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Η αλληλεπίδραση μεταξύ της ενδοθηλιακής λειτουργίας και της νευροπάθειας στην ανάπτυξη αγγειακής δυσλειτουργίας στον διαβήτη

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Diabetes and oxidative stress

Diabetes is currently recognized as an oxidative stress disorder. Oxidative stress per se is characterized by high accumulation of reactive oxygen species (ROS) that is impossible to be coerced by the endogenous circulating neutralizing agents and antioxidants. Hyperglycemia can induce oxidative stress through four mechanisms: (a) increased production of advanced glycation end-products (AGEs), (b) increased flux through the polyol/aldose pathway, (c) activation of protein kinase C (PKC), and (d) increased flux through the hexosamine pathway. Excessive superoxide production by the mitochondrial transport chain during hyperglycemia may be the initiating factor of all the above mentioned processes. The formation of superoxide and the subsequent increase in oxidative stress decreases NO bioavailability resulting in endothelial dysfunction and ultimately leading to atherosclerosis and cardiovascular disease.

Hyperglycemia promotes oxidative stress by conducing to the production of advanced glycation end products (AGEs) which are non-enzymatically glycated proteins or lipids susceptible to oxidation after expose to aldose sugars. AGEs can produce ROS, and trigger mechanisms that generate the production of intracellular oxidants. In addition, AGEs have been found to alter extracellular matrix protein function, cause vascular leak, decrease the bioavailability of endothelium derived nitric oxide (NO) and promote inflammation.

Hyperglycemia can also promote oxidative stress by increasing polyol pathway flux. Although aldose reductase usually presents low affinity to glucose, in a high glucose concentration environment, the increased intracellular glucose results an increased activity of aldose reductase and a consequent increase of the glucose reduction to sorbitol. This procedure, which consumes NADPH, decreases the reduced glutathione increasing subsequently the oxidative stress.

Furthermore, hyperglycemia and elevated free fatty acids, increase protein kinase C (PKC) activity which promotes oxidative stress through activation of mitochondrial NADPH oxidase. Increased PKC activity promotes vascular occlusion, and vascular inflammation by decreasing NO production, and increasing endothelin-1 (ET-1) production and vascular permeability. In addition, hyperglycemia shunts excess glucose through the

hexosamine pathway. Excessive intracellular glucose results in conversion of fructose 6-phosphate to glucosamine-6- phosphate, promoting a series of reactions that increase oxidative stress by NADPH depletion, TGF-beta and plasminogen activator inhibitor-1 (PAI-1) gene expression increase and endothelium nitric oxide synthase (eNOS) activity inhibition.

Oxidative stress and endothelial dysfunction

Endothelial dysfunction which first was described in early 80s by Furchgott and Zawadzki reflects an imbalance between release of vasodilator and vasoconstrictor endothelium-derived factors. This imbalance is thought to principally involve the reduced bioavailability of NO resulting from it's rapid inactivation by endothelial production of reactive oxygen species (ROS). Reductions in vascular NO signaling mediated by ROS may be accompanied in diabetes by a reduced synthesis of prostacyclin coupled with an undiminished or even enhanced formation of vasoconstrictor agents, which is also a factor in restricted vasodilation. With increased oxidative stress, tetrahydrobiopterin (BH4), a cofactor that tightly regulates NO production, is oxidized resulting in the uncoupling of eNOS and reduced NO production. Elevated levels of asymmetric dimethylarginine (ADMA), an endogenous inhibitor of eNOS through competition with L-arginine, may further reduce NO production. This perpetuates a cycle of vascular oxidative stress through the transfer of electrons to molecular oxygen, forming oxidant species such as superoxide and peroxynitrite, which further consumes NO and increases oxidative stress leading to endothelial dysfunction.

