

Endothelial Dysfunction in Hyperglycemia as a Trigger of Atherosclerosis

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Abstract: Type 2 diabetes is associated with a two to fourfold increased risk of both coronary heart disease and stroke. Dysfunction of endothelial cells (EC) is known to promote abnormal vascular growth such as that in atherosclerosis and arteriosclerosis and has been postulated as an initial trigger of the progression of atherosclerosis in patients with diabetes mellitus, and hyperglycemia is an independent risk factor for the development of cardiovascular disease. We and others have previously demonstrated that high D-glucose induced apoptosis through activation of the bax-caspase proteases pathway in human EC and the potential contribution of hepatocyte growth factor, as an anti-apoptotic factor, to the pathogenesis of endothelial dysfunction. The anti-apoptotic action of HGF was due to bcl-2-upregulation and the phosphatidylinositol 3-kinase pathway, which is involved in Akt activation. Although it has been known for years that cardiovascular tissues 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 work strongly suggests that NADPH oxidase is a major source of superoxide in cardiovascular cells, and oxidative stress can be involved in the process of endothelial dysfunction. NADPH oxidase can be activated in hyperglycemia through the protein kinase C pathway. From the viewpoint of these molecular mechanisms, HMG-CoA reductase inhibitors (statins) might inhibit the high glucose-induced NADPH oxidase activation through inhibition of Rac activity and finally prevent the increase in ROS production in diabetes. A recent clinical trial suggested that statins prevent several vascular events in patients with type 2 diabetes without a high concentration of LDL-cholesterol. These pleiotropic effects of statins can be expected to improve endothelial dysfunction through nitric oxide production and/or an anti-oxidant effect in diabetic patients.

Keywords: Hyperglycemia, endothelial cell, atherosclerosis, NADPH oxidase, hepatocyte growth factor, HMG-CoA reductase inhibitors.

INTRODUCTION

Diabetes is characterized by the premature development of microvascular and macrovascular disease [1-4]. In addition, hyperglycemia is an independent risk factor for the development of cardiovascular disease. The fact that glucose uptake by vascular cells is largely insulin-independent renders vascular cells vulnerable to glucose-induced injury when the extracellular glucose concentration is elevated [1-4]. Hyperglycemia has been postulated to accelerate atherosclerosis by induction of endothelial dysfunction.

In this review, we focus on endothelial dysfunction in hyperglycemia, and also describe the role of reactive oxygen species and NADPH oxidase in atherosclerosis associated with diabetes mellitus.

ENDOTHELIAL CELL DEATH IN HYPERGLYCEMIA

Cell 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 [5, 6]. We and others [7-9] 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 [10]. 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, but mannitol and L-glucose as controls for osmolarity did not [9, 11, 12]. 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 [9]. It has also been reported that bax accelerated the death of retinal cells in hyperglycemia *in vivo* [13] and that hyperglycemic conditions increased the expression of bax as early as the preimplantation blastocyte stage in the mouse [14]. In the latter report, blastocysts from bax-deficient mice were protected from glucose-induced apoptosis. This result suggests that bax may be a key modu-

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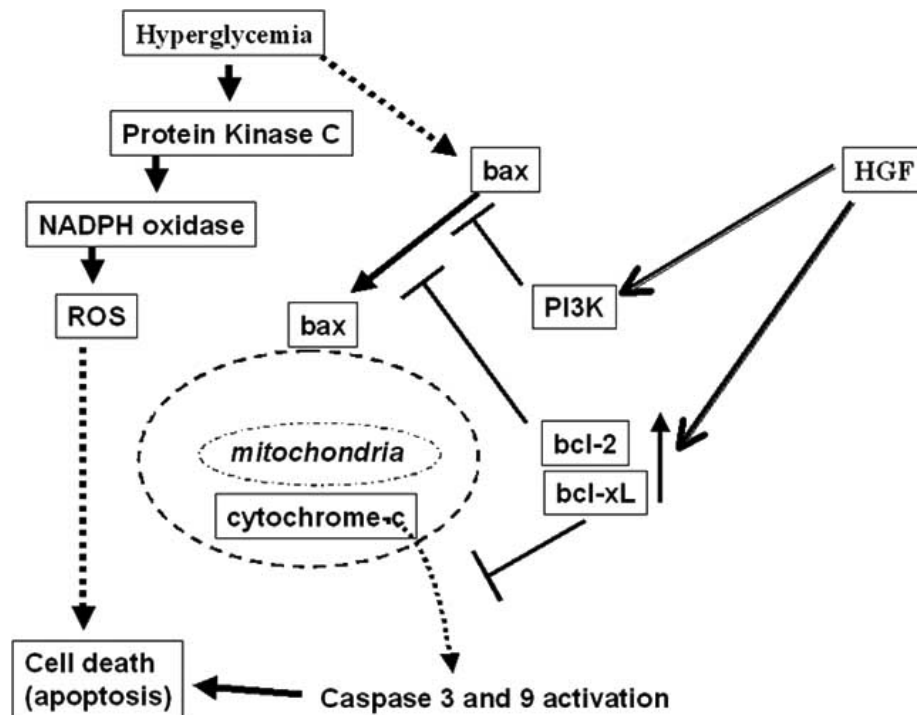


