ETIOLOGY OF DIABETIC MICROVASCULAR DISEASE AND SCIENTIFIC RATIONALE FOR NEW THERAPEUTIC TARGETS*

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ABSTRACT

The macrovascular and microvascular complications of diabetes have a greater impact on a patient’s quality of life than diabetes. Therefore, efforts to develop new therapies to prevent these complications or slow their progression are crucial.

Because previous therapies aimed at ameliorating the clinical symptoms of these complications have had only limited success, current efforts are aimed at intervening at the level of pathogenesis. Several studies in the United States and Europe have established that diabetic microvascular complications are linked to blood glucose levels. Considerable research examining this link strongly suggests that oxidative stress is the unifying mechanism that underlies glucose-mediated damage to the retinas, the kidneys, and the nerves.

Glucose metabolism leads to oxidative stress in several ways and uses several different pathways. High glucose alters mitochondrial respiration through the glycolytic pathway, causes vascular occlusion and ischemia through the hexosamine pathway, inhibits the ability of glutathione to function as an antioxidant within the cell through the polyol pathway, causes the formation of advanced glycation end products (AGEs) through the AGE-mediated injury pathway, and produces inflammation and capillary occlusion through the stress response to hyperglycemia. It also leads to oxidative stress by altering the gene expression of key proteins; decreasing blood flow; promoting angiogenesis, capillary occlusion, and inflammation; and damaging lipids and proteins through the protein kinase C (PKC) pathway.

Drugs that have been developed to target individual oxidative stress pathways include the aldose reductase inhibitors to block the polyol pathway, aminoguanide to block the AGE-mediated injury pathway, and ruboxistaurin to block the PKC pathway. Ruboxistaurin is currently in phase III clinical trials involving patients with diabetic retinopathy, diabetic macular edema, diabetic nephropathy, and diabetic peripheral neuropathy and appears to be very promising in reducing glucose-mediated injury.

Antioxidants, which are applicable to all of the oxidative stress pathways, have shown varying degrees of efficacy. One antioxidant in particular, lipoic acid, appears to be effective in treating diabetic neuropathy and is currently under study in a large phase III clinical trial.

Neurotrophic factors, which can serve as antioxidants, have been shown to prevent slowing of nerve conduction and increase nerve regeneration in experimental diabetes. However, these factors had no effect on diabetic peripheral neuropathy in a large clinical trial involving 1000 patients.

With regard to diabetic peripheral neuropathy, blocking multiple pathway components may be critical in preventing nervous system injury. Coupling antioxidant therapy and blockade of the oxidative stress pathways with neurotrophic support to promote nerve regeneration may result in the first effective treatment of diabetic peripheral neuropathy.

Diabetes mellitus affects approximately 171 million people worldwide, with 25% of them living in industrialized countries. Even more distressing is the projected increase in the global prevalence of diabetes mellitus in the near future: 220 million cases by 2010 and 366 million by 2030.

Annual costs of diabetes, already high, are likely to increase as well. Current estimates put the annual direct and indirect medical costs of diabetes in the United States at $132 billion, with direct medical costs accounting for $91.8 billion. Similarly, a survey of 8 European countries found the direct medical costs of diabetes to be €29 billion. Of the $91.8 billion estimate for direct medical costs, $23.2 billion was for diabetes care, $24.6 billion for chronic complications attributable to diabetes, and $44.1 billion for the excess prevalence of general medical conditions related to diabetes.

The complications of diabetes, macrovascular or microvascular, have a greater impact on a patient’s quality of life than diabetes. Although macrovascular complications, such as coronary artery disease, peripheral vascular disease, and stroke, are associated with considerable mortality, morbidity, and diminution of quality of life, diabetic microvascular complications of diabetes are just, if not more, devastating to the patient.

Specifically, diabetic retinopathy accounts for 25,000 cases of blindness in the United States each year, diabetic nephropathy is the most common reason for dialysis and the leading cause of complete renal failure, and diabetic peripheral neuropathy leads to foot ulceration, often serious enough to require amputation, in 15% of patients with diabetes in industrialized countries.

Multiple studies in the United States and Europe have established that diabetic microvascular complications are linked to blood glucose levels. This link raises an important question: Is there one unifying mechanism or hypothesis that underlies glucose-mediated damage to the retinas, the kidneys, and the nerves? The answer is yes, and the mechanism is thought to be oxidative stress. The sections that follow explain why this hypothesis is most likely correct, how oxidative stress damages small vessels in general and small vessels in nerve tissue in particular, and what treatments have been investigated thus far to reduce oxidative stress.