Endothelial dysfunction in diabetes

Endothelial dysfunction has been demonstrated in T2DM, in both the resistance and conduit vessels of the peripheral circulation as well as in the coronary circulation. The soluble adhesion molecules E-selectin, vascular cellular adhesion molecule (VCAM)-1 and intercellular adhesion molecule (ICAM)-1 are elevated in subjects with T2DM. Similarly, increased levels of von Willebrand factor (vWF), a measure of endothelial cell damage and activation, are found in diabetes. Microalbuminuria is an independent predictor of ED and may indicate widespread vascular dysfunction in diabetes.

Although the pathogenetic mechanisms underlying the development of ED in diabetes are not fully identified yet, according to the above mentioned data they mainly involve the increased oxidative stress and the subsequent restriction in NO bioavailability.

Vascular disease in diabetes

Endothelial dysfunction in both the micro- and macro-circulation is the final result of oxidative stress initiated, self perpetuating cascade of events. Progressive capillary changes including neovascularization in retinopathy, and narrowing and/or microthrombosis in peripheral neuropathy are the result of hyperglycemia induced increases in endothelial cell permeability, vascular inflammation, and other structural changes. A reduction in hyperglycemia by intensive glycemic control protocol has been shown in two separate landmark trials to decrease progression and occurrence of microvascular complications (retinopathy, neuropathy, and nephropathy) in *both* type 1 and 2 diabetes.

In contrast, glycemic control has been demonstrated to *conclusively* improve macrovascular outcomes in only *type 1 diabetes*. Despite this, macrovascular disease such as myocardial infarction (MI), cerebrovascular accidents, and peripheral arterial disease continues to account for a substantial portion of the mortality and morbidity in both type 1 and 2 diabetes. Improved glycemic control in type 1 diabetes has been associated with dramatically lower rates of macrovascular disease (42% decrease). However, despite reductions in all cause mortality associated with tighter glycemic control macrovascular event rates in type 2 diabetes are not improved with tighter glycemic control unless metformin was part of the regimen. Patients with metformin included as part of their regimen are better able to maintain glycemic control over 3 years compared to other regimens and have greater improvements in all cause mortality and decrease in stroke rates. Thus, treatment of usual cardiovascular risk factors such as hyperlipidemia and hypertension in type 2 diabetes plays a larger role in lowering the risk of macrovascular events, suggesting that oxidative stress induced by these traditional cardiovascular risk factors appears more important than that induced by hyperglycemia in such patients.

The interaction between peripheral neuropathy and skin microcirculation

In 1927, T. Lewis described the triple flare response (also known as the Lewis triple flare response). He demonstrated that the spreading of vasodilation was an axon reflex that depended on the integrity of the C nociceptive fibers. Subsequent studies indicated that acetylcholine could also produce this flare as it has a direct excitatory action on the C fibers that is related to its nicotinic action. It is currently realized that stimulation of the nociceptive C fiber results in both orthodromic conduction to the spinal cord and antidromic conduction to other axon branches, that is, the axon reflex. One function of this reflex is the secretion of several active peptides, such as substance P and calcitonin gene-related peptide, which cause vasodilation and increased permeability both directly and indirectly (through mast cell release of histamine). In addition, recent evidence suggests that these neurotransmitters play an important role in angiogenesis during wound healing.

The magnitude of the nerve axon reflex-related vasodilation is approximately one third of the maximal endothelium-dependent vasodilation that can be achieved after the iontophoresis of acetylcholine. Diabetic peripheral neuropathy, as any peripheral small fiber neuropathy, greatly affects this response to the point that is virtually absent in patients with severe disease. As a result, under conditions of stress, the capillary blood flow in the affected area is reduced, leading to increased vulnerability of the neuropathic limb to development of severe diabetic foot problems and failure to heal existing foot ulcers. The fact that successful bypass surgery, that restores blood flow in the large vessels, does not reverse the nerve axon reflex-related vasodilation abnormalities, further emphasizes the point that neuropathy itself leads to impaired vasodilation under conditions of stress and, therefore, functional ischemia. Treatments that can prevent the development of DPN or reverse its course may therefore, improve the wound healing capacity and reduce the rate of lower limb amputations.

