuals are exposed to the same degree of hyperglycaemia. We postulate that the variable degree of dicarbonyl and oxidative stress generated by hyperglycaemia in different individuals may play an important role in susceptibility to diabetic complications. To test this hypothesis, we have been investigating the production and detoxification of dicarboxylics in three distinct populations with diabetes where progression of complications has been carefully documented.

**Production and detoxification of α-dicarbonyls**

**MG (Scheme 1A)**

The production of MG occurs primarily from the triose phosphate intermediates in the glycolytic pathway (Scheme 1A), which include dihydroxyacetone phosphate and glyceraldehyde 3-phosphate [13,14]. MG is generated by two processes: (A) a spontaneous non-enzymatic elimination of the phosphate group, or (B) decomposition of an ene-diol triose phosphate intermediate that ‘leaks’ from the active site.
of triose phosphate isomerase [15]. Other possible sources of MG include the cytochrome P4502E1-catalysed oxidation of acetone from ketone bodies [12], cleavage of Amadori products to 3DG and fragmentation to MG by a reverse aldol reaction [16], and catabolism of threonine via aminoacetone [17]. MG detoxification occurs through activation of the GSH-dependent glyoxalase system. This pathway has been shown to be the major factor responsible for oxidative detoxification of MG, and results in the production of D-lactate [18].

3DG (Scheme 1B)
Our understanding of 3DG metabolism has been improved significantly by the discovery of the enzyme fructosamine 3-kinase, which appears to be the major source of 3DG. Fructosamine 3-kinase phosphorylates fructoselysine and other fructosamines [19,20] to fructosamine 3-phosphate, which subequently decomposes to produce 3DG. Phosphorylation of fructose to fructose 3-phosphate by the same enzyme [21,22] and direct auto-oxidation from glucose [23–25] are also sources of 3DG, but these are not as significant as the decomposition of fructosamine 3-phosphates. Both oxidative and reductive pathways exist for the detoxification of 3DG. The reductive pathway results in the production of 3-deoxyfructose, while a less well characterized oxidative pathway leads to the production of 3-deoxy-2-ketogluconic acid (see Scheme 1B). Both MG and 3DG are extremely reactive as glycating agents for proteins, phosphatidylethanolamine and possibly nucleic acids [21,26–28] and have been shown to be toxic to cultured cells [28–30]. They react with lysine and arginine residues in proteins under anaerobic conditions to form specific AGEs, such as pyrraline, imidazolones, argpyrimidine, N-carboxymethyllysine and various cross-linking structures [MOLD (MG lysine dimer) and others] [31]. Although oxidation is not required for modification of protein by these oxoaldehydes, oxidation does accelerate the degree of chemical modification of proteins, while metal-catalysed and non-metal-catalysed oxidation and peroxidation of lipids can also lead to a multitude of reactive carbonyl compounds [31].

Toxicity of MG
Several studies have shown that MG is significantly elevated in plasma from diabetics [32–36] and correlates with glycaemic control [32,37–39]. MG can also lead to increased AGE formation, which appears to be associated with diabetic vascular and neuropathic complications [39–41]. MG may also play a role in the development of a number of diabetic complications, including diabetic nephropathy, as suggested by several lines of evidence, including studies of the early stages of diabetic renal dysfunction showing that glomerular hyperfiltration is associated with elevated MG levels [42], and that elevated levels of MG and its metabolites may be associated with early albuminuria [43].

Toxicity of 3DG
3DG levels are also elevated in diabetes [32,33,44] and a strong association between tissue levels of 3DG and diabetic nephropathy has been found [45]. For instance, glomerular hyperfiltration, an early manifestation of diabetic nephropathy, correlates with plasma 3DG levels, and the relationship remains significant even after adjustment for age, duration of diabetes and glycaemic control. A positive relationship between plasma levels of 3DG and urinary albumin excretion has also been shown in subjects with Type II diabetes. A role for 3DG in diabetic nephropathy has also been suggested by Niwa et al. [46–48], who have identified elevated levels of the 3DG-derived AGE, imidazolone, in
Role of α-dicarbonyl metabolism in determining propensity to diabetic nephropathy

We have shown that α-dicarbonyl levels are significantly elevated in subjects with diabetes who show more rapid progression of diabetic nephropathy using three populations.

