Involvement of Glycation and Oxidative Stress in Diabetic Macroangiopathy

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Under diabetic conditions, the Maillard reaction facilitates the production of reactive oxygen species, and the activity of antioxidant enzymes such as Cu,Zn-superoxide dismutase is decreased, resulting in a remarkable increase of oxidative stress. The oxidative stress attacks DNA, lipids, and proteins and is also thought to be involved in the pathogenesis of diabetic complications, including the progression of macroangiopathy. Proliferation of smooth muscle cells (SMCs) is known to be associated with progression of macroangiopathy and is modulated by several growth factors. At least three mitogens for SMCs, platelet-derived growth factor (PDGF), fibroblast growth factor, and heparin-binding epidermal growth factor-like growth factor (HB-EGF), are known to be produced by SMCs themselves and are considered to be the most potent growth factors in the progression of macroangiopathy as seen in diabetes. HB-EGF, but not PDGF, is regulated at the transcriptional level by 3-deoxyglucosone (3-DG), a major and highly reactive intermediate in the glycation reaction. The induction seems to be triggered by the increase of reactive oxygen species produced by 3-DG. Taken together, glycation reactions under diabetic conditions may be highly associated with the pathogenesis of diabetic macroangiopathy by enhancing the gene expression of HB-EGF. *Diabetes* 45 (Suppl. 3):S81–S83, 1996

OXIDATIVE STRESS BY GLYCATION

Involvement of the glycation reaction in the pathogenesis of diabetic complications has been suggested (1). Schiff base adducts or Amadori products produced through the glycation reaction generate reactive oxygen species. Furthermore, there seems to be a possible connection between the production of reactive oxygen species and atherogenesis or diabetic complications, including macroangiopathy. Transition metals, especially Cu^{2+} and Fe^{3+}, have also been implicated in the production of reactive oxygen species by enhancing the Fenton chemistry.

INACTIVATION OF ANTIOXIDANT ENZYME BY GLYCATION

Cu,Zn-superoxide dismutase (Cu,Zn-SOD) is an antioxidant enzyme that is abundant in most tissues. Levels of glycated Cu,Zn-SOD are increased in patients with diabetes. Our previous studies indicated that human Cu,Zn-SOD is inactivated by glycation at specific lysine residues, Lys^{122} and Lys^{128}, and that glycation of Cu,Zn-SOD initially brings about a site-specific cleavage of the molecule between Pro^{62} and His^{63} followed by random fragmentation (2,3). We speculated that reactive oxygen species produced from the glycated protein were involved in both fragmentation steps. Thus, oxidative stress is brought about by the inactivation of this antioxidant enzyme as well as by the increased production of reactive oxygen species in diabetic conditions.

DNA DAMAGE INDUCED BY REACTIVE OXYGEN SPECIES PRODUCED DURING GLYCATION REACTION

Recently, the significance of reactive oxygen species in damage to many biological molecules, including DNA, has drawn much attention. Cleavage of purified DNA has been induced efficiently by direct treatment with reactive oxygen species. Several reducing sugars and advanced glycation end products (AGEs) have also been shown to have mutagenic effects. Reactive oxygen species are produced during the glycation of Cu,Zn-SOD. Therefore, we investigated the effects of glycated Cu,Zn-SOD on cloned DNA fragments and analyzed the formation of 8-hydroxydeoxyguanosine (8-OH-dG), one of the oxidative DNA products (4). Incubation of cloned DNA fragments with Cu,Zn-SOD and reducing sugars resulted in cleavage of the DNA. The extent of the cleavage corresponded to the reducing capacity of the sugar. Metal-chelating reagents (EDTA and bathocuproine) and an H_2O_2 scavenger (catalase) inhibited the DNA cleavage. Hydroxyl radical scavengers and aminoguanidine, an inhibitor of glycation, also inhibited the reaction. In this cleavage process, Fenton chemistry is implicated because a hydroxyl radical is produced by Cu^{2+} and H_2O_2 released from the glycated Cu,Zn-SOD. These results indicate that oxidative stress produced by glycation of metal-containing proteins may partly explain the deterioration of organs under diabetic conditions.

3-DEOXYGLUCOSONE (3-DG)

An intermediate in glycation reaction. 3-DG has been known to be a major and highly reactive intermediate in the glycation reaction and a potent cross-linker responsible for the polymerization of proteins to form AGEs (Fig. 1). Plasma 3-DG levels are increased under diabetic conditions (5,6). The enzyme that reduces 3-DG has been identified as an aldehyde reductase (7), and the aldehyde reductase itself undergoes glycation and inactivation (8).

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AGE, advanced glycation end product; Cu,Zn-SOD, Cu,Zn-superoxide dismutase; 3-DG, 3-deoxyglucosone; FGF, fibroblast growth factor; HB-EGF, heparin-binding epidermal growth factor-like growth factor; HUVEC, human umbilical vein endothelial cell; 8-OH-dG, 8-hydroxydeoxyguanosine; PDGF, platelet-derived growth factor; SMC, smooth muscle cell.