Methods of assessing endothelial function

Prior to the development of macrovascular and microvascular clinical disease early changes in endothelial function can be measured. These changes reflect alterations in the regulation of vascular tone or reactivity which is influenced by endothelial NO production (endothelial-*dependent* vasoreactivity) as well as vascular smooth muscle relaxation in response to NO (endothelial-*independent* vasoreactivity). In endothelial dependent vasodilation, acetylcholine, shear stress or hypoxia can activate endothelial cells to release NO. The stimuli of shear stress and hypoxia are utilized in the flow mediated dilation (FMD) technique to produce endothelium-dependent vasodilation. In contrast, endothelium-independent vasodilation occurs as a result of smooth muscle cell relaxation in direct response to exogenous NO (from NO donors such as nitroglycerin or nitroprusside). Vasoreactivity, which refers to both endothelial dependent and independent vasodilation in response to a stimulus, is a means to quantify endothelial cell and vascular smooth muscle function.

Macrocirculatory measurements

Macrovascular disease is most commonly assessed by ultrasound measurements of brachial artery diameter and the common carotid intima-media thickness (IMT). Changes in brachial artery diameter after stimuli measure early functional changes associated with atherosclerosis. Endothelium-*dependent* vasodilation of the brachial artery can be assessed by intra-arterial infusion of substances that act on the endothelium to release NO, such as acetylcholine, or by FMD. FMD is induced by occluding the brachial artery with a pneumatic tourniquet to the upper limb for a total of 5 minutes. Tissue hypoxia and pH changes in the area distal to the occlusion cause reactive vasodilation in the skin and muscle microcirculation immediately after release of the occlusion. This process causes a brief period of high blood flow and increased shear stress in the brachial artery that stimulates the endothelial production of NO and vasodilation that can be measured on high resolution ultrasound. Endothelium-*independent* vasodilatory function of the brachial artery can be assessed by intra-arterial or sublingual administration of NO donors such as nitroglycerin or nitroprusside.

In contrast, common carotid IMT identifies anatomic changes consistent with early atherosclerosis. Carotid artery intima-media thickness (IMT) is an ultrasound measure of the distance between the intima to the outer edge of the media. In-

creased intima-media thickness occurs early in the process of atherosclerotic plaque formation prior to luminal narrowing. IMT is associated with the presence of conventional atherosclerotic risk factors and can predict the development of cardiovascular events.

Microcirculatory measurements

Microcirculatory vascular reactivity is most commonly assessed by Laser Doppler flowmetry to measure blood flow in the skin. Blood flow is estimated from the combination of number and velocity of moving red cells within arterioles, capillaries, and postcapillary venules. A Laser beam is delivered to the skin via a fiber optic light guide, and reflected light is gathered by a second set of photodetectors. Light reflected by moving objects, such as red blood cells, is reflected at a different frequency. The Doppler shifted fraction of the light signal and the mean Doppler frequency shift is calculated to generate a value in mV, which is proportional to the quantity and velocity of red blood cells with the measured superficial skin microcirculation.

The microcirculation can be studied without systemic side effects by using iontophoresis and microdialysis techniques that allow for precise, local delivery of vasoactive agents. Iontophoresis uses a small charge to facilitate transcutaneous delivery of charged substances into the skin without trauma or pain. The length of stimulation, strength of current used, and area of delivery determine the number of molecules transported. Endothelium-dependent vasodilation is assessed by delivery of acetylcholine using anodal current given its positive charge, whereas endothelium-independent vasodilation is assessed by the delivery of the anion sodium nitroprusside using cathodal current. Microdialysis can be used to deliver larger, water-soluble vasoactive agents that lack a charge. These techniques allow for non-invasive measurement of abnormal endothelial function prior to the development of overt clinical disease.