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THE ROLE OF ENDOTHELIAL DYSFUNCTION IN THE PATHOGENESIS OF VASCULAR COMPLICATIONS OF DIABETES MELLITUS - A HIGH PRIORITY AREA OF INVESTIGATION

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Abstract

Endothelium, the inner layer of the vasculature, represents the interface between blood and organ systems and it is active in the process of contraction and relaxation of vascular smooth muscle and in functions like secretion of vasoactive substances. Endothelial dysfunction is an important cause of cardiovascular disease. The function of the endothelium can be assessed by invasive and noninvasive methods. Endothelial cells produce vasoactive substances like endothelium derived relaxing factor, prostacyclin, nitric oxide, and endothelium derived hyperpolarizing factor. Diabetes mellitus is associated with an increased risk of cardiovascular diseases. Hyperglycemia leads to cardiovascular damage through different pathways, including the polyol and hexosamine pathways, generation of advanced glycation end products, and activation of protein kinase C. Together with hyperglycemia induced mitochondrial dysfunction and endoplasmic reticulum stress, all these can promote the accumulation of reactive oxygen species. The oxidative stress induced by hyperglycemia promotes endothelial dysfunction with an important role in micro and macro vascular disease. Insulin-resistance could be independently predictive of cardiovascular disease. Life style modification and pharmacotherapy could possibly ameliorate the effect of insulin resistance

key words: *insulin-resistance, oxygen reactive species, cardiovascular disease, nitric oxide.*

Endothelium as an endocrine organ

Endothelium, the internal lining of the blood vessels, is also an endocrine organ since it secretes numerous vasoactive substances. There are various endothelium derived constrictive factors like angiotensin, catecholamines, vasopressin, tromboxan A2, prostaglandins, neuropeptide Y and also relaxing factors like kinins (bradykinin and kallidin),endothelium derived relaxing factor, and nitric oxide [1].

Nitric oxide is the main endothelium derived relaxing factor and is continuously produced in endothelial cells by nitric oxide synthase that uses L arginine as a substrate.

The kinin system includes two main peptides: bradykinin and kallidin which are derived from the proteolysis of kininogen, an inactive precursor, by means of enzymes named kininogenases [1]. Bradykinin acts on beta 1 receptors, determining smooth muscle constriction and relaxation, and also on beta 2

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receptors producing release of autocrine and paracrine factors (endothelium –derived relaxing factors, prostaglandin), vascular muscle relaxation, increased vascular permeability, etc. Bradykinin is a vasoprotector with vasodilatory, antithrombotic, fibrinolytic and antiproliferative effects mediated by nitric oxide and prostacyclin [1]. Bradykinin also mediates the release of catecholamines and histamine. The cardioprotective, vasculoprotective and reno protective effects of angiotensin converting enzyme (ACE) inhibitors are due in a great part to the potentiating effect of bradykinin [1].

Fiorentino et al. concluded that oxidative stress induces phenotypic alterations in vascular smooth muscle cells, contributing to the progression of atherosclerotic lesions. Thus, apoptosis of vascular smooth muscle cells can lead to atherosclerotic plaque instability and rupture [2].

Methods of measuring endothelial function

We can measure nitric oxide activity (as a marker of endothelial function) in plasma and urine but this is affected by dietary habits [3]. Brachial artery and forearm arteries ultrasound scanning is a noninvasive assessment of endothelial function, measuring the capacity of dilatation in response to nitric oxide. Another invasive technique is represented by quantitative coronary angiography. The most accurate method for the noninvasive measurement of endothelial function consists of flow mediated dilation assessment via ultrasound [4].

Impaired endothelial function leads to cardiovascular events and type 2 diabetes. It represents one of the most important risk factors for the development of cardiovascular disease, promoting atherosclerosis and endothelial dysfunction [5]. The reduction of the bioavailability of nitric oxide represents the main feature of endothelial dysfunction [6].

The role of insulin resistance in cardiovascular disease

A review by Reddy et al. [7] underlined that there are anthropometric traits like hyperinsulinemia, impaired glucose tolerance, dyslipidemia, endothelial dysfunction, hypertension and fat distribution of the body which increase the risk for cardiovascular disease and type 2 diabetes, the main pathologic mechanism being insulin resistance. The authors showed that insulin resistance, assessed by homeostasis model assessment, is independently predictive of cardiovascular disease risk, one unit increase in insulin resistance being associated with 5.4% increase in cardiovascular risk. In conclusion insulin resistance is the principal factor in the development of endothelial dysfunction, metabolic syndrome and cardiovascular disease [7]. Life style modification and pharmacotherapy could possibly ameliorate the effect of insulin resistance [7].

