Advanced glycation end-products in diabetic nephropathy

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Abstract. Advanced glycation end-products (AGEs) are the pigmented and fluorescent adduct formed by a non-enzymatic reaction between sugar and protein. Since AGEs are generated in high glucose milieu, then induce the structural and functional alteration of matrix proteins, and have biological effects on various kinds of cells including mesangial cells, AGEs have been implicated in tissue damage of diabetic nephropathy. To elucidate the factor(s) that determine the AGEs level in diabetic nephropathy, we quantitated the plasma pentosidine level of different status of diabetic nephropathy by HPLC assay. The plasma pentosidine level in diabetic nephropathy was found to be determined by factors such as renal function, control of glucose and the patient's age; of these, renal function was the most critical factor. For a better understanding of the pathological role of AGEs in diabetic nephropathy, we then examined renal tissues of diabetic nephropathy immunohistochemically using antibodies specific for AGE proteins. Immunohistochemistry revealed the positive immunostaining for AGEs in the expanded mesangial area of diffuse diabetic glomerulosclerosis. The degree of staining was stronger than that in patients of IgA nephropathy with a similar degree of mesangial expansion. The nodular lesions, characteristic of diabetic nephropathy, were also stained positive for AGEs. These findings suggests a potential link of AGEs accumulation, which may be determined by renal function, control of glucose and age, to renal tissue damage in diabetic nephropathy.

Key words: advanced glycation end-products; diabetes mellitus; end-stage renal failure; nephropathy

Introduction

Diabetic nephropathy is one of the most serious complications associated with diabetes mellitus and is becoming one of the major causes of end-stage renal failure. It is reported that 30–45% of patients with insulin-dependent diabetes mellitus ultimately develop diabetic nephropathy, and that in the USA and Japan 30% of newly dialysed patients are diabetics. The cause(s) and process of renal tissue damage in diabetes remain unknown. However, the Diabetic Control and Complications Trial study demonstrated that tight control of blood glucose slowed or prevented the development of diabetic complications, including renal involvement [1]. Furthermore, histological studies demonstrated a significant correlation between the control of blood glucose and the morphological change [2]. These findings indicate that high blood glucose is closely linked to the occurrence of diabetic nephropathy.

Glucose reacts non-enzymatically with the protein amino group, in what is known as the Maillard reaction, to reversibly form Schiff base, and upon rearrangement converts into a more stable Amadori product [3]. Some Amadori products are then converted into advanced glycation end-products (AGEs) over the month, through a series of chemical rearrangements, dehydration and fragmentation reactions. AGEs constitute a heterogeneous class of structures that are brown in colour, fluoresce and cross-link [3]. Since the AGE modification induces structural and functional alteration of matrix proteins and also has biological effects on various kinds of cell, AGEs have been postulated to be molecules with pathological significance in the development of diabetic complications. This article discusses the pathophysiology of AGEs in patients with diabetic nephropathy.

Accumulation of AGEs in diabetic nephropathy

The rate of AGEs accumulation is assumed to be proportional to time-integrated blood glucose. Monnier et al. first reported, by fluorospectrometrical analysis, that the glycation of skin collagen increased with the severity of diabetic complications and ageing, thereby suggesting that the degree of collagen-linked fluorescence represents long-term glycaemic control, and that non-enzymatic glycation may be
Fig. 1. Immunohistochemical detection of AGEs in renal tissue from a patient in the early stage of diabetic nephropathy (68-year-old non-insulin-dependent diabetic male with normal renal function and macroalbuminuria). Tissue sections were stained with the anti-AGE antibody. The anti-AGE antibody was raised by immunizing AGE-modified keyhole limpet haemocyanin (incubating keyhole limpet haemocyanin with glucose for 60 days at 37°C) into rabbits and the affinity-purified IgG fraction was used. Note the positive immunostaining for AGEs in the expanded mesangial area (A) and capillary wall (B). The nuclei were counterstained with Meyer's haematoxylin (A). Original magnification: A, x400; B, x1000.

Partly involved in the pathogenesis of diabetic complications [4]. Pentosidine, which is a fluorescent cross-link formed between arginine and lysine residues, is postulated to be one of the AGE structures [5]. Recent studies have demonstrated the presence of pentosidine in skin collagen [6], glomerular basement membrane [7] and plasma proteins of diabetic patients [8]. Pentosidine in skin correlates well with the severity of diabetic complications [9,10]. Sell et al. reported that pentosidine in the glomerular basement membrane was elevated in the majority of diabetic patients regardless of the type of diabetes [11]. Carboxymethyllysine, which is formed by the oxidative cleavage of fructoselysine, is also shown to accumulate in skin collagen of diabetic patients [6]. All these studies indicate that there is a close relationship between the accumulation of AGEs in the extracellular matrix and diabetic complications.

The mechanism of AGEs accumulation is due either to the increased generation or decreased removal of AGE-modified proteins. Therefore, the mechanism of AGEs accumulation in patients with diabetic nephropathy is complicated, since both mechanisms exist simultaneously in these patients. Recently, we found that the plasma pentosidine in diabetic patients was determined by several factors, such as the patient's age, renal function and control of glucose level [S. Sugiyama and T. Miyata, unpublished observation]. Among these, the renal function is found to be the most critical factor. This provides a new insight into the pathogenesis and treatment of diabetic nephropathy. In the early stage of diabetic nephropathy, increased plasma glucose concentrations might accelerate the formation of AGEs and trigger the accumulation of AGEs in the renal matrix tissue. However, once the renal function deteriorates, it might further accelerate the accumulation of AGEs and result in a more rapid development of renal lesions. At this stage, attempts to control glucose might not benefit the prevention of AGEs accumulation. Thus, it seems important to
normalize glucose and prevent the accumulation of AGEs in the matrix proteins before renal failure develops. This coincides with our clinical experience.

