Mechanism-based antioxidant therapies promise to prevent diabetic complications?

Intensive blood glucose control can prevent the initiation and progression of diabetic complications. However, the impacts of intensive therapy against diabetic complications might be limited, because of difficulty in maintaining blood glucose concentrations close to the normal range or other unknown reasons. Thus, another approach based on the elucidation of mechanisms of diabetic complications might be required to prevent the progression of the complications.

Production of reactive oxygen species (ROS) and lipid peroxidation are increased in diabetic patients, especially in those with poor glycemic control. Oxidative stress can be crucial for the development of diabetic vascular complications. Thus, there is interest in determining whether antioxidant therapy can complement intensive blood glucose control. In fact, a large number of studies evaluating the efficacy of antioxidants have been carried out. However, the efficacy of these antioxidant-based therapies is still uncertain in relation to preventing diabetic complications in clinical practice.

Certainly, a number of experimental studies suggest that some natural antioxidants, such as α -tocopherol (vitamin E), ascorbate (vitamin C), coenzyme Q (CoQ), taurine, glutathione or α -lipoic acid, showed beneficial effects on diabetic complications. However, the results from large, long-term clinical trials using α -tocopherol were disappointing. Among the various antioxidant-based therapies, only α -lipoic acid might be somewhat useful in preventing diabetic complications. Meta-analysis has provided evidence that intravenous treatment with 600 mg/day α -lipoic acid over 3 weeks significantly improves both positive neuropathic symptoms and neuropathic deficits in diabetic patients with symptomatic polyneuropathy. α -Lipoic acid is approved in Germany as an agent for the treatment of diabetic neuropathy.

The effectiveness of natural antioxidants in preventing diabetic complications is still uncertain. Therefore, new strategies for controlling oxidative stress, such as development of new mechanism-based antioxidants, will be required to prevent diabetic complications. In addition, to develop these agents, we should investigate the mechanisms that underlie the association between diabetes and oxidative stress. There are several potential mechanisms by which hyperglycemia can lead to oxidative stress¹:

1. Glucose, in its enediol form, might be auto-oxidized in a transition metal-dependent reaction to an enediol radical anion, which is then converted to ketoaldehyde, which can yield the superoxide anion.
2. Activation of the polyol pathway results in a decrease of reduced nicotinamide adenine dinucleotide phosphate

(NADPH) and oxidized nicotinamide adenine dinucleotide. In contrast, regeneration of reduced glutathione, which is an important intracellular antioxidant, also requires NADPH. Thus, glucose can increase ROS production by activation of the polyol pathway.

3. Non-enzymatic glycosylation (glycation) of proteins begins with the interaction of glucose with protein to form early glycosylation products, known as Schiff bases and Amadori products. Schiff bases and Amadori products are sources of the superoxide radical. In addition, glycation of Cu-Zn-superoxide dismutase (CuZnSOD), which is a major enzyme of the intracellular antioxidant system, led to gradual inactivation of the enzyme.
4. Hyperglycemia can activate NADPH oxidase through the activation of protein kinase C (PKC). NADPH oxidase generates superoxide by transferring electrons from NADPH inside the cell across the membrane.
5. The electron transport system of mitochondria is the primary energy producer of the cell, but this system is also thought to be the major source of intracellular ROS under normal physiological conditions.

We previously reported that hyperglycemia could increase the production of ROS from the mitochondrial electron transport chain (mitochondrial ROS)². In addition, the normalization of the mitochondrial ROS production prevented the glucose-induced activation of PKC and polyol pathway, and the formation of advanced glycation end-products (AGEs), all of which are known to be involved in the development of diabetic complications (Figure 1).

Because the production of mitochondrial ROS is thought to be one of the key events in the pathogenesis of diabetic complications and other mitochondria-related diseases, mitochondria-targeted antioxidants, such as idebenone and mitoquinone, have been developed. Idebenone is a synthetic short chain analog of CoQ₁₀, initially patented to provide a suitable medical composition for treating and improving the after-effects of cerebral infarction. It was reported that idebenone acts as an antioxidant and protects the mitochondrial membrane against lipid peroxidation. Interestingly, it was reported that in clinical trials, idebenone is useful in controlling cardiac hypertrophy in Friedreich's ataxia (FRDA), recovery of visual acuity in Leber's hereditary optic neuropathy (LHON), and improvement of mitochondrial oxidative metabolism in the brain of mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes (MELAS).

Figure 1 | Schematic showing the mechanism-based strategies and relationship between mitochondrial reactive oxygen species (ROS) and biochemical dysfunction. Mitochondrial ROS might be a causal link between hyperglycemia and the four main metabolic dysfunctions: (a) activation of the polyol pathway; (b) activation of the hexosamin pathway; (c) activation of the protein kinase C (PKC) pathway; and (d) advanced glycation end-products (AGEs) formation. In contrast, activation of the pentosephosphate pathway might also counter metabolic dysfunction. Mitochondria-targeted antioxidants or nuclear factor E2-related factor 2 (Nrf2) activators might ameliorate metabolic dysfunction by inhibition of mitochondrial ROS or activation of transketolase, respectively. TCA, tricarboxylic acid cycle.

Furthermore, a phase 3 study of idebenone in Duchenne muscular dystrophy (DMD) is ongoing now.