In type 2 diabetes and diet induced obesity, insulin resistance is associated with an altered endothelial cell phenotype i.e. endothelial dysfunction. The bioavailability of nitric oxide is reduced and this has an impact on large blood vessels, contributing to atherosclerosis and cardiomiopathy, and on small blood vessels, contributing to retinopathy, neuropathy and nephropathy [8].

Thus, in insulin resistant subjects there are disturbances of activation of the rennin-angiotensin system, hyperglycemia, lipotoxicity, oxidative stress and an increase of proinflamatory cytokines [8]. The bioavailability of nitric oxide is decreased because of its increased degradation or decreased synthesis or both [9]. In addition, Sukumar et al. [10] demonstrated that insulin resistance is associated with a higher production of the superoxide anion O_2^-. Reactive oxygen species are highly reactive metabolites of oxygen [11]. Cellular sources of reactive oxygen species are xantin oxidase,

NADPH dependent oxidases, mitochondrial oxidases, NO synthases and lypo-oxygenases [12]. NADPH oxidase represents in insulin resistant humans and mice a major source of O_2^- [13].

All these might have important clinical implications since Boyle et al. [14] estimated an increase to 33% by 2050 of the prevalence of diagnosed and undiagnosed cases of type 2 diabetes characterized by insulin resistance and endothelial dysfunction.

Endothelium derived relaxing factors

Endothelial dysfunction is characterized by a reduction in the bioavailability of vasodilators, particularly nitric oxide [15]. Nitric oxide, endothelium derived hyperpolarizing factor and prostacyclin are the substances which cause relaxation of the vascular smooth muscle cells. The endothelial cell membrane contains many receptors reacting to a variety of endogenous substances that activate nitric oxide synthesis by various kinds of coupling proteins.

Cathecolamines like epinephrine and nor-epinephrine have a vasodilatation effect mediated in part by their endothelial alfa2 adrenergic component. Vasopressin and oxytocin have a particular striking effect on cerebral arteries. They release endothelium derived hyperpolarizing factor by acting on endothelial vasopressin receptors and cause endothelium dependent relaxation in certain arteries [15].

Bradykinin is a potent stimulator of the release of endothelium dependent relaxation factor and of nitric oxide [1]. Nitric oxide decreases platelet adhesion and aggregation, increases relaxation of vasculature by activating cytosolic guanylate cyclase, so it is very important against thrombosis and vascular occlusion [1].

Acetylcholine, bradikinine, histamine, and substance P stimulate the formation of nitric oxide at the endothelial level, having endothelium dependent (mediated by nitric oxide) vasodilatation effects [15]. Meanwhile, nitroglycerin and papaverin have endothelium independent (not mediated by nitric oxide) vasodilatation effects.

Wennmalm et al. [16] showed that in atherosclerosis, hypercholesterolemia, essential hypertension and diabetes mellitus, endothelial dysfunction correlates to impaired tissue perfusion, more frequent thrombus formation, and increased local vascular resistance, all these representing aggravating factors for atherosclerosis.

In micro-vascular angina exists a particular form of endothelial dysfunction limited to the arterial resistance vessels [16].

In another article, Hadi et al. [17] showed that in patients with diabetes or insulin resistance, endothelial dysfunction may be a critical early target for preventing cardiovascular disease and atherosclerosis. Despite the fact that the biochemical link between vascular lesions and hyperglycaemia remains incompletely understood, control of hyperglycemia remains the best way to improve endothelial function [17].

Similarly, Schalkwijik et al. [18] showed that dysfunction of the vascular endothelium is regarded as an important factor in the pathogenesis of diabetic macro and micro angiopathy. In both type 1 and type 2 diabetes complicated by micro and macro-albuminuria, endothelial dysfunction is generalized [18]. Schalkwijik et al. [18] support the idea that endothelial dysfunction in diabetes has three main sources: (1) hyperglycemia by its direct action on endothelial function, (2) synthesis of growth factors, cytokines and vasoactive agents in other cells and (3) the components of the metabolic syndrome.

