Aging of Blood Vessels

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Abstract

William Osler stated that “A man is as old as his arteries.” Now, it has been known that attenuation of the production of reactive oxygen species and inhibition of inflammatory pathways play a central role in the anti-aging therapy for vasculature. Dysfunction of endothelial cells (EC) has been known to promote abnormal vascular growth such as that in atherosclerosis and arteriosclerosis and postulated as an initial trigger of the progression of atherosclerosis in patients with diabetes mellitus, hypertension and hyperlipidemia. We and others have previously demonstrated high D-glucose directly induced apoptosis through activation of the bax-caspase proteases pathway in human EC. Although it has been known for years that vascular cells can release a large amount ROS, including superoxide, hydrogen peroxide, and nitric oxide, the role of oxidative stress in atherogenesis has received increasing attention in recent years. Recent works strongly suggest that NADPH oxidase is a major source of superoxide in cardiovascular cells oxidative stress can be involved in the process of endothelial dysfunction. From a view of these molecular mechanisms, HMG-CoA reductase inhibitor (statins) might inhibit the NADPH oxidase activation through inhibition of Rac activity and finally prevent the increase in ROS production in diabetes. Actually, recent clinical trial suggests that statins prevent several vascular events in patients with type 2 diabetes without high concentration of LDL-cholesterol. This pleiotropic effect of statins can improve endothelial dysfunction through Nitric Oxide production and/or antioxidant effect in diabetes patients.

KEY WORDS: atherosclerosis, aging, endothelial dysfunction, NADPH oxidase

Introduction

About one hundred years ago, William Osler stated that “A man is as old as his arteries.” 1) Now, in clinical and basic vascular research, the importance of endothelial function has been proposed as an aging of blood vessels and postulated as an initial trigger of the progression of atherosclerosis in patients with diabetes mellitus, hypertension and hyperlipidemia. For example, Diabetes is characterized by the premature development of microvascular and macrovascular disease, and hyperglycemia has been postulated to accelerate atherosclerosis by induction of endothelial dysfunction. 2-5)

In this review, we focus on endothelial dysfunction in molecular biology, and also describe the role of reactive oxygen species and NADPH oxidase in atherosclerosis as an aging of blood vessels.

Pathophysiological aging of vessels

Aging of blood vessels has become the most common and important issue as an atherosclerosis. 6-9) In his historic textbook, William Osler stated, “Longevity is a vascular question, which has been well expressed in the axiom that a man is only as old as his arteries. To a majority of men death comes primarily or secondarily through this portal. The onset of what may be called physiological arteriosclerosis depends, in the first place, upon the quality of arterial tissue (vital rubber) which the individual has inherited, and secondly upon the amount of wear and tear to which he has subjected it.” 10) Many studies of aging focus on factors that affect the endothelium and intima, hence cannot distinguish the effects of aging from the effects of disease, nor the effects of damage from the effects of repair or remodeling. Studies on intima-media thickness show progressive thickening with age. 9,10) However, clinical studies are limited by inability to distinguish intima from media. While the media dose not appreciably thicken with age, individual elastin lamellae actually thin and become separated by increasing amounts of non-load-bearing material. 11) Since the aorta and major elastic arteries dilate with age, tension in the wall is borne by the thinner elastin lamellae and remaining collagen.
Increased arterial stiffening with age is apparent as an increase in pulse wave velocity (PWV), which is the speed travel of the pulse wave along the wall of the artery. It is estimated non-invasively from the delay of pressure wave front at the femoral site compared to the carotid site, and from the distance travelled by the pulse. A typical value in a 20-year-old is 5 m/s and in an 80-year-old is 12 m/s (i.e. a 2.4-fold increase over 60 years). The aging process can be well monitored by change in the PWV rather than by reliance on the cuff sphygmomanometer.

