Carbonyl Stress in the Pathogenesis of Diabetic Nephropathy

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Diabetic nephropathy is a major chronic complication of diabetes mellitus and an important cause of increased morbidity and mortality in diabetic patients. Although several lines of evidence have suggested that poor glycemic control undoubtedly plays a significant role, the metabolic events responsible for its development are not understood well. Possible mediators of untowards effects of hyperglycemia include the advanced glycation end products (AGEs). AGEs, carboxymethyllysine and pentosidine, whose formation is closely linked to oxidation, accumulate in the characteristic diabetic glomerular lesions, such as the expanded mesangial matrix and nodular lesions, in co-localization with other oxidation-specific protein adducts, such as malondialdehyde-lysine, 4-hydroxynonenal-protein adduct, and acrolein-protein adduct. These five biomarkers are formed under oxidative stress by carbonyl amine chemistry between protein amino group and carbonyl compounds derived from carbohydrates, lipids, and amino acids. This article focuses on new aspects of the pathology of diabetic nephropathy, implicating an increased oxidative stress and carbonyl modification of proteins by autooxidation products of carbohydrates, lipids, and amino acids in diabetic glomerular tissue damage ("carbonyl stress"). (Internal Medicine 38: 309–314, 1999)

Key words: advanced glycation end products (AGEs), oxidation, glycooxidation, carbonyl compounds

Introduction

With rapid development of therapy for diabetes mellitus, the mortality of acute complications of diabetes mellitus has decreased, but the mortality of chronic complications of diabetes mellitus has increased. Diabetic nephropathy is one of the major chronic complications of both type 1 and type 2 diabetes mellitus and also an important cause of increased morbidity and mortality among these patients. Furthermore, the increase in number of patients with chronic renal failure due to diabetic nephropathy is a major social and economic problem in several countries: diabetic nephropathy has been the most common cause leading to end-stage renal disease in both the United States and Japan (more than one-third of the number of patients with newly end-stage renal disease). The elucidation of the molecular pathogenesis of diabetic nephropathy is therefore necessary for the development of effective treatment modalities to prevent onset of diabetic nephropathy or to retard development of diabetic nephropathy. This article reviews recent studies on the pathogenic mechanisms of diabetic nephropathy, especially the increased glomerular matrix protein modification with carbohydrates and lipids, best described as carbonyl stress.

Pathogenesis of Diabetic Nephropathy

Several pathogenic mechanisms for diabetic glomerular damage have been proposed. Brenner and Anderson (1) demonstrated that glomerular hyperfiltration and/or hyperperfusion is a causal factor in the development of diabetic nephropathy in type 1 diabetes mellitus. It has also been suggested that the etiology of diabetic complications is associated with the activation of the polyol pathway (2), myo-inositol metabolism abnormalities (3), high activity of protein kinase C (4, 5), hypercoagulability (6), and hereditary factors (7). We have recently reported that interleukin 6 and transforming growth factor β are important factors in the development of diabetic nephropathy (8, 9), and several investigators have demonstrated that cytokines and growth factors play an important role in the pathogenesis of diabetic nephropathy (10–12). Furthermore, we showed that alternation in balance between production and degradation of renal extracellular matrix were implicated in the pathogenesis of diabetic nephropathy (13, 14). There are thus a variety of mechanisms accounting for the pathology of diabetic nephropathy (summarized in Table 1).

Poor glycemic control undoubtedly plays a significant role as shown by both clinical (15) and pathological studies (16). Possible mediators of untowards effects of hyperglycemia in-

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Table 1. Pathogenesis of Diabetic Nephropathy

1. Hemodynamic factors	glomerular hyperfiltration, hypercoagulability
2. Metabolic alterations	activation of polyol pathway, myo-inositol metabolism abnormalities, activation of protein kinase C (PKC)
3. Cytokines and growth factors	TGF- β , TNF- α , IL-6, PDGF, HGF, IGF, endothelin, etc.
4. Alteration of balance between production and degradation of ECM	
5. Nonenzymatic glycation	advanced glycation end products (AGEs), glycated protein
6. Carbonyl stress	
7. Genetic factors	
8. Others	

clude the advanced glycation end products (AGEs) known to accumulate in diabetic subjects (17, 18). AGEs have thus been proposed as one of important factors in the pathogenesis of diabetic nephropathy (18–20).