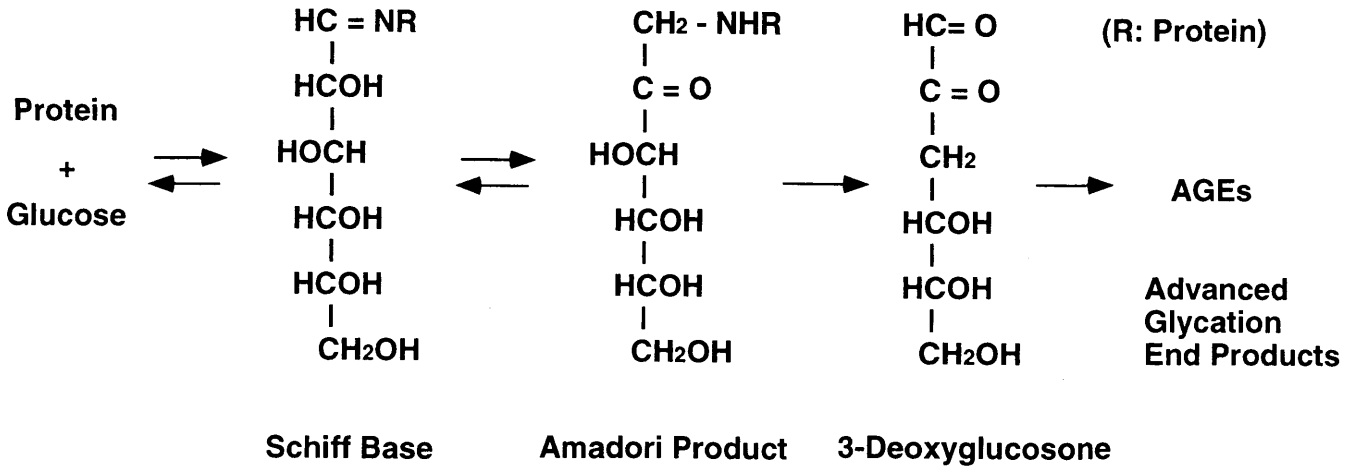

FIG. 1. General scheme of glycation reaction.

PROLIFERATION OF SMOOTH MUSCLE CELLS (SMCs) IN ATHEROGENESIS

The process of atherogenesis in the arterial wall is characterized by the formation of fibrous fatty lesions by inflammatory processes such as the infiltration of macrophages and T-cells, and the proliferation of neointimal SMCs that have migrated in from the media. It has been suggested that cells such as SMCs in the atherosclerotic lesions produce growth factors. Proliferation of SMCs in atherogenesis is modulated by several growth factors. At least three mitogens for SMCs (platelet-derived growth factor [PDGF], fibroblast growth factor [FGF], and heparin-binding epidermal growth factor-like growth factor [HB-EGF]) are known to be produced by SMCs themselves and are considered to be the most potent growth factors in the progression of macroangiopathy in diabetes.

HB-EGF

HB-EGF is a potent SMC mitogen and chemoattractant that was originally identified in human macrophage-conditioned medium (9). HB-EGF is a member of the EGF family and is synthesized as a transmembrane precursor (pro–HB-EGF), which is processed to the mature 87-amino acid form of HB-EGF (Fig. 2). HB-EGF is similar to PDGF and FGF in that it is synthesized by macrophages and SMCs and is also a potent mitogen and chemoattractant for SMCs. To elucidate whether HB-EGF is implicated in the pathogenesis of human atherosclerosis, immunohistochemical localization of HB-EGF in human aortic walls and atherosclerotic plaques was examined (10). The medial SMCs of the aorta in babies and children synthesized HB-EGF protein, while the number of SMCs producing HB-EGF was dramatically decreased in young and middle-aged adults. In atherosclerotic plaques, however, a marked production of HB-EGF protein was detected in SMCs and macrophages of the plaques. Furthermore, EGF receptors, to which HB-EGF is known to bind, were detected in plaques of SMCs. These data suggest that HB-EGF may be implicated in the migration and proliferation of SMCs that occur in normal development of arterial walls and in the formation of atherosclerotic plaques.

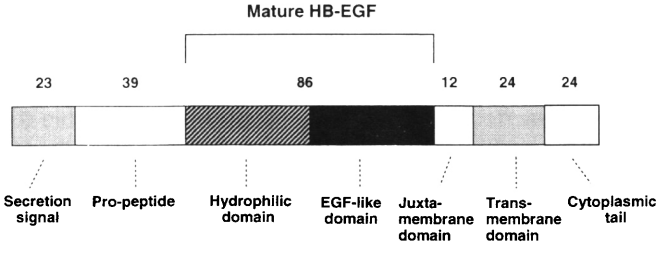

FIG. 2. Primary structure of HB-EGF.

INDUCTION OF HB-EGF mRNA BY 3-DG IN RAT AORTIC SMCs

To elucidate the mechanism by which atherosclerosis is accelerated under diabetic conditions, we have investigated the effects of 3-DG, a major and highly reactive intermediate in the glycation reaction, on the expression of genes encoding two growth factors (PDGF and HB-EGF) in cultured rat aortic SMCs and human umbilical vein endothelial cells (HUVECs) (M.A. et al., unpublished observations). Low levels of PDGF and HB-EGF mRNA were detected in rat aortic SMCs by Northern blot analysis. Treatment of SMCs with 3-DG resulted in a marked increase in HB-EGF mRNA levels in a dose-dependent manner, but the PDGF mRNA level was not increased. In contrast, after treatment of HUVECs with 3-DG, the mRNA levels of these two growth factors were not increased. It has been suggested that the HB-EGF mRNA level is modulated partly by intracellular peroxides (Y.K. et al., unpublished observations). We investigated the effects of 3-DG on intracellular peroxide levels with flow cytometric analysis using an oxidation-sensitive fluorescent probe 2′,7′-dichlorofluorescin diacetate. 3-DG increased the levels of intracellular peroxides rapidly, preceding the increase of HB-EGF mRNA level in rat aortic SMCs. Based on these results, it is conceivable that oxidative stress induced by 3-DG might trigger the induction of HB-EGF mRNA expression.

These results indicate that increased oxidative stress may be highly associated with the pathogenesis of diabetic macroangiopathy by enhancing the gene expression of HB-EGF, one of the potent mitogens and chemoattractants for aortic SMCs.

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