**Endothelial cell death hyperglycemia**

In recent molecular analysis, endothelial dysfunction can be understood based on endothelial cell dysfunction or dysregulation of gene expression. Proliferation and cell death are considered two mechanically related phenomena. An emerging body of evidence has revealed that cells are programmed to commit suicide by default and require specific extracellular factors to survive. We and others have demonstrated that high D-glucose treatment induced endothelial cell death in a culture model. In particular, a recent report has documented the presence of apoptosis in endothelial cells treated with high D-glucose. Disruption or dysfunction of endothelial cells, causing loss of multiple endothelium-derived substances (PGI2, NO, CNP), has been hypothesized to play a pivotal role in the progression and/or development of vascular disease in diabetes. We demonstrated that high D-glucose treatment induced endothelial cell death through the induction of apoptosis, not mannitol and L-glucose as controls for osmolarity. Of importance, our studies revealed a significant increase in bax, a proapoptotic factor, by high D-glucose treatment, which indicated that apoptosis induced by high D-glucose may be attributable to an inappropriate increase in the ratio of bax to bcl-2 induced by high D-glucose. It has also reported that in vivo bax accelerated death of retinal cells in hyperglycemia and that hyperglycemic conditions increased the expression of bax as early as the preimplantation blastocyst stage in the mouse. In the latter report, the blastocysts from bax-deficient mice were protected from glucose-induced apoptosis. This result suggests that bax may be a key modulator of hyperglycemia-induced apoptosis and high-rate congenital malformations and spontaneous miscarriages induced by hyperglycemia at early stages after conception. Interestingly, the blastocysts from bax-deficient mice were protected from glucose-induced apoptosis, and in streptozotocin-induced hyperglycemic rats, TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling) staining in aortic sections showed a six fold increase of positive cells in the media of diabetic aorta, whereas electron microscopy demonstrated the typical apoptotic cells and bodies in the media of aorta from diabetic, but not control, rats. Moreover, high glucose increased apoptosis in cultured endothelial cells and programmed cell death of retinal microvascular cells occurs in situ in human and experimental diabetic retinopathy. From these evidences, endothelial cell death, especially apoptosis, in hyperglycemia condition may cause endothelial dysfunction.

**Role of reactive oxygen species (ROS) and NADPH oxidase in vascular tissues of diabetes**

The role of oxidative stress in atherogenesis has received increasing attention in recent years. It has been known for years that cardiovascular tissues can release a large amount ROS, including superoxide, hydrogen peroxide, and nitric oxide. Recent works strongly suggest that NADPH oxidase is a major source of superoxide in cardiovascular cells. NADPH oxidase is a membrane-associated enzyme that catalyzes the 1-electron reduction of oxygen using NADPH or NADH as the electron donor. NADPH oxidase in leukocytes has been thoroughly studied and is found in phagocytes and B-lymphocytes. Recently 5 components have been identified in the core of the enzyme: p40FAD (PHOX for Phagocyte Oxidase), p47FAD, p67FAD, p22PHOX and gp91PHOX. In the resting cell, three of these five components, p40PHOX, p47PHOX and p67PHOX, exist in the cytosol, forming a complex. The other two components, p22PHOX, and gp91PHOX, are bound to the membranes. When these two groups of components are separated by their distribution in different subcellular compartments, as in the resting cell, the enzyme is inactive. Various stimuli, such as protein kinases A or C, lead to the phosphorylation of the cytosolic components and the entire cytosolic complex then migrates to the membrane (Figure 1). Not only the core subunits are required for activation, but also two low-molecular-weight guanine nucleotide-binding proteins, Rac and Rap. In the resting cell, Rac is located in the cytoplasm in a dimeric complex with Rho-GDI (Guanine nucleotide Dissociation Inhibitor) and Rap is located in membranes from which it can be copurified with the cytochrome. During activation, Rac binds guanosine triphosphate (GTP) and migrates to the membrane with the core cytosolic complex. Therefore, it has been suggested that Rac may be involved in the activation of cardiovascular NADPH oxidase.

Recent reports have indicated that exposure of cultured vascular cells to high glucose level increased ROS production and treatment of the cells with PMA, a protein kinase C (PKC) activator, or angiotensin II also increased it. In addition, such increases by high glucose level or PMA were restored to control values by diphenylene iodonium (an NADPH oxidase inhibitor) and captopril C or GF109203X (a PKC inhibitor). In contrast, other inhibitors of flavoproteins, such as xanthine oxidase (oxypyrin), nitric oxide synthase (L-N-monomethyl arginine), and mitochondrial electron transport chain oxidase (rotenone), were ineffective. These results suggest that high glucose level stimulates ROS production via a PKC-dependent activation of NADPH oxidases in vascular cells and renal mesangial cells. In parallel with the NAD(P)H oxidase activity, high glucose level or angiotensin II induced an activation of Rac-1 and this activation was inhibited by PKC. Several reports have recently shown that the expression of NADPH oxidase subunit proteins (p22PHOX, p47PHOX, or p67PHOX) is upregulated in aorta from animal models of atherosclerosis and in saphenous vein and internal mammary artery from patients with diabetes and coronary artery disease. These results further support the idea that vascular NADPH oxidase may play a role in the pathogenesis of macroangiopathy associated with diabetes.