Role of AGEs in Diabetic Nephropathy

AGEs are generated by the Maillard reaction through non-enzymatic glycation and oxidation (“glycoxidation”) of protein amino groups (18). AGEs comprise a variety of molecular structures, such as *N*^ε-(carboxymethyl) lysine (CML) (21), pentosidine (22), and pyrraline (23), characterized by different formation mechanisms. AGEs accumulate in plasma and tissue proteins of diabetic patients (17, 24). Their accumulation is correlated with the severity of diabetic complications (25, 26). We recently reported that a monoclonal anti-AGE antibody reacts with the mesangial area in patients with diabetic nephropathy, and that the intensity of AGE staining gradually increases in parallel with the degree of mesangial expansion (19). Furthermore, we showed that monoclonal antibody against the reduced glycated lysine reacts with glomerular basement membrane in the glomeruli of patients with diabetic nephropathy (20).

Several lines of evidence suggest that AGEs may be involved in the development of diabetic glomerular lesions (19, 27, 28). AGEs stimulate matrix production and inhibit mesangial proliferation (29), and the glycated glomerular basement membrane is more resistant to digestion by protease (30). Doi et al (31) showed that mouse mesangial cells exhibit an increase in mRNA and protein synthesis of type IV collagen, laminin and heparan sulfate proteoglycan after exposure to AGEs. Yang et al (32) demonstrated that AGEs induce an increase in mRNA expression for extracellular matrix proteins such as type IV collagen, laminin, and transforming growth factor β (TGF- β). On the other hand, an AGE inhibitor, aminoguanidine, reduced these morphological changes (32). More interestingly, AGE-modified proteins stimulate a variety of cellular responses (33–35) via a specific cell-surface receptor (36, 37) on several cell types including glomerular mesangial cells (38, 39).

Carbonyl Stress: Alteration Non-Enzymatic Chemistry in Diabetic Nephropathy

Under oxidative stress, proteins may be modified either directly by reactive oxygen species with the eventual formation of oxidized amino acids or indirectly by reactive carbonyl compounds formed by autooxidation of carbohydrates, lipids, or amino acids.

Autooxidation of carbohydrates yields reactive carbonyl compounds, precursors of AGEs, such as glyoxal (40), methylglyoxal (41), and glycolaldehyde (42), as well as dehydroascorbate formed by oxidation of ascorbate (43, 44). Lipid peroxidation of polyunsaturated fatty acids yields other reactive carbonyl compounds: some are identical to those formed from carbohydrates (45), such as glyoxal and methylglyoxal, and others are characteristic of lipids, such as malondialdehyde (MDA), 4-hydroxynonenal (HNE), and acrolein (46, 47). The latter reactive carbonyl compounds produce lipoxidation products, MDA-lysine, HNE-protein adduct, and acrolein-protein adduct, also termed advanced lipoxidation end products. The hydroxy-amino acids, L-serine and L-threonine, are also oxidized and converted to reactive carbonyl compounds, such as glycolaldehyde and acrolein, which are highly reactive with proteins, leading to formation of CML and acrolein-protein adducts (48), respectively. Therefore, products are formed by carbonyl amine chemistry between protein amino group and carbonyl compounds derived from autooxidation of carbohydrates and lipids (40–42, 49, 50).

We recently demonstrated that glycoxidation products, a subclass of AGEs which requires both glycation and oxidation for their formation such as CML and pentosidine, accumulate in expanded mesangial matrix and nodular lesions in diabetic nephropathy, in co-localization with MDA-lysine, a lipoxidation product (27). However, pyrraline, another AGE structure whose deposition is rather independent of oxidative stress, was not found within diabetic glomeruli (27). We therefore hypothesized that the localizations of glycoxidation products and MDA-lysine (both formations are closely related to oxidative process) are independent evidence for local oxidative stress and the increased carbonyl modification of proteins

Figure 1. Carbonyl stress pathway.

in diabetic glomerular lesions ("carbonyl stress") (Fig. 1).

Evidence for Carbonyl Stress in Diabetic Glomerular Lesions

In order to examine the presence of carbonyl stress in diabetic nephropathy, human renal tissues from patients with diabetic nephropathy and control samples were examined, with specific antibodies, to characterize carbonyl modification of proteins by autoxidation products of carbohydrates, lipids, and amino acids: CML (derived from carbohydrates, lipids, and amino acid), pentosidine (derived from carbohydrates), MDA-lysine (derived from lipids), HNE-protein adduct (derived from lipids), and acrolein-protein adduct (derived from lipids and amino acid) (51). These antibodies recognize distinct structures and do not cross-react with the other structures, as demonstrated by immunoblot analysis (27).