Fig. (1). Potential mechanisms of anti-apoptotic action of HGF.

Hyperlipidemia activates NADPH oxidase in endothelial cells through the protein kinase C pathway. High D-glucose treatment increased Bax protein without affecting Bcl-2 protein, and also stimulated the translocation of Bax to the mitochondrial heavy membrane. On the other hand, since HGF could significantly increase Bcl-2 protein without affecting Bax protein, it could block the translocation of Bax. These changes in Bax released cytochrome c from mitochondria, resulting in activation of the caspase cascade. Therefore, upregulation of Bcl-2 induced by HGF can block the release of cytochrome c through both a direct action on mitochondria and blockade of bax translocation.

lator of hyperglycemia-induced apoptosis and the high rate of congenital malformations and spontaneous miscarriages induced by hyperglycemia in the early stages after conception. Interestingly, blastocysts from bax-deficient mice were protected from glucose-induced apoptosis [14], 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 aortic media of diabetic rats, while electron microscopy demonstrated typical apoptotic cells and bodies in the aortic media of diabetic, but not control, rats [15]. Moreover, high glucose increased apoptosis in cultured endothelial cells [9, 10], and programmed cell death of retinal microvascular cells occurred in situ in human and experimental diabetic retinopathy [16]. From these findings, endothelial cell death, especially apoptosis, in a hyperglycemia condition may cause endothelial dysfunction.

ANTI-APOPTOTIC ACTION OF HEPATOCYTE GROWTH FACTOR IN ENDOTHELIAL CELLS

We have also focused on the role of hepatocyte growth factor (HGF) because it is a novel member of the angiogenic growth factors [17, 18]. Local vascular HGF production was downregulated by high D-glucose through the activation of transforming growth factor- β [8]. Importantly, recombinant HGF significantly increased bcl-2 protein without affecting

bax protein and attenuated the high glucose-induced caspase 3 and 9 activation [19]. The anti-apoptotic action of HGF through bcl-2 induction may be effective against not only high glucose conditions, but also other stimulation involved in activation of the mitochondrial-mediated apoptotic pathway, because HGF attenuated caspase 3 activation induced by tumor necrosis factor- α through the phosphatidylinositol 3-kinase pathway, which was involved in Akt activation [20]. These anti-apoptotic actions of HGF are not unique as vascular endothelial growth factor (VEGF) and fibroblast growth factor also exhibited such actions. Expression of VEGF and its receptor was also decreased in the myocardium of diabetic rats [21], similar to HGF [22]. However, a potential unique mechanism of HGF is the ability of direct association between bcl-2 and c-met (specific receptor of HGF) via bag-1 protein. The bag-1 protein has been reported to interact with the bcl-2 protein and to cooperate with the bcl-2 protein to suppress apoptosis [23]. Of importance, the bag-1 protein appears to inhibit cell death by binding to bcl-2, the raf-1 protein kinase, and c-met [24]. The cooperative activation of these bcl-2-related genes may also participate in the prevention of cell death by HGF, although further studies are necessary. It has been suggested that bcl-2 exerts anti-apoptotic activity by two mechanisms: sequestration of the performs of two major caspases—pro-caspase 9 and pro-caspase 8—and inhibition of apoptogenic mitochondrial changes, including cytochrome c release and loss, resulting in apoptosis inducible factor release from isolated

mitochondria [25, 26]. It has also been reported that HGF can protect against cell death through the phosphorylation of bad via phosphatidylinositol 3-kinase and increase bcl-xL [27], and bax translocation can be regulated by a conformational change resulting in the exposure of its BH3 domain, and phosphatidylinositol 3-kinase prevents apoptosis through the inhibition of conformational change of the bax BH3 epitope [28] (Fig. (1)). From these findings, endothelial cell death, especially apoptosis, in hyperglycemia can be attenuated by addition of growth factors, which are powerful anti-apoptotic factors.

