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The role of advanced glycosylation end-products in the pathogenesis of atherosclerosis

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Abstract. Coronary artery disease and cerebrovascular disease due to the rapid progression of atherosclcrosis is the principal cause of death in diabetes mellitus. Modification of low-density lipoproteins (LDL) by advanced glycosylation end-products (AGE) may play a central role in the development of atherosclerosis, especially in diabetic patients. An AGE-modified form of LDL (AGE-LDL) has been found to circtulate in human plasma, and AGE modifications have been identified as being present on both the apoprotein (ApoB) and the phospholipid components of LDL. By utilizing an AGE-specific ELISA, we measured the AGE attached to the ApoB and lipid components of LDL from normal controls and diabetic patients with or without end-stage renal disease (ESRD), as well as lipid oxidation. AGE-ApoB, AGE-lipid and oxidized LDL (Ox-LDL) in diabetic patients were significantly higher than those in patients without diabetes. The correlation between AGE-ApoB and AGE-lipid were highly significant. An especially marked elevation of AGE-LDL was found in diabetic patients with ESRD. The correlation between the serum total cholesterol and the AGE-LDL (AGE-ApoB and AGE-lipid) was significant. In addition, based on the known biological properties of AGE-modified peptide (AGE-peptide), we have proposed that these chemically reactive circulating AGE-peptides contribute to tissue injury by reattaching to susceptible target proteins both within and outside the vasculature, and that this process accelerates vascular pathology in diabetic patients. These data indicate that AGE-modified LDLs may represent a particularly atherogenic form of LDL, and AGE-LDLs as well as AGE-peptides are likely to contribute to the development of atherosclerosis in diabetic patients.

Key words: advanced glycosylation end-products; atherosclerosis; diabetes mellitus; low-density lipoproteins

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Introduction

Diabetic patients suffer a high incidence of atherosclerotic disease, including coronary heart disease (CAD) and cerebrovascular disease. The principal cause of death in diabetic patients is atherosclerotic macrovascular disease. Although this high incidence and morbidity in diabetic patients has yet to be explained, persistent hyperglycaemia, which is a major hallmark of diabetes, might play an important role in the acceleration of atherosclerosis. There is a missing link between chronic hyperglycaemia shown in diabetic patients and diabetic complications, including macro- and microvascular complications. A growing body of evidence has linked the accumulation of the late products of glucose–protein interaction (AGEs) to a variety of chronic complications [1,2]. In contrast to the 'early' Amadori products, which do not result in pathological changes, a significant correlation has been shown between the tissue AGEs and the presence and severity of diabetic complications [1,2]. Recent research findings suggest that circulating AGE-peptide and intermediate substances such as 3-deoxyglucosone play an important role in the formation and acceleration of AGE *in vivo*. Also, there is substantial evidence to indicate that modified low-density lipoproteins (LDL), including oxidized LDL (Ox-LDL) and AGE-LDL, contribute to atherogenesis by a number of mechanisms. The elevated plasma AGE-LDL in diabetic patients due to persistent hyperglycaemia and increased AGE-peptide is likely to act to produce the rapidly progressive vasculopathy of diabetes or end-stage renal disease in concert with Ox-LDL [3,4].

Circulating AGE-peptide and AGE-LDL

The development of radio receptor [5,6] and enzyme-linked immunoassay methods [7] for measuring AGEs led to the observation that circulating serum AGEs increase markedly in diabetic patients with end-stage

renal disease (ESRD) [8]. Circulating AGEs comprise both serum protein-bound AGEs and low-molecular-weight AGEs (AGE-peptide) which might be degradation products of tissue AGEs [6,8]. The existence of AGE-peptide in circulation has been confirmed by two independent laboratories [9,10]. Current renal replacement therapies including haemodialysis and CAPD have been shown to be insufficient for removing AGEs that bind rapidly with proteins [8]. Increased AGE-peptide in diabetic ESRD might explain the rapid progression of atherosclerosis shown in diabetic ESRD. Also, these circulating AGE-peptides have been postulated to be an important class of uraemic toxic substances.

We recently described that amino-containing phospholipids as well as apoproteins react with glucose to initiate AGEs, forming lipid-linked AGEs (AGE-lipid) and apolipoprotein-linked AGEs (AGE-ApoB) [3]. AGE-lipids have also been found to promote fatty acid oxidation of LDL. This process, termed AGE oxidation, appears to result from the inter- and intramolecular oxidation–reduction reactions that are an inherent feature of AGEs [3,4]. The predominant site of AGE was found to lie within a single, 67-amino-acid region located 1791 residues amino-terminal of the putative LDL receptor binding domain [11]. These data provide further evidence for important structural interactions between the LDL receptor binding domain and remote regions of the ApoB polypeptide.

Figure 1 shows a current scheme for the formation of AGE *in vivo*. AGE formation occurs not only through Amadori products but also through reactive intermediates on decomposition of reactive sugar, Schiff base or Amadori products [12]. Also, AGE-peptide, which is a degradation product from tissue AGE, might act as an accelerator for the rapid formation of AGE. This pathway and mechanism of AGE reaction may explain the relatively rapid formation of AGE *in vivo*.