In the first study (n = 14), one group consisted of a complication-resistant cohort (n = 7) who had no evidence of nephropathy after 25 years’ duration of diabetes (protected group), while the other group (n = 7) was a complication-susceptible cohort who had developed overt diabetic nephropathy prior to 15 years’ duration of Type I diabetes (accelerated group).

The second and largest study group comprised 110 subjects with Type I diabetes whose degree of progression of kidney damage over 5 years had been measured via kidney biopsies in a study of the ‘natural history’ of diabetic nephropathy. This group was unique, in that it consisted of individuals who had no clinical evidence of renal damage at the time of study.

The third study group consisted of 45 Pima Indians, a population with an unusually high incidence of nephropathy associated with Type II diabetes. Each subject underwent a renal biopsy to determine renal morphology and was also categorized on the basis of the urinary albumin/creatinine ratio as normoalbuminuric (<30 mg/g), microalbuminuric (30–300 mg/g) or macroalbuminuric (>300 mg/g). GBM (glomerular basement membrane) width, fractional mesangial area and epithelial podocyte number were determined by electron microscopy. Increases in GBM width and fractional mesangial area, and a decrease in epithelial podocyte number, were noted as albuminuria increased.

In each person, MG production and GAPDH (glyceraldehyde-3-phosphate dehydrogenase) activity were measured using blood levels or by the erythrocyte response to incubation with high glucose. 3DG levels and oxidative stress were also measured.

Results of studies

MG production in erythrocytes

In the first study, erythrocytes from each subject were incubated sequentially in 5 and 30 mM glucose for 24 and 48 h respectively, and the production of MG and D-lactate was measured in cells and media. As shown in Figure 1, we found that levels of MG and D-lactate in these incubations were significantly increased for the accelerated group compared with the protected or control groups.

In the natural history study, MG production by erythrocytes incubated in 30 mM glucose was significantly higher (251.1 ± 111 pmol/g of erythrocytes) for subjects in the upper quintile of rate of GBM thickening compared with that for subjects in the lower quintile (183.7 ± 67.5 pmol/g of erythrocytes; P = 0.04). This relationship was independent of age, duration of diabetes or HbA1c, when analysed by analysis of covariance. Similar results were seen for D-lactate production (upper quintile of GBM thickening, 504.9 ± 155.4 nmol/g of erythrocytes; lower quintile, 424.2 ± 135.2 nmol/g of erythrocytes), although this did not achieve statistical significance (P = 0.12). Similar patterns were seen in vivo, where urinary MG levels showed a significant relationship with increased GBM width when adjusted for duration of diabetes and HbA1c levels (P = 0.036) by multiple regression analysis.

In the Pima Indian study, plasma MG levels showed a strong relationship with GBM width (R = 0.37, P = 0.016), which remained significant when adjusted for HbA1c or glomerular filtration rate by multiple regression analysis. Epithelial podocyte number was correlated inversely with MG levels (P = 0.02), indicating that higher MG levels were associated with decreased podocyte numbers. Overall glycemic control, based on HbA1c, did not correlate with renal morphological changes.

These findings strongly support our hypothesis that cells ‘nephropathy progressors’ produce more MG, and its product D-lactate, when exposed to high glucose levels in vitro. These results were confirmed by our in vivo studies, which showed a significant correlation of nephropathy progression with levels of MG. These apparent effects of MG were independent of glycemic control, suggesting that individuals that are prone to diabetic nephropathy exhibit higher levels of MG stress for a given level of glycemic stress. Since MG is a potent precursor of AGEs, and is toxic in its own right, increased levels in nephropathy progressors support the possibility that MG could play a causal role in renal damage.

The increased MG levels observed in the described studies could be the result of increased production and/or decreased detoxification. To date we have found no concrete evidence of impaired detoxification, suggesting that the differences in MG levels are more likely to be due to differences in production.