ROLE OF REACTIVE OXYGEN SPECIES (ROS) AND NADPH OXIDASE IN VASCULAR TISSUES OF DIABETICS

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 suggests that NADPH oxidase is a major source of superoxide in cardiovascular cells [29]. NADPH oxidase is a membrane-associated enzyme that catalyzes the 1-electron reduction of oxygen using NADPH or NADH as an electron donor. NADPH oxidase in leukocytes has been thoroughly studied and is found in phagocytes and B-lymphocytes. As shown in Figure 2, five components have been identified in the core of the enzyme:

p40^{phox} (phox for PHagocyte OXidase), p47^{phox}, p67^{phox}, p22^{phox} and gp91^{phox}. In the resting cell, three of these five components, p40^{phox}, p47^{phox} and p67^{phox}, exist in the cytosol, forming a complex. The other two components, p22^{phox} and gp91^{phox}, 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 (Fig. (2)). 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 [30, 31].

Recent reports have indicated that exposure of cultured vascular cells to a high glucose level increased ROS production and treatment of the cells with PMA, a protein kinase C (PKC) activator, also increased them. In addition, such increases by a high glucose level or PMA were restored to control levels by diphenylene iodonium (an NADPH oxidase inhibitor) and calphostin C or GF109203X (PKC

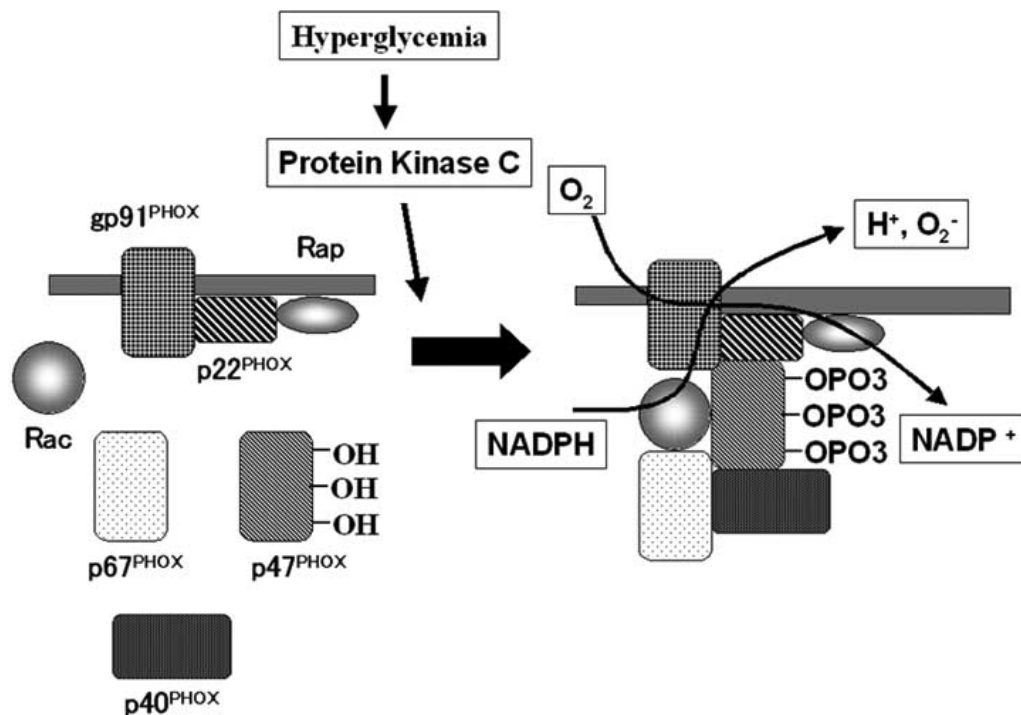


Fig. (2). NADPH oxidase. The core enzyme comprises five components: p40^{phox} (phox for PHagocyte OXidase), p47^{phox}, p67^{phox}, p22^{phox} and gp91^{phox}: In the resting cell, three of these five components, p40^{phox}, p47^{phox} and p67^{phox}, exist in the cytosol, forming a complex. The other two components, p22^{phox} and gp91^{phox}, are located in the membranes. When it is stimulated by hyperglycemia through the protein kinase C pathway, the cytosolic component becomes heavily phosphorylated and the entire cytosolic complex migrates to the membrane. Activation requires participation, not only of the core subunits, but also of two low-molecular-weight guanine nucleotide-binding proteins, Rac and Rap. During activation, Rac binds guanosine triphosphate (GTP) and migrates to the membrane along with the core cytosolic complex.

inhibitors). In contrast, other inhibitors of flavoproteins, such as xanthine oxidase (oxyprinal), nitric oxide synthase (1-N-monomethyl arginine), and mitochondrial electron transport chain oxidase (rotenone), were ineffective. These results suggest that a high glucose level stimulates ROS production via PKC-dependent activation of NADPH oxidases in vascular cells and renal mesangial cells. In parallel with NAD(P)H oxidase activity, a high glucose level induced activation of Rac-1 and this activation was inhibited by PKC. Several reports have recently shown that the expression of NADPH oxidase subunit proteins (p22^{phox}, p47^{phox}, and p67^{phox}) is upregulated in the aorta of animal models of diabetes [32, 33] and in the saphenous vein and internal mammary artery from patients with diabetes and coronary artery disease [34]. Furthermore, this enzyme-driven superoxide production was reported to be involved in vascular dysfunction such as impaired endothelium-dependent vasodilation found in a type 2 diabetes animal model [33]. 