Subcellular localization of idebenone is thought to be in mitochondria. However, because it distributes through the extracellular and intracellular compartments, its effectiveness might be uncertain. Thus, mitoquinone, which is a mitochondria-targeted antioxidant analog of idebenone, has been newly developed. Because mitoquinone possesses a terminal triphenylphosphonium group instead of a hydroxyl group, it is accumulated several hundred-fold within mitochondria, enhancing the protection of mitochondria from oxidative damage. A phase 2 study of mitoquinone in chronic hepatitis C virus (HCV) infection was carried out, and mitoquinone could decrease the liver damage associated with chronic HCV infection³. Disappointingly, to our knowledge, there is no clinical evidence of idebenone and mitoquinone in preventing diabetic complications. However, FRDA, LHON and MELAS are clinical syndromes of mitochondrial disorders, in which respiratory chain functions are defective. In addition, molecular pathology of DMD is reported to be associated with increased ROS production and mitochondrial dysfunction. Furthermore, in HCV infection, there is considerable evidence for increased mitochondrial ROS and damage leading to cell death and tissue fibrosis. Therefore, similar to those mitochondria-related diseases, both idebenone and mitoquinone might show benefits in preventing diabetic complications (Figure 1).

By the way, in classical natural antioxidants, why could α -lipoic acid show some effectiveness in preventing diabetic complications when α -tocopherol could not? One possible explanation for the difference might be as a result of the difference in antioxidant capacity between α -lipoic acid and α -tocopherol. α -Lipoic acid is believed to be a powerful antioxidant compared with α -tocopherol. Another explanation might be that α -lipoic acid has additional effects in preventing diabetic complications. We previously reported that metformin and 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR) activate adenosine monophosphate-activated protein kinase (AMPK), normalize hyperglycemia-induced mitochondrial ROS production and promote mitochondrial biogenesis in cultured human umbilical vein endothelial cells. Because an overexpression of dominant negative AMPK α 1 (T172A) attenuated metformin-induced and AICAR-induced inhibition of mitochondrial ROS and production of mitochondrial biogenesis, the effects of metformin and AICAR were dependent on the activation of AMPK⁴. AMPK might be one of the molecular targets for attenuating hyperglycemia-induced overproduction of mitochondrial ROS. Interestingly, it was recently reported that α -lipoic acid could activate AMPK in multiple peripheral tissues, including skeletal muscle, liver and adipocytes⁵. In addition, it was reported that α -lipoic acid improves mitochondrial dysfunction and oxidative damage in aging. Taking all this together, administered α -lipoic acid might reduce

hyperglycemia-induced mitochondrial ROS through AMPK activation in peripheral tissues, and prevent diabetic complications.

Because oxidative stress is generally defined as an imbalance that favors the production of ROS over the antioxidant defense system, there is another strategy to reinforce the antioxidant defense system. Nuclear factor E2-related factor 2 (Nrf2) is one of the most important cellular defense mechanisms to cope with oxidative stress. Thus, Nrf2-targeted agents, such as bardoxolone methyl and sulforaphane, have been developed to prevent or slow down the progression of oxidative stress-related diseases. Nrf2 is a transactivator of genes containing an antioxidant response element (ARE) in their promoter. Such genes code for a number of antioxidative enzymes including NADPH: quinone oxidoreductase, glutathione S-transferases, aldo-keto reductases and heme oxygenase-1. Under normal physiological conditions, Nrf2 is anchored in the cytoplasm by binding to Kelchlike ECH-associated protein 1 (Keap1), which promotes the ubiquitination and subsequent proteolytic degradation of Nrf2; whereas both bardoxolone methyl and sulforaphane interact with cysteine residues on Keap1, allowing Nrf2 translocation to the nucleus and subsequent upregulation of a number of genes of antioxidative enzymes.

Recently, the 52-Week Bardoxolone Methyl Treatment: Renal Function in CKD/Type 2 Diabetes (BEAM) study was carried out in patients with moderate to severe chronic kidney disease (CKD) and type 2 diabetes as a double-blind, randomized, placebo-controlled phase 2 clinical trial⁶. After 24 weeks of the treatment, the patients treated with bardoxolone methyl showed a significant increase in the mean estimated glomerular filtration rate compared with those treated with placebo. Although it is unknown whether bardoxolone methyl could reduce hyperglycemia-induced mitochondrial ROS, it was reported that heme oxygenase-1 regulates cardiac mitochondrial biogenesis through Nrf2-mediated transcriptional control of nuclear respiratory factor-1 (NRF-1). In addition, activation of Nrf2 by sulforaphane increased ARE-linked gene expression of transketolase and glutathione reductase, and ameliorated hyperglycemia-induced production of ROS, activation of hexosamine and PKC pathways, and prevented increased cellular accumulation and excretion of the glycation agent methylglyoxal. Furthermore, Nrf2 was reported to regulate promoter activity of the aldose reductase gene, which is the key enzyme of the polyol pathway. Because activation of Nrf2 might prevent hyperglycemia-induced ROS production and metabolic dysfunctions, such as activation of hexosamine, PKC and polyol pathways and

accumulation of intracellular AGEs, bardoxolone methyl or sulforaphane might have promise for the treatment of diabetic complications (Figure 1).

Considered together, although oxidative stress has been implicated in the pathology of diabetic complications, the efficacy of classical natural antioxidants in preventing diabetic complications is still uncertain. However, mechanism-based antioxidants, such as idebenone, mitoquinone, bardoxolone methyl and sulforaphane, have been developed. These mechanism-based strategies might suggest the potential for better treatment approaches to reduce the burden of oxidative stress and to prevent diabetic complications in clinical practice. In particular, because mitochondrial ROS production in response to hyperglycemia might be the central in the pathogenesis of diabetic complications, reduction of mitochondrial ROS might be the important therapeutic strategy to prevent diabetic complications.

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