**GLUCOSE-MEDIATED OXIDATIVE STRESS**

High glucose results in oxidative stress, which results in energy depletion and the formation of free radicals. The free radicals—superoxides that are also known as reactive oxygen species (ROS)—are extremely active molecules that break down energy stores, genetic materials, proteins, and lipids. By literally eating up the essential components of the cell, ROS mediate cellular damage and death. The end result of these processes is cellular dysfunction and loss of cells in the kidneys, loss of pericytes in the eyes, and loss of neurons that underlie diabetic microvascular complications.

When glucose-mediated oxidative stress damages nerve cells in diabetic neuropathy, the nerves are no longer able to function properly, leading to loss of normal axonal transport to the distal axons, a gradual distal-to-proximal loss of axons, and a “dying back” of the nerves. As shown in Figure 1, each nerve fiber in a normal sensory nerve is surrounded by insulating myelin. However, as neuropathy progresses from mild to moderate to severe, distal-to-proximal axonal loss increases.

Clinically, the signs and symptoms of diabetic neuropathy follow a “stocking-and-glove” pattern of distribution that reflects the distal-to-proximal pattern of axonal loss. The loss of sensation begins distally, in the feet and hands, before it moves proximally to affect the legs and arms.

**Figure 1. Normal Nerve and Progressive Axonal Loss in Mild, Moderate, and Severe Diabetic Neuropathy**

Nerve biopsy cross-sections showing a normal sensory nerve, with each clear area denoting a single, myelinated nerve fiber (upper right panel), and progressive axonal loss in mild, moderate, and severe neuropathy (upper left, lower right, and lower left panels, respectively).
HOW GLUCOSE METABOLISM LEADS TO OXIDATIVE STRESS

Although there are no study results demonstrating how glucose metabolism leads to oxidative stress, researchers have found that high glucose alters mitochondrial respiration, causes vascular occlusion and ischemia, inhibits the ability of glutathione to function as an antioxidant within the cell, causes the formation of advanced glycation end products (AGEs), and produces inflammation and capillary occlusion.10-12 As summarized in Figure 2, several different pathways are utilized to produce these effects and ROS.

GLYCOLYTIC (MITOCHONDRIAL OXIDATIVE STRESS) PATHWAY

Under normoglycemic conditions, glucose is converted to pyruvate through the process of glycolysis, the pathway that is most familiar to clinicians. Pyruvate enters the mitochondria, the energy source of the cell, and the production of adenosine triphosphate (ATP) usually ensues.

However, under hyperglycemic conditions, excess glucose floods the pathway and the tricarboxylic acid cycle converts glucose into reducing equivalents, such as nicotinamide adenine dinucleotide (NADH); ATP is ultimately produced through electron transfer. When electron transfer occurs too quickly, there are too many electrons that need to be moved through the system, and the system becomes clogged, much as a tube becomes clogged when it is overloaded. The back-up of electrons leads to the formation of superoxides, which then cause cellular damage.11,13 In other words, when excess glucose tries to go through the system too quickly, the enzymes needed to shuttle glucose through the system become depleted, the system becomes clogged, and superoxides are formed; cellular damage then ensues.

POLYOL PATHWAY

A second way in which glucose can be metabolized to produce ROS is the polyol pathway.7-10,12-14 Excess glucose is converted by aldose reductase to sorbitol. However, the process depletes the cell of reduced NADH phosphate (NADPH), an essential cofactor in the regeneration of glutathione, a key antioxidant that the cell needs to defend itself against the formation of ROS. In short, without NADPH, the cell cannot regenerate glutathione, and without glutathione, the cell cannot protect itself against oxidative stress.

HEXOSAMINE PATHWAY

Shunting of excess intracellular glucose into the hexosamine pathway may also contribute to the development of diabetic complications.9,13 In this pathway, glucose is converted to fructose-6-phosphate, but instead of entering the mitochondria, fructose-6-phosphate provides substrates for reactions that require uridine diphosphate-N-acetylg glucosamine, including the synthesis of proteoglycans and O-linked glycoproteins. In parallel, this pathway activates transcription factors such as tumor-related growth factor-β and plasminogen activator inhibitor-1, both of which cause macrovascular and microvascular occlusion and ischemia.