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 diabetic vascular complications in animal models. However, the 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 antioxidants but also as pro-oxidants. 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 increased albumin excretion and abnormal retinal hemodynamics [35]. Clinical trials are now ongoing to assess the effects of a PKC- beta inhibition on diabetic retinopathy and neuropathy. The beneficial effects of a PKC-beta specific inhibitor might be also due, at least in part, 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 ANTI-OXIDANTS

The effect of 3-hydroxy-3-methylglutaryl CoA reductase inhibitors (statins) on cardiovascular disease 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 level. In the cholesterol biosynthetic pathway, reduction of HMG-CoA to mevalonate by HMG-CoA reductase is the rate-limiting step. Inhibition of this enzyme by statins leads to not only a reduction of cholesterol but also a reduction of the synthesis of several isoprenoid intermediates (Figure 1). These intermediates, such as farnesylpyrophosphate (FPP) and geranylgeranylpyrophosphate (GGPP), serve as important lipid attachments for posttranslational modification of 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 [36]. Thus, protein isoprenylation allows covalent attachment, sub-cellular localization, and intracellular trafficking of mem-

brane-associated proteins. Importantly, members of the Ras and Rho GTPase family are major substrates for posttranslational modification by prenylation [36, 37]. Ras translocation from the cytoplasm to the plasma membrane is dependent on farnesylation, whereas Rho translocation is dependent on geranylgeranylation [38, 39]. Notably, recent reports have revealed that statins may inhibit ROS production in vascular cells probably via inhibition of angiotensin II-induced NADPH oxidase activation [40, 41]. Therefore, statins might also inhibit 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 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 by atorvastatin in type 2 diabetes in the Collaborative Atorvastatin Diabetes Study (CARDS): a multicenter randomized placebo-controlled trial [42]. The aim of this study was to assess the effectiveness of atorvastatins at 10 mg daily for primary prevention of major cardiovascular events in patients with type 2 diabetes without a high concentrations of LDL-cholesterol. The subjects were 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 acute coronary heart disease events by 36%, coronary revascularization by 31%, rate of stroke by 48%, and mortality by 27%. No excess of adverse events was noted in the atorvastatin group. These beneficial effects of statins on diabetic vascular complications may be explained by their antioxidative properties rather than their cholesterol-lowering effect.

CONCLUSION

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 destabilization. This can lead to a reduction of risk of cardiovascular events and strokes. The anti-atherosclerotic effects of statins can be expected to improve endothelial dysfunction through nitric oxide production and/or an anti-oxidant effect in diabetic patients. Furthermore, novel therapies are expected to stabilize the endothelial cell layer, and make a great contribution to diabetic patients.

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