Complications of Diabetes Mellitus and Oxidative Stress

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Abstract: It has been reported that oxidative stress is enhanced in response to hyperglycemia in vascular tissues of patients with diabetes mellitus, leading to the peroxidation of cellular membrane lipids as well as the increased oxidative modification of amino acids and DNA. This can be explained by the molecular mechanisms of active oxygen overproduction and impaired antioxidant defense. Overproduction of active oxygen under hyperglycemic conditions is reported to be associated with the following factors: 1) Auto-oxidation of glucose; 2) effects of protein glycation products; 3) activation of protein kinase C; and 4) active oxygen produced by mitochondria. On the other hand, regarding the antioxidant defense system, decreases in SOD activity in hydrogen peroxide detoxifying activity in the glutathione redox cycle, or in the contents of the ascorbate and glutathione (GSH) lead to impaired antioxidant function. These abnormalities initiate oxidative stress to vascular tissues resulting in the activation of transcriptional factors (NF-B, AP-1) in vascular cells, which subsequently induces the expression of genes of adhesion molecules and growth factors, leading to the progression of atherosclerosis. In this context, examinations of the significance of antioxidants as strategy for preventing vascular disorders in diabetic patients have been conducted, and the clinical usefulness of these agents have been reported in small-scale clinical studies.

Key words: Hyperglycemia; Glycation/oxidation; Anti-oxidation; Vascular disorders

Introduction

Diabetes mellitus is accompanied by vascular disorders, which is relatively specific to diabetes, and caused primarily by hyperglycemia. In contrast, atherosclerosis in diabetes advances 10 years earlier in diabetic patients as compared to patients not suffering from diabetes and is accelerated by hyperglycemia, hypertension, hyperlipidemia, obesity, and smoking, which are commonly observed in diabetic patients.

Recent studies have reported that the various mechanisms accompanying hyperglycemia cause more oxidative damage (increased oxidative stress) in the blood and tissue of diabetic patients, as compared with healthy individuals.
In fact, Dandona et al. reported that increases in 8-hydroxydeoxyguanosine, content in monocytes considered to be an indicator of oxidative degeneration, are observed in monocyte DNAs of patients with type I diabetes mellitus.

We also reported that phospholipids in plasma lipoprotein undergo oxidative modification in patients with type II diabetes mellitus, leading to an increase in contents of peroxide lipids and lysophosphatidylcholine, and that an accumulation of peroxide lipids is observed in cardiac and vascular tissues in diabetic animals.

Enhanced oxidative stress in the blood and tissue is thought to play an important role in the onset and progression of atherosclerosis and microvascular complications in diabetic patients. Thus, the molecular mechanisms of the enhancement of oxidative stress in diabetes have been studied in two topics such as 1) increased production of active oxygen and 2) impaired antioxidant defense (function of scavengers).

Overproduction of Active Oxygen Due to Hyperglycemia

Why does active oxygen overproduction occur in patients with diabetes mellitus? Intracellular carbohydrate metabolism is impaired under hyperglycemic conditions, which is followed by the overproduction of active oxygen via the various mechanisms associated with hyperglycemia, leading to the specific vascular disorders (Fig. 1).

1. Glycation and oxidation of proteins and lipids, and active oxygen production

Non-enzymatic glycation of proteins under hyperglycemic conditions is accompanied by the production of active oxygen. Using the electron paramagnetic resonance method, Mullarkey et al. demonstrated that non-enzymatic glycation...
2. Production of active oxygen induced by RAGE-dependent intracellular signal transduction

The AGE (RAGE) receptor has been identified, and its significance in the onset of vascular disorders in diabetic patients examined. The protein structure recognized by the RAGE is considered to be CML. When AGE binds to RAGE, intracellular production of active oxygen occurs leading to increased expression of cell adhesion molecules such as VCAM-1 in the vascular endothelial cells, in addition to increased expression of monocyte tissue factor, resulting in an acceleration in the onset and progression of vascular disorders.

Cell damage is mediated by the accumulation of AGES whose formation is linked to oxidative stress. Furthermore, AGES also promote the production of the vascular endothelial growth factor (VEGF) leading to an increase in blood vessel permeability and the induction of neovascularization.

3. PKC activation and active oxygen production

PKC (protein kinase C) is a phospholipid-dependent serine/threonine kinase. In diabetes mellitus, diacylglycerol (DAG) is synthesized de novo utilizing excess glucose taken up by cells, and activates PKC via the glycolysis system. It has been reported that PKC acti-
vation is observed in many vascular tissues such as retina, heart, aorta, and glomeruli which are isolated from diabetic animals. PKC activation is related to vasoconstriction, proliferation and overgrowth of smooth muscle cells as well as accelerated synthesis of extracellular matrix proteins, and thus plays significant roles in the onset and progression of vascular cell dysfunction in diabetes mellitus (Fig. 2).

Recently, it has been reported that, a PKCβ isoform-specific inhibitor (LY 333531) has been developed and its usefulness in inhibiting the onset and progression of diabetic complications has been demonstrated.15,16

It is also indicated that PKC is activated by generated active oxygen, and that the activated PKC induces the activation of phospholipase A₂ (PLA₂) resulting in enhanced prostaglandin metabolism, which is associated with increased production of active oxygen.17

4. Abnormal mitochondria and active oxygen production

In a recent study,18 it has been reported that the production of active oxygen is increased when the oxidative phosphorylation in mitochondria is enhanced. The mitochondrion has been shown to play an important role in active oxygen production particularly under hyperglycemic conditions. Hyperglycemia-induced activation of PKC, AGE production, sorbitol accumulation and activation of NF-κB (nuclear factor-κB) have been reported to be reversed after inhibiting active oxygen production caused by mitochondria in aortic endothelial cells, suggesting that mitochondria plays an important role in the production of active oxygen under high glucose conditions.