Finally, Hamdy et al. [19] concluded that adipocytokines (leptin, adiponectin, tissue

necrosis factor alpha, interleukin-6 and resistin) may represent the missing link between insulin-resistance and cardiovascular disease. They say that "endothelial dysfunction is an early step in atherosclerosis which has been reported in patients with type 2 diabetes, in individuals at high risk for type 2 diabetes, in those with impaired glucose tolerance, in obese non diabetic patients and even in normoglycaemic first degree relatives of type 2 diabetic patients" [19].

Possibilities of intervention in endothelial dysfunction

Recent cell based studies identified paroxetine (an antidepressant agent) as an inhibitor of diabetic endothelial dysfunction [20]. Thus, Gero et al. [20] exposed endothelial cells to elevated extra cellular glucose and tried to identify compounds that prevent formation of reactive oxygen species induced by hyperglycemia. Paroxetine, without interfering with the mitochondrial electron transport chain, reduced the hyperglycemia induced formation of reactive oxygen species inside mitochondria. Paroxetine can maintain the ability of vessels to respond acetylcholine (an endothelium dependent relaxant) both in vitro and in vivo (in rats with streptozotocin induced diabetes) [20].

The association between obesity, insulin resistance and endothelial dysfunction may become a key target in the prevention of type 2 diabetes and cardiovascular disease [21]. Central or abdominal obesity leads to insulin resistance and endothelial dysfunction by means of cytokines and metabolic products that derived from fat. Some recent studies demonstrated that it is possible to ameliorate endothelial function and, in a proportion, also inflammation by means of pharmacological or non-pharmacological treatment of obesity or insulin resistence [21]. Both diabetes and insulin resistance cause endothelial dysfunction which affect intracellular matrix homeostasis, smooth muscle physiologic and vascular permeability [22].

Wennmalm et al. [16] showed that restriction of dietary intake of lipids, increased intake of antioxidants like vitamin E and fish oil might restore endothelial dysfunction. In another article, Turan et al. [23] showed that inhibiting the overproduction of super oxides and peroxides would prevent cardiac dysfunction in diabetes, but this is difficult to achieve using conventional antioxidants like vitamin E. The use of catalytic antioxidants will be important [23].

Fiorentino et al. [2] said that currently there are contrasting clinical evidence regarding the benefits of antioxidant therapies in the prevention or treatment of diabetic cardiovascular complications. However, we can counteract oxidative stress by means of reducing episodes of hypoglycemia and hyperglycemia, by treatment of hypertension, dyslipidemia, kidney dysfunction and obesity [2].

Do incretins improve endothelial function?

Incretin hormones are secreted from the gastrointestinal tract in response to food intake. Two incretin hormones were identified, one derived from the L cells of the distal small intestine and large bowel (Glucagon Like Peptide 1 - GLP-1), the second secreted by the K cells of the proximal small intestine (Glucose dependent Insulinotropic Polypeptide GIP) [5]. Some studies showed that endothelial function was improved by using GLP-1 and GLP-1 related drugs [24-26]. Thus, exenatide was shown to improve endothelial function assessed by flow mediated dilation technique in subjects with type 2 diabetes. This effect is still debated because almost all these studies were non randomized trials with a small number of patients. Jun-ichi Oyama et al. [5] concluded in a recent review that large scale randomized

prospective clinical studies will provide the evidence whether incretin therapy have clinical benefits on vascular protection for type 2 diabetes patients at risk of cardiovascular disease [5].

Conclusions

Diabetes mellitus, especially type 2, is associated with an increased risk of cardiovascular disease and mortality from cardiovascular disease. Endothelial dysfunction represents an early step in the development of atherosclerosis. Endothelial dysfunction present in diabetes mellitus and in cardiovascular diseases is represented by decreased endothelium-dependent vasorelaxation, increased vascular permeability and increased leukocyte-endothelial cell adhesion, and an altered production of vasoactive substances. Impaired endothelial function is closely associated with insulin resistence and may contribute to insulin resistance. In type 2 diabetes endothelial dysfunction is present from the onset of the disease.

Several recent studies have demonstrated favorable effects of lifestyle changes in improving endothelial function and insulin sensitivity. Some trials have demonstrated that statin therapy and the therapy with angiotensin converting enzyme inhibitors may improve endothelial function in diabetes. Incretin therapy provides clinical benefits of vascular protection but future large studies are needed in the future.

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