MINI REVIEW

Hyperglycemia and the pathogenesis of atherosclerosis: lessons from murine models

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Diabetes is associated with major mortality and morbidity from late vascular complications, both microvascular (retinopathy, nephropathy, neuropathy) and macrovascular (accelerated atherosclerosis (ATS)). Since hyperglycemia defines diabetes, it would seem an unescapable conclusion that hyperglycemia also induces these complications. However, despite remarkable efforts, research into the mechanisms of glucose-induced vascular damage has so far proven rather inconclusive.

One important limitation is the lack of a suitable animal model of diabetic complications. The basic problem, common to many vascular disorders, is to recapitulate a chronic, progressive process in a shorter time frame so that it is amenable to experimental observation. Murine models would be attractive, but normal mice are resistant to ATS (1). One exception is the C57BL/6 strain, in which female mice develop ATS lesions, but only after prolonged exposure (>20 weeks) to a high-fat diet. A recent publication has reported studies on diabetes-induced ATS in mice (2). Diabetes was induced by streptozotocin (STZ, a toxin specific for insulin-producing cells) in both balb/c and C57BL/6 female mice. Diabetes per se failed to induce ATS in either mouse strain. However, C57BL/6 mice fed a fat-enriched diet developed lesions resembling fatty streaks. The extent and severity of these lesions were unaffected by diabetes. In contrast, when balb/c mice were fed a fat-enriched diet, only diabetic animals developed ATS lesions. The diabetic animals were clearly hyperglycemic and hypoinsulimemic, and were not treated with exogenous insulin, ruling out hyperinsulinemia as a confounding factor. Interestingly, diabetes-induced fatty streaks in balb/c mice contained both lipid-laden macrophages and smooth muscle cells, whereas only macrophages were seen in the C57BL/6 mice. The dependence on concomitant fat feeding can be explained by the fact that normal mice, as opposed to humans, have a predominance of high density lipoprotein and barely detectable low density lipoprotein (LDL) and very low density lipoprotein (VLDL) circulating particles; this profile is ‘humanized’ by the fat-enriched diet (1). This study provides the first experimental evidence for a role of glucose in the pathogenesis of ATS. Further, the strain-specificity points to the involvement of additional genetic factors. Unfortunately, the balb/c model has limitations in that the lesions are of small size and unusual location. They are seen in the aortic sinus, but spare the aortic arch and arterial branching points, which are the usual sites of human atheromas. The small size and slow progression are limiting factors for the observation of lesions in intervention studies.

New models of ATS have been generated with the advent of gene deletion techniques, the so-called ‘knock-out mice’. Deletion of the apolipoprotein E (ApoE) gene results in minor ATS lesions (3). However, when these mice are treated with STZ (at age 6 weeks), they develop significant lesions, now located in the branching points in the aortic arch, at age 12 weeks (i.e. only 6 weeks after the induction of diabetes) (4). This model again demonstrates a pathogenic role for hyperglycemia in ATS. It should be emphasized, though, that the model combines two atherogenic stimuli, hyperlipidemia as well as hyperglycemia. Indeed, these animals display an increase in VLDL, VLDL remnant and LDL particles induced by the deletion of the ApoE gene. ApoE normally being required for the clearance of these particles. Nonetheless, this model has already allowed studies into the pathogenic mechanisms of glucose-induced vascular damage, in particular the role of advanced glycation end products (AGEs) (4).

