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Morphological characteristics of Endothelial Dysfunction in Association with Insulin Resistance

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Between 120 and 140 million people suffer from diabetes mellitus (type 1 and type 2) worldwide, and this number may well double by the year 2015. It has been known for years that diabetes increases risk for developing atherosclerosis. Type 2 diabetes is a state of insulin resistance, hyperglycemia and dyslipidaemia characterized with high cardiovascular risk and accelerated atherosclerosis. Many mechanisms by which hyperglycaemia can result in endothelial dysfunction have now been identified. Concurrently, dysfunction of the endothelium leads to increase of the vascular tone and permeability and a prothrombotic environment. The cellular events responsible for plaque rupture allow for an increase of cell death from necrosis or apoptosis. This review summarizes the current knowledge of the processes that may be responsible for increased plaque rupture and occlusion associated with diabetes.

Key words: endothelium, insulin resistance, type 2 diabetes.

Type 2 diabetes mellitus is increasing in prevalence and is a potent risk factor for the development of atherosclerotic vascular disease and increased risk of adverse cardiovascular events. Approximately 15-25 per cent of patients with ischaemic heart disease have a history of diabetes mellitus [9]. Unfortunately, cardiovascular complications remain the leading cause of death among patients with type 2 diabetes, accounting for 70 per cent of all case-fatalities. Patients with type 2 diabetes develop an abnormal endothelial function, platelet hyperactivity, aggressive atherosclerosis, a propensity for adverse arterial remodelling, enhanced cellular and matrix proliferation following arterial injury, and impaired fibrinolysis with a tendency for thrombosis and inflammations.

Mechanisms of endothelial dysfunction in diabetes

Hyperglycaemia may contribute to endothelial dysfunction in diabetes in several ways. Limited availability of nicotinamide adenine dinucleotide phosphate (NADPH), a necessary cofactor for endothelial nitric oxide synthetase (eNOS), may occur as a result of decreased activity of the pentose phosphate pathway and result in decreased nitric oxide (NO) production. Increased transport of glucose across the endothelial cell membranes leads to increased activity of the sorbitol (aldose reductase) pathway, resulting in an increase in the nicotinamide adenine dinucleotide ($NADH/NAD^+$) ratio [16]. The ob-

tained environment is one of oxidative stress which promotes generation of superoxide (O^{2-}), an anion generated by a number of pathways which can quench NO, reducing its availbility despite normal (or even increased) production [1]. This situation is aggravated in hyperglycaemia by formation of glycation end-products and autooxidation of glucose [16]. The endothelial dysfunction is an early marker for the development of both micro- and macrovascular complications of type 2 diabetes. Hyperglycemia leads to the formation of advanced glycation end products (AGEs), which in their turn lead to inflammatory cell recruitment and cell proliferation. Modulation of the AGE and AGE-receptor axis results in a significant reduction of the neointima proliferation which induces restenosis in the Zucker rat – a model of insulin resistance [23].

Lesion initiation

It is generally believed that infiltration of monocytes into the subendothelial space and subsequent accumulation of lipid-loaded macrophages initiates the formation of atherosclerotic lesions. In young humans, accumulation of lipid-loaded macrophages is often seen in areas with intimal thickening, called intimal cell masses [17, 19]. This suggests that products of these smooth muscle cells may be critical to lesion localization [20].

