Roles of advanced glycation end-products in the progression of diabetic nephropathy

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Abstract. Available data indicate that the development of diabetic nephropathy is linked to hyperglycaemia. Glucose reacts nonenzymatically with proteins to form Schiff base and Amadori products. Further incubation of these early products leads to the formation of advanced glycation end-products (AGEs). AGEs seem to play a central role in the progression of diabetic nephropathy. Immunohistochemically, AGEs were also detected in an expanded mesangial matrix, especially in nodular lesions from patients with diabetic nephropathy. AGEs staining was noted in the Bowman’s capsule, periglomerular fibrosis in sclerosing glomeruli. In our ultrastructural study of mesangial matrix from patients with diabetic nephropathy by high-resolution scanning electron microscopy after cellular removal, the meshwork structure was evident at higher magnification. In nodular lesions, the loose meshwork structure appeared to be composed of various sized strands, ranging from 6 to 24 nm (mean ± SD: 11.4 ± 3.8 nm). The pore sizes were variable, ranging from 4 to 70 nm (mean ± SD: 23.6 ± 12.3 nm), and were statistically larger than those of normal controls. As the AGEs are localized most notably in nodular lesions, advanced glycations play a role in the progression of diabetic nephropathy through impairment of the assembly of matrix proteins in vivo. Because type V and type VI collagens are the major components of nodular lesions, increases in these interstitial and fibril or microfibril collagens may contribute to the formation of wider strands in the mesangial matrix of a nodular lesion. As no metalloprotease that is specific for type VI collagen has been identified thus far, AGEs formation might occur preferentially in type VI collagen-rich nodular lesions, which are sites of slow turnover.

Key words: advanced glycation end-products; diabetic nephropathy; glomerulosclerosis; glycation; mesangium

Introduction

Diabetes mellitus is one of the major causes of renal failure. Nephropathy in diabetes mellitus follows a period of clinical silence in which only subtle functional changes are found. After 15–20 years, hyperfiltration is gradually followed by proteinuria and declining renal functions [1] that ultimately progress to nephrotic syndrome and renal failure.

Morphologically, diabetic nephropathy is characterized by thickening of the glomerular basement membrane (GBM) and expansion of the mesangial matrix, namely an increase in the glomerular extracellular matrix [2]. The intercapillary glomerulosclerosis or Kimmelstiel–Wilson nodules (nodular lesion; Figure 1), composed of increased mesangial matrix, is pathognomonic of diabetic nephropathy.

Although much evidence exists in animals and man that the nephropathy is related to the diabetic state [3,4], the precise mechanism of diabetic nephropathy remains unknown.

Glycation in diabetes and localization of advanced glycation end-products (AGEs) in diabetic nephropathy

Glucose reacts non-enzymatically with proteins to form Schiff base and Amadori products. Further incubation of these early products leads to the formation of AGEs that are characterized by fluorescence, a brown colour and cross-linking [5,6]. Protein modification by AGE is expected to occur in proteins with relatively long half-lives, such as collagen [7], lens crystallins and...
myelin [6]. Such a modification occurs with normal ageing and is accelerated in patients with diabetes [4].

Recent immunohistochemical studies with anti-AGE antibodies have demonstrated their presence in serum [8] and in several types of human tissues, including arterial wall, brain and renal cortex [9,10]. However, AGEs have not been demonstrated directly in diabetic glomerular lesions.

Concerning the role of glycation in the diabetic kidney, Mitsuhashi et al. [11] have reported that AGEs are increased in the renal cortex of a streptozotocin-induced diabetic rat. Miyata and Monnier [10] have demonstrated the presence of pyrraline only in the fibrous crescent areas of the glomeruli or obliterated glomeruli. On the other hand, our antibody [12,13], although it did not react with pyrraline [12], detected AGEs in non-obliterated glomeruli and an expanded mesangial matrix, especially in nodular lesions (Figure 2A) [14]. We also observed AGE staining in the Bowman's capsule, and periglomerular fibrosis in sclerosing glomeruli (Figure 2B).

Ultrastructure of glomerular extracellular matrices

Physiological studies have suggested that the GBM is a heterologous structure with pores varying in size from 3.8 to 5.2 nm [15]. However, ultrastructural studies have revealed the pores to be larger, 4–6 nm, which may be related to the different techniques employed. In this regard it is well known that the ultrastructure of the GBM varies with the particular technique employed. For instance, conventional transmission electron microscopy shows the GBM to have an amorphous appearance, whereas the meshwork structure of the GBM is readily visualized after chemical treatment [16]. Moreover, using negative staining [17], freeze etch [18], deep etch and tissue negative staining [19], a fibrillar network of the GBM has been elucidated.