AGEs have long been suspected of playing a role in the pathogenesis of diabetic complications (reviewed in reference 5). AGE generation is a multi-step process. The first step is non-enzymatic glycation of proteins, by reaction of glucose (or other reactive sugars) with free amino groups in proteins. This reaction, which occurs in direct proportion to the prevailing glycemia, underlies the clinical glycohemoglobin assay. Further rearrangements of the carbohydrate moieties (including redox reactions) lead to irreversible advanced glycation products. AGEs forming on a variety of proteins from a variety of sugars have common antigenic determinants, suggesting structural similarities. AGE formation may affect the function of numerous proteins. Glycation of extracellular matrix components such as collagen and laminin could affect matrix assembly and other processes in the vascular wall. Glycation of LDL particles could also increase their atherogenic potential. However, it now appears that an essential mechanism for AGE activity is the activation of ligand-specific cell surface receptors (6). One receptor, named RAGE (receptor for AGEs), is a member of the immunoglobulin-like superfamily with a predicted molecular mass of 55 kDa.
and is expressed in macrophages, endothelial and vascular smooth muscle cells. A second, 80 kDa, protein, identified as a homolog of the iron-binding protein, lactoferrin (lactoferrin-like polypeptide), is postulated to bind both AGEs and RAGE in a ternary complex, independently of its iron content. The function of RAGE can now be assessed both in vitro and in vivo by inhibition studies using either anti-receptor antibodies or the soluble form of the receptor (sRAGE) (6). The cellular effects of AGEs appear to be mediated by an oxidant stress. Exposure of endothelial cells to AGEs induces the formation of peroxidized lipids, and activation of the oxidation-inducible transcription factor NF-κB. This transcription factor, which is also activated by cytokines, controls the expression of numerous genes thought to be involved in atherogenesis. Infusion of AGEs in rats increases vascular permeability, an effect blocked by simultaneous treatment with anti-oxidants (7–9). The mechanism of receptor-mediated oxidant stress is still poorly defined. Receptor binding could activate an NADH oxidase-like enzyme and generate intracellular superoxide anions. Alternatively, AGEs themselves could be the direct source of extracellular reactive oxygen intermediates (ROIs); receptor binding would then simply dock AGEs and thus localize the oxidant stress to the cell membrane. Whatever the mechanism, the available evidence suggests that AGEs, via receptor-mediated ROI generation, may play a pathogenic role in atherogenesis.

The latter conclusion is now supported by in vivo experiments using the diabetes model in the ApoE-deficient mice just described (4). Shortly after the induction of chemical diabetes, the animals were treated with a daily i.p. injection of sRAGE or a control protein, murine serum albumin (MSA). In contrast to MSA, sRAGE caused a dose-related inhibition of atheroma formation. There was a decrease not only in size and number, but also in the complexity of the lesions. Indeed, there was a decrease in both fatty streaks and complex lesions, defined by necrosis or fibrous cap formation. By immunohistochemistry it was shown that diabetes was associated with increased AGE deposition in the vascular wall, as well as increased RAGE expression. Both AGE deposition and RAGE expression were inhibited by sRAGE. This suggests that sRAGE prevents AGE accumulation by forming complexes with AGEs in the circulation, thus preventing their transfer across the endothelial barrier. Incidentally, this interpretation implies that AGE formation on plasma proteins is quantitatively more important than on insoluble components of the vascular wall. Diabetes caused a three-fold increase in serum cholesterol levels compared with the control ApoE-deficient mice, but the lipid profile was unaffected by sRAGE treatment. Thus, changes in the lipid profile cannot account for the beneficial, atheroprotective effect of sRAGE. Nor did sRAGE improve the average glycemia in diabetic mice. Further experiments are awaited to determine the mechanisms of the atheroprotective effect of sRAGE, in particular whether it prevents AGE-mediated oxidative stress.

Thanks to the development of a suitable murine model, this work provides important evidence for a direct effect of hyperglycemia on the development of atherosclerosis and for the role of AGE formation in this process. Clinical studies have so far failed to demonstrate a convincing effect of tight glycemic control on macrovascular complications in diabetic patients. The Diabetes Control and Complications Trial Research Group trial demonstrated a clear reduction in the incidence of microvascular complications in Type 1 diabetes with intensified insulin therapy, but the incidence of cardiovascular events in the study population was too low to detect an effect on this endpoint (10). More recently, the United Kingdom Prospective Diabetes Study (UKPDS) of Type 2 diabetes also failed to demonstrate a major beneficial effect of intensified therapy on the incidence of macrovascular disorders (11, and see Lopez-Liuchi & Meier (12)). These disappointing results may simply indicate that the quality of glycemic control presently achievable is insufficient to be effective. Treatment with inhibitors of AGE formation and/or function represents a promising avenue for the prevention of diabetes-related vascular disorders, and provides an alternative – or rather a complement – to intensified glycemic control.

References


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