Impairments of the Antioxidant Defense Mechanism Due to Hyperglycemia

1. Endogenous antioxidants

Endogenous antioxidants including ascorbate, vitamin E, reduced glutathione, β-carotene, various amino acids, proteins, uric acid, bilirubin, etc. directly scavenge active oxygen. Under hyperglycemic conditions, the intracellular concentrations of reduced ascorbate, and of reduced
glutathione and vitamin E are reported to be decreased. Accordingly, it can be said that antioxidant supplementation is desirable in patients with diabetes mellitus.

2. Endogenous scavenger enzyme systems

The metabolic pathway comprising the enzyme system involved in the scavenging of active oxygen generated in cells is shown in Fig. 3. Such enzymes include the following: 1) Superoxide dismutase (SOD), the enzyme that converts endogenous $O_2^-$ into hydrogen peroxide, including Cu, Zn-SOD, Mn-SOD, and extracellular SOD; and 2) catalase and the glutathione redox cycle (GR cycle), which convert hydrogen peroxide (H$_2$O$_2$) into water. Hydrogen peroxide itself does not have an unpaired electron but is converted into a highly reactive hydroxy radical ($\cdot$OH), and accordingly, hydrogen peroxide detoxification mechanism plays an important role in protecting cells and tissue from oxidative stress. The activity of the GR cycle is dependent on the activity of the enzymes participating in the cycle including glutathione peroxidase and glutathione reductase, on the intracellular contents of NADPH (reduced nicotinamide adenine dinucleotide phosphate), and on the activity of the pentose phosphate pathway, an NADPH regeneration system.

Hyperglycemia has been reported to impair these antioxidant defenses. Under hyperglycemic conditions, the SOD is glycated and the peptide chain is cut, resulting in a decrease in its activity. In addition, the activity of the GR cycle is decreased due to the impaired activation of the pentose phosphate pathway.

Oxidative Stress and Vascular Disorders

Enhanced oxidative stress under hyperglycemic conditions causes an increase in peroxide lipids in the cell membrane, which induces the intracellular expression of specific genes. To
date, it has been understood that the activity of two transcriptional factors, NF-κB and AP-1 (activator protein-1), is regulated by intracellular redox states.22) When activated, these transcriptional factors bind to the specific binding sites in the regions upstream of various genes such as VCAM-1, ICAM-1, as well as cytokines and growth factors including MCP-1 and PDGF and then regulate the expression of those genes. Vascular disorders progress through the expression of these proteins which are involved in cell-cell interactions in the vascular wall (Fig. 4).

Strategy for Preventing the Onset of Diabetic Vascular Complications Using Antioxidants

As described above, oxidative stress may play an important role in the onset and progression of diabetic vascular complications. This suggests the possibility that such complications can be prevented and treated with antioxidants. For example, increased MCP-1 mRNA expression in response to the plasma lipoproteins of diabetic patients is observed in cultured endothelial cells, but its expression does not increase when the endothelial cells are pretreated with an antioxidant (probucol or α-tocopherol).2) The usefulness of antioxidants and the PPARα activator (fibrate) has been suggested in the control of NF-κB activity.23) Furthermore, in experiments using cardiac and vascular tissue taken from diabetic rats, the activation of transcriptional factors induced by active oxygen is noted, but such activation is inhibited by the 4-week administration of food containing 1% probucol.3) Moreover, the administration of α-tocopherol has been demonstrated to improve decreased retinal blood flow24) and glomerular hyperfiltration25) in diabetic animal models.

Although all of these findings have been obtained in non-clinical studies, it has also been reported that probucol improves the endothelium-dependent relaxation of coronary arteries in humans through mechanisms other than lowering cholesterol.26) In addition, regarding diabetic microvascular complications in humans, Bursell et al. administered a large dose (1,800 IU/day) of vitamin E to patients with type I diabetes mellitus for 4 months, and found that decreased blood flow was improved, and that enhancement of glomerular filtration and of urinary excretion of albumin was inhibited, without any change in the status of blood glucose control.27)

While it may be difficult to completely inhibit the onset and progression of vascular complications in diabetic patients, the usefulness of antioxidants in the treatment of impaired vascular functions induced by oxidative stress has been demonstrated. Large-scale clinical studies on the efficacy of antioxidants in the treatment of such complications are anticipated. At present, the usefulness of various antioxidants in the treatment of diabetic neuropathy is being examined.

Conclusion

Under hyperglycemic conditions, the generation of active oxygen is increased and antioxidant defense is impaired, which leads to the activation of redox-sensitive transcriptional factors including NF-κB and AP-1, resulting in promoted synthesis of basement membrane proteins, decreased vasodilation, glomerular hyperfiltration, as well as the activation of endothelial cells, platelets, and leukocytes that accompanies increased cell-cell interaction. The extent of the involvement of such molecular mechanisms and the resultant vascular responses vary in relation to the sites in vessels. By identifying those molecular mechanisms in the site of their occurrence, it is possible to develop therapeutic agents which inhibit the onset and progression of microvascular disorders even if blood glucose is not completely controlled, and to identify diabetic patients with vascular disorders at an early stage.
REFERENCES