**Immunohistochemical detection of AGEs in diabetic nephropathy**

To better understand the pathological role of AGEs in diabetic nephropathy, the determination of the precise localization of AGEs in diabetic renal tissue is useful. Renal tissue from diabetic patients was thus immunostained using the antibody specific for AGEs.

In renal tissues from patients in the early stage of diabetic nephropathy, positive immunostaining for AGEs was observed in the expanded mesangial area (Figure 1A) and capillary wall (Figure 1B). The immunoreactivity of the anti-AGE antibody was completely inhibited in the presence of excess AGE-modified bovine serum albumin (BSA) during the immunoreaction (data not shown), indicating that the immunostaining was specific. The expansion of the mesangial matrix and increased width of the capillary wall are the most prominent characteristics in diabetic nephropathy. This mesangial expansion is thought to be a principal cause of renal dysfunction [12]. It is not conclusive at present whether the accumulation of AGEs in the mesangial area represents merely the accumulation of the post-translational modification of tissue proteins or the active participation in renal tissue damage. It is also unknown whether the AGE receptors (RAGEs) on the mesangial cells [13] are involved in the AGE accumulation within the mesangial area, by taking up circulating AGE-modified proteins or AGE-modified peptides.

In the advanced stages of diabetic nephropathy, the immunostaining was positive in the nodular lesion (Figure 2A) and arterial wall (Figure 2B), but completely inhibited in the presence of excess AGE-modified BSA. Our preliminary immunohistochemistry using the anti-pentosidine antibody revealed positive immunostaining for pentosidine with a staining pattern similar to that of the anti-AGE antibody [K. Horie and T. Miyata, unpublished observation]. These findings are compatible with previous observations by Makino et al. that strong immunostaining for AGEs was detected in the nodular lesion [14]. A previous immunohistochemical study using the anti-pyrraline antibody demonstrated that the immunostaining for pyrraline was positive in the crescent-shaped extracellular matrix in sclerosed glomeruli and the arterial wall but negative in the nodular lesion [15]. This difference in the immunostaining pattern may be due to the difference in the AGE structures. Table 1 summarizes the results of immunohistochemistry by our and other research groups.

**Pathological role of AGEs in diabetic nephropathy**

Several lines of evidence from in vitro studies have suggested a role of AGE-modified proteins in the development of diabetic nephropathy. The AGE modification results in the alteration of structure and function of proteins [3]. AGE-modified proteins are also known to stimulate cellular responses [16-18] via receptors specific to AGE-modified proteins [19,20], or to generate reactive oxygen intermediates [21,22]. It is of note that the mesangial cells express the RAGE on the cell membrane [13]. This RAGE forms a complex consisting of a 60 and a 90 kDa protein [13], which is different from the one expressed on the endothelial cells and monocytes/macrophages, i.e. a complex consisting of a lactofenin and a 35 kDa protein of the immunoglobulin superfamily [19]. In fact, AGE-modified proteins are shown to stimulate cultured mesangial cells to synthesize matrix proteins [13], or to up-regulate the transcription, translation and secretion of type IV collagen in normal mouse kidney cell lines 1231.

The pathological involvement of the Maillard reaction in diabetic nephropathy is also suggested by in vivo studies using experimental animals. The administration of aminoguanidine, a well-known inhibitory drug of the Maillard reaction, reduced the urinary excretion of albumin and inhibited mesangial expansion in diabetic mice [24]. Furthermore, the intravenous administration of the monoclonal antibody specific to Amadori-modified albumin prevented mesangial expansion in diabetic mice [25].

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### Table 1. Summary of the results of immunohistochemistry using anti-AGE antibodies

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Early stage</th>
<th>Advanced stage</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Mesangial area</td>
<td>Capillary wall</td>
<td>Nodular lesion</td>
</tr>
<tr>
<td>Anti-pyrraline</td>
<td>Not reported</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Anti-AGE (monoclonal)</td>
<td>+</td>
<td>-</td>
<td>+</td>
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<tr>
<td>Anti-AGE (polyclonal)</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Anti-pentosidine</td>
<td>+</td>
<td>+</td>
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*Crescent-shaped extracellular matrix in sclerosed glomeruli.
**Intimal and perivascular sclerosed region.
***K. Horie and T. Miyata, unpublished observation.
Conclusion
The facts that (i) the AGEs accumulation increased in accordance with the severity of diabetic nephropathy and (ii) AGEs were present in the characteristic lesion of diabetic nephropathy, e.g. the expanded mesangial area, nodular lesion and arterial wall, suggest a potential link between AGEs accumulation and the pathogenesis of diabetic nephropathy. However, it should be emphasized again that additional studies are needed to answer the question of whether the AGEs accumulation represent merely the accumulation of the post-translational modification in the diabetic renal tissue or whether they have pathological significance in the development of diabetic nephropathy. Further studies to elucidate the role of AGEs in diabetic nephropathy will be necessary.

References