Therefore, the hexosamine pathway not only potentiates the damaging effects of high glucose, it also activates growth factors that cause vascular ischemia, which in turn triggers free radical formation and more oxidative stress.

AGE-MEDIATED INJURY PATHWAY

In this pathway, excess glucose undergoes auto-oxidation and randomly attaches to, oxidizes, and glycates various proteins in the cell and in the plasma.7-9,12-14 These proteins then become AGEs and form large mats within the cell that cause the cell to become more rigid, less mobile, and less permeable. In this way, AGEs cause nerves to become fat and rigid, impairing axonal transport and cell adhesion and enhancing the formation of ROS.

Figure 2. How Glucose Metabolism Results in Oxidative Stress

AGE = advanced glycation end product; GSSG = oxidized glutathione; NADH = reduced nicotinamide adenine dinucleotide; ROS = reactive oxygen species.
**PROTEIN KINASE C PATHWAY**

An important pathway that has been intensely investigated in recent years is the protein kinase C (PKC) pathway (Figure 3). In this pathway, high glucose activates diacylglycerol (DAG), which then activates PKC. As in the hexose pathway, where the metabolism of glucose eventually activates transcription factors that promote vascular ischemia and free radical formation, DAG-activated PKC alters the gene expression of key proteins, leading to decreased blood flow, capillary occlusion, and inflammation.

The cell has been assaulted and, as a result, begins to manufacture inappropriate proteins that allow damaging events to occur and negate the cell’s ability to react appropriately to superoxides. The end result is the formation of more ROS and more oxidative stress.

**THE IMPACT OF Glucose**

In addition to the 5 oxidative stress pathways described above, glucose plays a role in producing oxidative stress by providing a sympathetic surge. Hyperglycemia increases the release of hormones and produces a stress response that causes capillary occlusion and inflammation.

Therefore, under high glucose conditions, complication-prone vascular cells of the eyes, kidneys, and nerves are assaulted by glucose, in addition to many other mechanisms through at least 5 different pathways.

**THERAPEUTIC TARGETS**

The knowledge that the damaging effects of hyperglycemia and oxidative stress occur at multiple different points in multiple pathways has led to the development or potential development of drugs that target the individual pathways (Figure 4).

One such group of these drugs is the aldose reductase inhibitors to block the polyol pathway. The rationale is that blocking the conversion of glucose to sorbitol spares the depletion of NADPH, thus preserving the ability of cells to regenerate glutathione and mount a strong antioxidant defense against oxidative stress.

Another drug is the AGE inhibitor aminoguanidine, which blocks the ability of glucose to attach itself to the proteins involved in AGE-mediated injury.

The PKC pathway is the focus of considerable attention, largely because of an active PKC-β inhibitor (ie, ruboxistaurin) that is currently in clinical trials involving patients with diabetic retinopathy, diabetic macular edema, diabetic nephropathy, or diabetic peripheral neuropathy and appears to be promising in blocking glucose-mediated injury. Furthermore, the thiazolidinediones are also active in blocking the PKC pathway.

Other agents in development or under consideration are uncouplers and mitochondrial antioxidant...
enzyme promoters to block the glycolytic pathway that permits excess mitochondrial activity; glutamine:fructose-6-phosphate aminotransferase inhibitors to block the hexose pathway; and AGE receptor inhibitors to block the AGE-mediated injury pathway.

**CURRENT STATUS OF CLINICAL TRIALS**

At present, there are several clinical trials evaluating the efficacy of antioxidants and other agents in mitigating diabetic microvascular complications. Although most of the aldose reductase inhibitors that were available in years past have been withdrawn from the market because of lack of efficacy or toxicity, 2 phase II trials and a phase III trial are evaluating this family of drugs again. It may be that one of these drugs alone will not be sufficient and that a combination of aldose reductase inhibitors and antioxidants or AGE inhibitors might be needed instead.

Results of previous animal and phase I/II trials of the PKC-β inhibitor ruboxistaurin have been promising. Two phase III trials are under way, and more are planned.

Multiple small trials of the AGE inhibitor aminoguanidine and various vitamins (eg, thiamine) and cofactors have been conducted involving patients with diabetic neuropathy. Results thus far show no benefit or are still unavailable.