High resolution scanning electron microscopy (HRSEM), equipped with a field emission source, a very short objective lens and a resolving power of 0.5 nm at 30 kV [20], made it possible to observe the three-dimensional ultrastructure of the GBM. These HRSEM studies from our laboratory [2,21–24] and others [25,26] clearly demonstrate the meshwork and pores of the basement membranes. In a previous investigation we observed the polygonal meshwork structures not only in the GBM [2,21–24] but also in the tubular basement membrane [21,24], Bowman's capsule basement membrane and peritubular capillary basement membrane [21]. The strands averaged 6–7 nm wide, whereas the pore sizes within the meshwork were variable and differed according to the basement membrane type. The strands and the pores in the mesangial matrix were a little wider than those of other basement membranes in the kidney from our HRSEM studies [14,27]. In humans [14], loose meshwork structures were seen, composed of relatively uniform strands ranging from 3 to 18 nm wide (mean ± SD: 10.4

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Fig. 1. Transmission electron micrograph showing nodular lesion (NOD) from a patient with diabetic nephropathy. CL: capillary lumen; US: urinary space; M: mesangial cell. x4400.
± 3.3 nm). The pores demonstrated fairly uniform diameters, ranging from 4 to 28 nm (mean ± SD: 13.0 ± 5.3 nm). By transmission electron microscopy, the mesangial matrix appears more coarsely fibrillar and less electron dense. These differences might be due to the dissimilarity of components of the extracellular matrix [28].

**Glomerular matrix proteins and their changes in diabetes**

Both the GBM and the mesangial matrix contain type IV collagen, laminin, entactin, nidogen and heparan sulphate proteoglycan [29]. The following additional components, including fibronectin, amyloid-P component, actomyosin, chondroitin sulphate proteoglycans [29] and MMP-50/100 [30] basement membrane associated collagen [31] are localized exclusively in the mesangial matrix.

In diabetics, various compositional changes of matrix proteins have been studied extensively. Biochemical and immunohistochemical studies have indicated an increase in the normal constituents of the matrix, such as type IV collagen and fibronectin [32,33], and a decrease [34] or no change [35] in heparan sulphate proteoglycans in cases of early diabetes. However, in cases of progressive glomerulosclerosis, decreases in type IV collagen [32,33] and heparan sulphate proteoglycans [35,36] have been observed in immunohistochemical studies. Immunohistochemically, in the late stages, minor components or components not normally present in the mesangial matrix (such as types III, V and VI collagen) may increase [31–33].

**Ultrastructural changes of extracellular matrices by glycation**

These structural changes are accompanied by an impairment of the associative properties of the basement membrane components that reduces their ability to interact with each other to form an ordered polymeric complex. Tarsio et al. [37] have found that non-enzymatic glycation of laminin and/or type IV collagen significantly reduces the cooperative binding with heparin. They have speculated that this is one of the causes of the decrease in heparan sulphate proteoglycan in relation to the proteinuria found in patients with diabetic nephropathy. Charonis et al. [38,39] showed impairment of self-assembly of laminin after *in vitro* non-enzymatic glycation. Glucose incorporation resulted in a drastic decrease of long-to-long laminin dimers, which normally form during the initial steps of assembly. Furthermore, non-enzymatic glycation of laminin reduced its ability to self-associate into complexes larger than dimers. Aminoguanidine, which has been suggested to inhibit cross-link formation, was shown to restore to a large extent the shape and assembly of laminin.

More recently, Anderson et al. [26] have examined structural changes in the bovine kidney tubular
Age in diabetic nephropathy

Fig. 3. HRSEM of an acellular nodular lesion from a patient with diabetic nephropathy. Note the loose meshwork composed of pores (arrowheads) and strands (arrows) of varying size. ×157 000.

basement membrane following in vitro non-enzymatic glycation. Using HRSEM, they identified an increase in the number of large openings in the meshwork and concluded that non-enzymatic glycation and cross-link formation among basement membrane components might lead to modifications of the basement membrane.

In our ultrastructural study of mesangial matrix from patients with diabetic nephropathy by HRSEM after cellular removal, the meshwork structure was evident at higher magnification [14]. The widths of these strands were relatively uniform (mean ± SD: 10.5 ± 2.7 nm). The strands generally branched and anastomosed with neighbouring strands at short intervals, but some straight and curvilinear strands were also observed. The diameters of the pores were variable (mean ± SD: 14.0 ± 6.1 nm); however, statistically they did not differ from those of the controls. In nodular lesion, the loose meshwork structure appeared to be composed of various-sized strands ranging from 6 to 24 nm (mean ± SD: 11.4 ± 3.8 nm) (Figure 3). Occasionally some thick fibres were noted running parallel among the strands. The pore sizes were variable, ranging from 4 to 70 nm (mean ± SD: 23.6 ± 12.3 nm), and were statistically larger than those of the controls. As the AGEs localized most notably in nodular lesions, advanced glycations play a role in the progression of diabetic nephropathy through impairment of the assembly of matrix proteins in vivo.

Because types V and VI collagens are the major components of nodular lesions [31,32], increases in these interstitial and fibril or microfibril collagens may contribute to the formation of wider strands in the mesangial matrix of a nodular lesion. As mesangial cells are known to produce these extracellular matrix components in vitro, phenotypic changes of the mesangial cell may be responsible for change in these components [40].

The preferential localization of AGEs in a nodular lesion suggests that AGEs might cause porosity of the extracellular matrix in vivo. As no metalloprotease that is specific for type VI collagen has been identified thus far [41], AGEs formation might occur preferentially in type VI collagen-rich nodular lesions, which are sites of slow turnover.

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