Skin fibroblasts and MG production

To assess the effect of elevated glucose concentrations on MG production in nucleated human cells, we have cultured human skin fibroblasts obtained from individuals with Type I diabetes. These individuals had not demonstrated clinical nephropathy at the time of sampling, and are participating in a study performed at the University of Minnesota [50]. With cells from the first three subjects, we observed wide variations in MG production in response to 25 mM glucose plus insulin, with values ranging from −0.4 to 16.3 pmol/mg of cellular protein on repeated sampling (n = 10). It was of particular interest that cells with high MG production demonstrated a rapid fall-off in viability, and a reduced numbers of passages (see Figure 2). We are currently investigating these
Figure 2 | MG production by fibroblasts from three individuals with Type I diabetes relative to cell viability over a specific number of passages

MG production: △, 0.32 pmol/mg of protein; ○, 12.3 pmol/mg; ●, 14.6 pmol/mg.

characteristics in subjects with well defined progression or non-progression of nephropathy.

Relationship between MG levels and GAPDH activity in erythrocytes

To evaluate the role of GAPDH in the overproduction of MG observed in nephropathy progressors, we have measured GAPDH activity in vitro in diabetic subjects, and in vivo in study population 2 (natural history study), where progression of nephropathy has been carefully documented. In both groups we have found that MG production has a close inverse relationship with GAPDH activity [51]. The reasons for the observed decrease in GAPDH activity are not entirely clear, but it is well known that this enzyme is very susceptible to a number of environmental factors that are perturbed in diabetes. These include oxidative stress [52], direct glycation [53] and altered redox state with reduced levels of NAD+ [54,55]. In addition, it is possible that these differences in enzyme activity could be a consequence of intrinsic genetic variations [56].

Oxidative stress markers

Many studies have demonstrated that the diabetic state is associated with increased oxidative stress, which in turn is felt to play a pathogenetic role in diabetes-related vascular damage [57,58]. In our investigations we also observed increased oxidative stress, based on higher levels of 8-isoprostanes and lower levels of GSH, in nephropathy progressors. Both of these metabolites have been shown to be sensitive markers of oxidative stress and are useful indicators of such stress in the clinical setting [59,60]. Although Hammes et al. [61] showed an association between diabetic retinopathy and increased accumulation of the oxidative product carboxy-methyl-lysine, our studies are the first to show statistically significant increased oxidative stress in association with documented early diabetic nephropathy. These observations are consistent with the histological demonstration of increased levels of markers of glycoxidative stress in glomeruli from diabetic subjects with diabetic nodular glomerulosclerosis [62]. Several mechanisms can potentially contribute to this increased oxidative stress. These include increased mitochondrial generation of reactive oxygen species [63], reduced activity of protective mechanisms [64,65] and increased generation of reactive oxygen species from Amadori products or Schiff bases [58].

3DG and diabetic nephropathy

In the ‘natural history’ study, levels of 3DG also showed a significant correlation with progression of diabetic nephropathy. These higher levels of 3DG could be generated by greater production of its major precursors, Amadori products or Schiff bases, under hyperglycaemic conditions, or could be secondary to an inability to detoxify these products by enzymatic mechanisms [19,66]. Our data confirm a strong relationship between 3DG levels and the Amadori product HbA1c, but also show that the relationship between nephropathy progression and 3DG is independent of glycaemic control. This suggests that other environmental or genetic factors could play a role in determining 3DG levels in those subjects with accelerated nephropathy, and further studies are needed to investigate this hypothesis.

Conclusion

Our studies, utilizing unique study populations of diabetic patients, are designed to provide a rigorous test of the hypothesis that resistance or susceptibility to development of diabetic complications may be due in part to variable production of MG and 3DG and the level of oxidative stress. Our results suggest a new paradigm for thinking about the process of non-enzymatic glycation and variation in the activity of processes that could account for variable rates of diabetic nephropathy on exposure to hyperglycaemia. If these observations are confirmed by future studies, they have the potential to have a major impact on the prevention and treatment of the complications associated with diabetes.

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References


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