An increasingly accepted hypothesis is that endothelial dysfunction appears to be a feature of type 2 diabetes. In fact, data obtained from several animal models of insulin resistance (including Zucker rats) indicate to an impaired endothelium-dependent vasodilatation [15, 21]. Diabetes appears to enhance foam cell lesion formation in experimental animals and in humans. In animal models of diabetes, like Zucker rats or diabetes induced by toxic substances (Alloxan or Streptozotocin) an increased formation of fatty streak lesions has been observed [11]. Type 2 diabetes is increasingly recognized to be associated with inflammation [3] and excess production of cytokines including tumour necrosis factor-alpha (THFα) by adipose tissue [10] which in turn may have direct effects on endothelial metabolism. Inflammation plays a key role in initiation of atherosclerosis and also in the plaque rupture processes leading to arterial occlusion. Diabetes most likely results in increased inflammation in advanced plaques. It has been shown that diabetic patients, undergoing coronary atherectomy, for symptomatic coronary artery disease display a larger content of macrophages than patients without diabetes. Increased inflammatory and immune reactions in diabetes could be explained with increased levels of modified light density lipoprotein (LDL). Glycation of LDL under hyperglycemic conditions is likely to result in increased formation of oxidized LDL [2].

Diabetes renders an increased expression of molecules that promote monocyte adherence to the endothelium and subsequent migration into the subendothelium space. Thus, high glucose levels and AGEs stimulate vascular cell adhesion molecule-1 (VCAM-1) expression in endothelial cells in culture [6], in animals [13] and patients with diabetes [12]. These studies show that diabetes may increase inflammation in advanced lesion of atherosclerosis and it is reasonable to posit that such changes would accelerate plaque rupture. One theory is that increased levels of modified LDL could explain increased inflammatory and immune reactions in diabetes. Glycation of LDL under hyperglycemic conditions is likely to cause an increased formation of oxidized LDL [2]. Both oxidized LDL and glycated LDL also induce formation of immune complexes, which at least in vitro, exert pro-inflammatory effects by stimulating cytokine release from macrophages [18]. It is considered that plaque rupture may be caused by an increase proteolytic activity within the lesion that would promote extracellular matrix degradation and thereby weakening of the fibrous cap [8].

Apoptosis

The cellular events responsible for plaque rupture suggest an increase of cell death by necrosis and/or apoptosis. A modern concept is that the apoptotic cell death induces a release of cytokines and an inflammatory response in the arterial wall [14]. A critical feature of the change from the macrophage foam cell lesion to a true atheroma is the formation of a necrotic core [19], which is due to macrophage death. One of the factors that cause macrophage death is free cholesterol [22]. These cells show spontaneous activation of caspases even before exposure to oxidized LDL. It is speculated that another group of proteases, the cathepsins, may be involved in causing plaque rupture. The cathepsins are cysteine proteinases that degrade elastin and fibrilar collagen. The events in the lesion are measured by TUNEL staining (indicative of less apoptosis), showing a larger lipid core and a thinner fibrous cap [7]. The third major hypothesis on the cellular events causing plaque rupture states that increased cell death, by necrosis and/or apoptosis, is responsible for the rupture. The effects of diabetes on apoptosis are unclear. It has been shown that streptozotocin-diabetic rats without atherosclerosis have an increased number of TUNEL-positive aortic smooth muscle cells (SMCs) compared to non-diabetic rats [4], and that hyperglycemia protects against medial SMC death in mice [5].

Conclusion

Detailed studies on the mechanisms leading to plaque rupture are not possible without relevant animal models. If the number of models available to study plaque rupture in non-diabetic animals is small, even fewer models are currently available to study plaque rupture in the diabetic setting. Rabbits, hamsters and rats, for instance, develop fatty streak lesions and atheromas, but plaque rupture and occlusion are rare or absent. The urgent need to develop animal models of diabetes-associated arterial occlusion remains. Recently, studies on the mechanisms leading to plaque rupture have become much more feasible due to the characterization of mouse models that exhibit features of plaque rupture. Understanding the mechanisms that cause plaque rupture and arterial occlusion in diabetes is very important. The vascular endothelium is a complex autocrine and paracrine organ which provides a first line defense against atherosclerosis. The recent characterization of murine models have increased the knowledge of the molecular mechanisms involved in endothelial dysfunction, plaque rupture in both diabetic and non diabetic animals and render the opportunity to learn a lot more about the effects of the diabetic syndrome on atherosclerotic lesions in humans.

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