21. ADVANCED GLYCATION END-PRODUCTS AND DIABETIC RENAL DISEASE

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Since there is chronic hyperglycaemia in diabetes, there is an acceleration of the Maillard or browning reaction [1]. This is a spontaneous reaction between glucose and proteins, lipids or nucleic acids, particularly on long-lived proteins such as the collagens [1]. There is a sequence of biochemical reactions, many of which are still poorly defined, leading to the formation of a range of advanced glycation end-products (AGEs), some of which are fluorescent. These modified long-lived tissue proteins are formed as a result not only of glycation but also oxidative processes and many of these AGEs are now considered glycoxidation products [2]. Over the last decade, an increasing number of AGEs have been identified [3]. However, the identity of the AGEs linked to diabetic complications and in particular to renal disease has not been clearly determined. Of particular interest is the hypothesis that diet derived AGEs may also be involved in tissue AGE accumulation [4].

Initial studies involved assessment of AGEs by measuring their specific fluorescence in tissue homogenates. It was clearly shown that these fluorescent AGEs were increased in the aorta from diabetic rats [5]. Further studies were performed in diabetic animals and confirmed increased AGEs in the diabetic kidney [6, 7] and retina [8], sites of diabetic microvascular disease. In clinical studies, Monnier et al were able to demonstrate increased AGE levels with age, diabetic patients having an even further increase in AGE levels, as assessed by specific fluorescence [9]. In addition, levels of collagen-linked fluorescence from human skin increased with the severity of retinopathy, suggesting that there is a link between the severity of complications and cumulative exposure to hyperglycaemia [9]. However, in that study, the trend for collagen-linked fluorescence to be linked with levels of proteinuria did not reach statistical significance.

More recently, other techniques have been developed to assay AGEs. Non-fluorescent AGEs such as carboxymethyllysine (CML) have been assayed and a relationship between this AGE and diabetic complications has been shown [10]. Using a radioreceptor assay, Makita et al have reported increased AGE levels in diabetic patients,
particularly in the setting of renal impairment [11]. Various antibodies to AGEs have
now been developed and using a variety of immunohistochemical techniques, increased
AGE levels have been reported in both human and experimental diabetes [12, 13]. Our
own group using a radioimmunoassay has detected increased AGE levels in the diabetic
kidney [13] and using immunohistochemistry we have localised this increase in AGE
levels to the glomerulus [14]. Beisswenger et al have used an ELISA technique to detect
AGEs in serum and noted increased AGE levels in diabetic patients with complications
including retinopathy and nephropathy [15].

**AGE RECEPTORS**

Over the last few years, a number of AGE binding sites have been identified. The first
binding site to be cloned has been termed RAGE and was initially identified in
endothelial cells [16]. Our own group has detected RAGE in various other sites
including the kidney, retina, nerve and blood vessels, sites of diabetes associated
vascular injury [14]. Further studies have suggested that RAGE has a central role in the
development of vascular disease in diabetes by influencing various pathological
processes including expression of adhesion molecules involved in mononuclear cell
recruitment and hyperpermeability [17, 18]. Vlassara's group has cloned at least 3
different proteins which bind to AGEs [19]. The role of these proteins remains an area
of intensive investigation and it has been postulated that they may mediate a range of
functions including clearance of AGEs and activation of intracellular messengers such as
protein kinase C [19]. These AGE-binding sites have been identified in cultured
mesangial cells [19]. Other proteins such as lysozyme can also bind AGEs [20] but the
significance of these ligand-receptor interactions has still not been fully delineated. Our
own group has identified AGE binding sites in proximal tubules which appear to be
upregulated in diabetes [21]. However, the molecular identity of these AGE binding
sites has not yet been determined. It is still uncertain whether these AGE-binding
proteins act primarily to clear AGEs which would be viewed as a beneficial effect or
whether they are mainly involved in activating a range of pathological processes which
lead to diabetic complications.

**AGES AND CYTOKINES**

*In vitro* AGEs have been shown to activate a range of cytokines which may be relevant
to diabetic complications. In the non-diabetic mouse, *in vivo* injection of *in vitro*
prepared AGEs has been shown to not only lead to increased gene expression of TGFB
and type IV collagen in the kidney [22] but also to lead to histological changes with
some resemblance to diabetic nephropathy [23]. These changes included mesangial
expansion and glomerulosclerosis. Our group has shown that activation of gene
expression of the proscerotic cytokine, TGFB1 is closely linked to AGE accumulation in
blood vessels [24]. Furthermore, the inhibitor of advanced glycation, aminoguanidine,
prevented diabetes associated overexpression of TGFB1 and type IV collagen in these
blood vessels [24]. AGEs have been also shown to activate vascular endothelial growth