AGE and diabetic atherosclerosis

Patients with diabetes or renal insufficiency suffer a high incidence of atherosclerotic disease. The decreased survival of diabetic patients treated by dialysis therapy is not surprising, in light of their extensive extrarenal vascular disease and their acceleration of atherosclerosis. There is a missing link in the explanation of the dramatic acceleration of atherosclerosis in diabetic patients with ESRD in contrast to non-diabetic patients with ESRD. The clue lies in the persistent hyperglycaemia found in diabetic patients. Recently, studies of atherosclerosis have been influenced by the hypothesis that human LDL oxidation may initiate the atherosclerotic lesion or worsen its course [12]. Ox-LDL could be the *in vivo* counterpart of chemically modified forms of LDL. For example, AGE-modified LDL may play an important role in the pathogenesis of atherosclerosis in diabetics. We have recently shown that

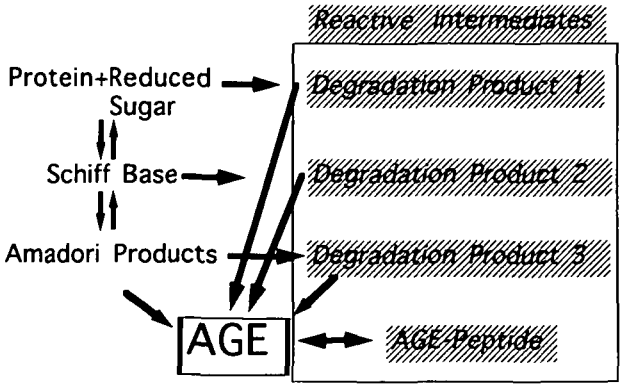

Fig. 1. Schematic representation of the formation of AGEs.

upon incubation of LDL with glucose, AGE formation occurs in both the apoprotein B (ApoB) and lipid components of LDL [3]. Also, *in vitro* glycation of lipids is shown to result in fatty acid oxidation [3]. Based on the known biological properties of AGEs, we have proposed that these chemically reactive circulating AGE-modified peptides contribute to tissue injury by reattaching to susceptible target proteins both within and outside the vasculature, and that this process accelerates vascular pathology in diabetic patients.

In order to confirm that the diabetic serum AGE-peptide can also react with LDL, we isolated the low-molecular-weight AGE-peptide fraction by passing the serum through an ultrafiltration device. When these human serum-derived AGE-peptides were exposed to normal LDL, a dramatic and rapid increase in AGE-LDL formation was observed compared with glucose-induced AGE-LDL. At the same time, LDL oxidation mimicked the AGE-LDL modification [4]. In the presence of the AGE-cross-link inhibitor aminoguanidine, the AGE development on either ApoB or the lipid component of LDL was inhibited [4]. This further confirmed the covalent attachment of AGE-peptide onto the LDL components. To address the potential mechanism for the lipid disorder of diabetes and ESRD, we investigated the possibility that circulating AGE-LDL formed by AGE-peptide prevents recognition by LDL receptors [4].

We first measured AGE-ApoB and AGE-lipid in circulation [3]. Marked elevations in AGE-ApoB as well as AGE-lipid were present in plasma from diabetic as well as non-diabetic patients with ESRD compared with patients with normal renal function. We evaluated the association between circulating AGE-LDL and renal function *in vivo*. The correlation between circulating AGE-ApoB or AGE-lipids and serum creatinine was significant.

Direct histochemical evidence for the accumulation of AGEs in coronary atheroma in diabetic haemodialysis patients was demonstrated by using AGE-specific antibody [14]. There is positive AGE staining throughout much of the plaque in transverse sections

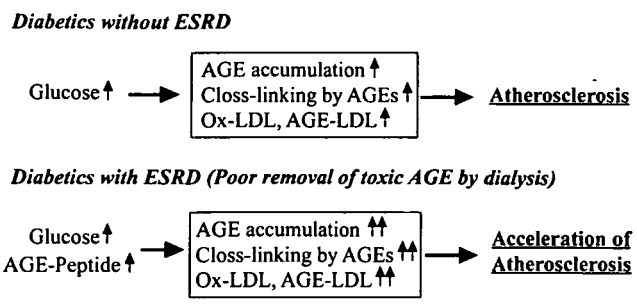

Fig. 2. Possible mechanism for diabetic atherosclerosis.

of a coronary artery from a 72-year-old diabetic haemodialysis patient. Control sections showed no positive staining.

Figure 2 shows our current hypothesis for the explanation of atherosclerosis in diabetic patients with or without ESRD. In diabetics without ESRD, persistent hyperglycaemia could cause increased AGE accumulation and protein–protein cross-linking by AGEs in their tissues. Enhanced oxidized LDL and AGE-LDL may also contribute to the formation of atherosclerosis. On the other hand, diabetics with ESRD have the additional burden of poor removal of toxic AGE. Elevated AGE-peptides and AGE-proteins worsen AGE accumulation, cross-linking by AGEs and increased oxidized AGE and AGE-LDL. These mechanisms may explain the dramatic acceleration of atherosclerosis found in diabetic patients with ESRD. Also, AGE inhibitors might represent a new class of drug which may interdict the diabetic complications including atherosclerosis.

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