Antioxidants, which represent an overarching therapy that is applicable to all of the oxidative stress pathways described earlier, are effective in experimental diabetes. There is also evidence that antioxidants decrease oxidative stress when used clinically.

In fact, clinical trials have shown that lipoic acid, a simple antioxidant that is available over the counter and is used quite commonly in Germany, is effective in patients with diabetic peripheral neuropathy. Currently, lipoic acid is under study in a large phase III clinical trial.

**NEUROTROPHIC SUPPORT**

Neurotrophic factors are growth factors that are essential to normal nerve function, nourishing the nerves similar to the way fertilizer nourishes the soil.

However, in the presence of diabetes, neurotrophic factors are decreased, and oxidative stress further decreases their expression. The reduced availability of nerve growth factors contributes to diabetic neuropathy by preventing nerve regeneration and reducing resistance to oxidative stress. Thus, hyperglycemia initiates a vicious feed-forward cycle in which damage to point A leads to more damage to point B, still more damage to point C, and so on.

Studies of neurotrophic factors in animals with experimental diabetes have shown that nerve growth factor and insulin-like growth factor-1 prevent the slowing of nerve conduction and increase nerve regeneration. Researchers have found that antioxidant therapy can restore neurotrophic factors and that these factors themselves can serve as antioxidants. However, in the 1 large clinical trial completed thus far, nerve growth factor was administered to 1000 patients, but it had no effect on diabetic peripheral neuropathy.

**CONCLUSIONS**

Many different variables are involved in the development of diabetic microvascular complications, with oxidative stress being the unifying mechanism that underlies glucose-mediated damage. Therefore, antioxidant therapy is a rational approach to prevention.

Although antioxidants appear to be helpful in this regard, the combination of an antioxidant and an agent that blocks 1 of the key pathways contributing to the development of complications and oxidative stress would be even more effective.

With regard to diabetic peripheral neuropathy in particular, blocking multiple pathway components may be critical in preventing nervous system injury. Coupling antioxidant therapy and blockade of the oxidative stress pathways with neurotrophic support to promote nerve regeneration may result in the first effective treatment of diabetic peripheral neuropathy.

**Q & A HIGHLIGHTS**

Member of the audience: My area of study is oxidative stress situations. I think it’s important to be
aware of the amount of insulin a patient receives because insulin may promote the oxidative stress that is triggered by enhancement of intracellular glucose. What are your thoughts about this?

Dr Feldman: That raises an important point. Why do patients get retinopathy, nephropathy, or neuropathy, but not myopathy or fat-opathy? Muscle isn’t affected, and neither is fat. The answer has to do with how glucose enters cells and the presence of different glucose transporters in tissues that are prone to complications.

The nerves have facilitated glucose transport. Therefore, whatever amount of glucose is present in the environment will enter the nerves, and that process is independent of insulin. In contrast, cells that are more resistant to complications use insulin and a different family of glucose transporters to allow a more regulated entry of glucose.

Stated another way, different glucose transporters allow different amounts of glucose into the cell. Nerve, kidney, and eye tissues are not as dependent on insulin as the mainstay tissues in the body that use glucose for energy.

Member of the audience: What are your thoughts about treatment with lipoic acid?

Dr Feldman: Lipoic acid is an extremely potent antioxidant and is known to act as a general sink for superoxides and hydrogen peroxide, which results when superoxides are catalyzed. Similar to the superoxides, hydrogen peroxide also causes a great deal of oxidative stress in the cell.

Because of its potency and ability to sop up superoxides and hydrogen peroxide, lipoic acid will likely be a successful treatment for diabetic neuropathy. Several studies done in Germany have shown lipoic acid’s potential therapeutic use in neuropathy, and it is currently being evaluated in a phase III clinical trial in the United States.

Member of the audience: Would you care to speculate why vitamins C and E never worked as antioxidant therapy for neuropathy?

Dr Feldman: Vitamins E and C are less potent as antioxidants than lipoic acid. Although vitamin E is effective at decreasing lipid peroxidation, it works downstream, whereas lipoic acid is more efficient because it works upstream. It is pretty much the same for vitamin C.

Clinicians should use lipoic acid plus vitamin C and E and compare the combination with single-antioxidant therapy. To my knowledge, that hasn’t been done.

REFERENCES