Open renal biopsy specimens were obtained from 17 non-insulin-dependent diabetes mellitus patients with diabetic nephropathy. Control samples were also obtained from 6 subjects using uninvolved portions of surgically removed kidneys afflicted with malignancies. These subjects had no urinary abnormalities and histological examination of control tissues excluded any glomerular diseases.

The pathological hallmarks of diabetic nephropathy are increased thickness of the glomerular basement membrane and

mesangial expansion (52–56). The latter is considered more significant than the former, as expansion of the mesangium has been reported to ultimately lead to renal insufficiency (55). Diabetic patients were thus divided into three grades by the degree of mesangial expansion using light microscopy findings: grade I had mild mesangial expansion, grade II had moderate mesangial expansion, and grade III had severe mesangial expansion with Kimmelstiel-Wilson nodules.

Results of immunohistochemical studies in renal tissues from patients with diabetic nephropathy and control samples are shown in Table 2. The antibodies for CML, pentosidine, MDA-lysine, HNE-protein adduct, and acrolein-protein adduct did not stain the glomeruli in normal renal tissues from all 6 control subjects (exemplified for pentosidine and HNE-protein adduct in left and right panels in Fig. 2A, respectively).

In renal tissues of diabetic nephropathy patients, glomeruli were stained positive for CML, pentosidine, MDA-lysine, HNE-protein adduct, and acrolein-protein adduct in patients with diabetic nephropathy (exemplified for pentosidine and HNE-protein adduct in left and right panels in Figs. 2B and 2C, respectively). Immunostaining was mainly observed in the expanded mesangial area and capillary walls. Nodular lesions within the glomeruli were also strongly stained positive for all the 5 biomarkers. The intensity of their immunostaining appeared to increase with the histological severities of diabetic nephropathy.

Table 2. Immunostaining for Glycoxidation and Lipoxidation Products in Normal and Diabetic Renal Tissues

		CML	Pentosidine	MDA-lysine	HNE-protein adduct	Acrolein-protein adduct
Normal kidney		trace	trace	trace	trace	trace
	:grade I	trace	weak	weak	trace~weak	trace~weak
Diabetic nephropathy	:grade II	weak	weak~strong	weak~strong	weak	weak
	:grade III	weak~strong	strong	strong	weak~strong	strong

Figure 2. Immunohistochemical detection of pentosidine (left panel) and HNE-protein adduct (right) in normal (A) and diabetic renal tissues (B and C). The pentosidine and HNE-protein product antibodies did not stain the glomeruli in normal subjects. By contrast, both pentosidine and HNE-protein product were stained positive in the expanded mesangial area (B) and in nodular lesions (C) ($\times 200$).

These data suggest a broad derangement in non-enzymatic biochemistry in diabetic glomerular lesions, and implicate an increased local oxidative stress and carbonyl modification of proteins in diabetic glomerular tissue damage.

Role of Carbonyl Stress in Diabetic Nephropathy

The pathological significance of carbonyl stress in diabetic glomerular lesions is an issue of particular interest. As already described, the final protein adducts resulting from carbonyl stress, such as AGE modified proteins, stimulate a variety of cellular responses including glomerular mesangial cells. Furthermore, carbonyl stress may also have direct biological effects.

Reactive carbonyl compounds and residual carbonyl groups on AGE-modified proteins react covalently with matrix tissue proteins and alter their structures and functions. Or, they react with cell surface proteins and stimulate cellular responses by

cross-linking cell surface proteins (57, 58). We recently demonstrated an increased intracellular protein-tyrosine phosphorylation in murine thymocytes and fibroblasts by interaction with carbonyl compounds, such as glyoxal and MDA (59). This increased protein-tyrosine phosphorylation may be attributed to the cross-linking of cell surface proteins and the subsequent activation of some protein tyrosine kinase, such as c-Src (59). The protein-tyrosine phosphorylation by interaction with carbonyl compounds is completely inhibited by pretreatment with 2-isopropylidenehydrazono-4-oxo-thiazolidin-5-ylacetanilide (OPB-9195), an inhibitor of carbonyl-amine reactions (50, 59). Numerous intracellular proteins are also phosphorylated on tyrosine residues in primary cultured human and rat mesangial cells exposed to carbonyl compounds (60).

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