Inhibition of oxidative stress using various antioxidants has shown some success at preventing the diabetic vascular complications in animal models. However, results of studies in humans have generally been negative. One possible reason for its ineffectiveness is that radical scavengers such as vitamin E may serve not only as an antioxidant but also as a pro-oxidant. For example, vitamin E reacts with radicals and subsequently
generates tocopheroxyl radicals. Presently, one of the most promising specific inhibitors of PKC-beta is LY333531. Oral administration of LY333531 to diabetic rats has been reported to prevent the increased albumin excretion, elevated glomerular filtration, and abnormal retinal hemodynamics. 31) The beneficial effects of PKC-beta specific inhibitor might be also at least in part due to its inhibitory effect on oxidative stress. Further investigation into the anti-oxidative properties of this agent is imperative.

3-hydroxy-3-methylglutaryl CoA reductase inhibitors (statins) as an anti-oxidants

The effect of the 3-hydroxy-3-methylglutaryl CoA reductase inhibitors (statins) on cardiovascular diseases is mainly attributed to their cholesterol-lowering properties, but accumulating evidence has shown that some beneficial effects of these agents may be independent of plasma cholesterol levels. In the cholesterol biosynthetic pathway, reduction of HMG-CoA to mevalonate by the HMG-CoA reductase is a rate-limiting step. Inhibition of this enzyme by statins not only leads to the reduction of cholesterol but also the reduction of the synthesis of several isoprenoid intermediates. These intermediates, such as farnesylpyrophosphate (FPP) and geranylgeranylpyrophosphate (GGPP), serve as important lipid attachments for posttranslational modification of a variety proteins, including the gamma subunit of heterotrimeric G proteins, heme-a, nuclear lamins, as well as Ras and Ras-like proteins, such as Rho and Rac. 32) Thus, protein isoprenylation allows the covalent attachment, subcellular localization, and intracellular trafficking of membrane-associated proteins. Importantly, members of Ras and Rho GTPase family are major substrates for posttranslational modification by prenylation. 32,33) Ras translocation from the cytoplasm to the plasma membrane is dependent on farnesylation, whereas Rho translocation is dependent on geranylation. 34,35) Notably, recent reports have revealed that statins may inhibit ROS production in vascular cells probably via inhibition of angiotensin II–induced NADPH oxidase activation. 36,37) Therefore, statins might also inhibit the high glucose–induced NADPH oxidase activation and finally prevent the increase in ROS production in diabetes. For activation of NADPH oxidase, active Rac has to be anchored in the membrane via its geranylgeranyl tail. Statins may inhibit the high glucose–induced activation of Rac by inhibiting the geranylgeranylation–dependent translocation of Rac from the cytosol to the cell membrane. This notion may be supported by a recent clinical trial showing primary prevention of cardiovascular disease with atorvastatin in type 2 diabetes in the Collaborative Atorvastatin Diabetes Study (CARDS): multicentre randomized placebo-controlled trial. 38) The aim of this study was to assess the effectiveness of atorvastatin 10 mg daily for primary prevention of major cardiovascular events in patients with type 2 diabetes without high concentrations of LDL-cholesterol. The trial was followed for 3.9 years, and 127 patients allocated placebo (2.46 per 100 person-years at risk) and 83 allocated atorvastatin (1.54 per 100 person-years at risk) had at least one major cardiovascular event (rate reduction 37%). Atorvastatin reduced the acute coronary heart disease events were reduced by 36%, coronary revascularizations by 31%, rate of stroke by 48%, and the death rate by 27%. No excess of adverse events was noted in the
atrovasstatin group. These beneficial effects of statins for diabetic vascular complications may be explained by their antioxidative properties rather than their cholesterol-lowering effect.

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**Anti-aging therapy for atherosclerosis**

In aging vessels, impaired endothelial function induces vasoconstriction and inflammatory and proliferative changes in the arterial wall and promotes atherosclerotic lesion growth. Prevention or normalization of endothelial function, conversely, contributes to the prevention of vascular lesion progression or stabilization. This can lead to risk reduction of cardiovascular events and strokes. Anti-atherosclerotic effects of statins and angiotensin converting enzyme inhibitors can be expected to improve endothelial dysfunction through Nitric Oxide production and/or anti-oxidant effect. Furthermore, novel therapies with anti-oxidants have been expected to stabilize the endothelial cell layer, which make great contributions as an anti-aging therapy in vasculature.

**References**


