Oxidative Stress: The Lead or Supporting Actor in the Pathogenesis of Diabetic Complications

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Oxidative stress is increased in both diabetic and insulin resistance states and may contribute to the development of microvascular and cardiovascular diseases associated with both of these syndromes (1–4). In addition, oxidative stress has been suggested to cause abnormalities of insulin secretion and actions (5,6). The increases in oxidative stress is probably due to the abnormal metabolic milieu such as hyperglycemia, dys-lipidemia, and elevated free fatty acids (FFA), which commonly occur in patients who have diabetes and less-than-perfect glycemic control and in insulin-resistant state (7–10). There are a great deal of evidence that oxidant productions are increased in vascular cells exposed to elevated levels of glucose and in various cardiovascular tissues derived from diabetic and insulin-resistant states. However, controversy exists as to the role of oxidative stress in the pathogenesis of diabetic microvascular and cardiovascular complications (11). In this article, we briefly provide an overview of the following questions: (1) Is oxidant stress increased in diabetic and insulin-resistant states? (2) What mechanisms is hyperglycemia using to produce the increases in oxidants? (3) Are the oxidants induced by diabetes involved in the specific vascular pathologies observed in clinical diabetes? (4) Can antioxidants ameliorate diabetic complications?

For the first question, there is a wealth of reports that many protein, lipid, and DNA markers of oxidative stress are increased in cultured vascular cells exposed to high glucose levels and vascular tissues from animals and patients with diabetes. In cultured vascular cells, elevating glucose levels in the media has been shown to increase oxidant production including gluco-oxidants, glycated compounds, oxidized LDL, superoxidents, and nitrotyrosine (7,12–15). Similarly, elevated levels of isoprostanes, 8-hydroxydeoxyguanosine, and lipid peroxides have been reported in diabetic animals and in patients with diabetes (14–17). Although numerous reports have substantiated that oxidant productions are increased in diabetics, clinical evidence for tissue damage as results of oxidative stress has not been clearly demonstrated because both plasma and cells contain a large reserve of antioxidants. In fact, the levels of the various antioxidants in the plasma and cells have not consistently been shown to be decreased in the diabetic states (18–20). Nevertheless, substantial evidence exists that diabetes and insulin resistance are states of increased oxidative stress, and the production of oxidants are significantly increased.

Increases in oxidant production, observed in diabetes and insulin-resistant states, are the products of metabolisms of hyperglycemia, FFA, and other metabolites (2,8–10), which are the results of insulin deficiency and resistance. For hyperglycemia, increases in oxidant productions are due to multiple processes. Glucose can undergo nonenzymatic reactions forming gluco-oxidants and glycated products, which can be oxidants (20,21). Metabolisms of the excessive intracellular glucose can occur by several processes such as aldose reductase, mitochondrial oxidative phosphorylation, activation of oxidases, and alteration of NADPH/NADP ratios (7,22–24). Among these possibilities, recent focus has been on mitochondrial metabolism and activation of NADPH oxidases (7,24). Suggestions have been made that most glucose-induced oxidants are derived from glycolysis and mitochondrial oxidative phosphorylation with the productions of superoxide (7). In addition, byproducts of this process will cause the activation of signaling cascades such as activation of protein kinase C (PKC), hexosamine productions, increase flux via aldose reductase (AR), and glycated products. However, other authors have reported that the metabolism of high glucose levels can activate NADPH oxidase in the vascular cells independent of mitochondrial metabolisms (24). One mechanism that can increase NADPH oxidase activity is the activation of PKC, which is elevated by glucose-induced elevation of diacylglycerol (DAG) via de novo synthesis pathway (24). Thus, it is very likely that hyperglycemia is increasing oxidant production by multiple pathways rather than a single dominant route. This is an important distinction because it is much simpler to design therapeutic agents for one target than for multiple pathways.

Besides glucose, elevation of FFA, which is present in both diabetic and insulin-resistant states, also can increase oxidant productions because the metabolism of FFA is dependent on β-hydroxylation, acetyl-CoA, and uncouple mitochondrial oxidative phosphorylation (8–10,25,26). In fact, recent reports have demonstrated that increases in malondialdehyde levels and NF-κB expression can be detected in insulin-resistant states without hyperglycemia not only in vascular tissues but also in muscle and adipose tissues (10,27). In addition, FFA...
infusion can reduce the levels of glutathione in the plasma (27).
Thus, increases in oxidant production in the diabetic and insulin-resistant states can originate from the metabolism of multiple metabolites such as glucose or FFA and from multiple pathways.

The third question is on the importance of oxidative stress in the pathogenesis of diabetic complications. This is clearly the most important question in this field of investigations. Although clear and direct answers are not available, it is likely that the importance of oxidative stress in causing tissue damage is tissue specific. Clinical evidence suggests that increases in oxidative stress could be very important for the acceleration in cardiovascular risks that are observed in both diabetes and insulin resistance in which either hyperglycemia or elevated FFA exist (1,2). However, it is difficult to assign oxidative stress the lead role in diabetic microvascular complications, including nephropathy and retinopathy, because classical pathologies of diabetic retina and glomeruli are rarely observed in insulin-resistant patients without diabetes, even though both hyperglycemia and FFA can increase superoxide productions from the mitochondrial metabolism (2,7,8,25). Thus, it is possible that oxidative stress could be playing a supportive but not the lead or initiation role in diabetic microvascular diseases.

**Therapeutic Trials by Antioxidants**

The last question raises the issue of whether antioxidant treatments can be effective to prevent or delay the onset of diabetic complications. This question has been tested in cultured vascular cell, animal models of diabetes and in patients with diabetes(28–33). It is impossible to review all of the literature in this brief overview. In general, many types of antioxidants have been studied, including vitamin C, vitamin E, β-carotene, lipoic acids, and many others (28–37).

The addition of antioxidants such as vitamins C and E, lipoic acid, antioxidative enzymes, taurine, acetylcystein, and others has been reported to prevent hyperglycemia-induced biologic changes such as cytokine induction, matrix synthesis, and cellular growth and turnover (36–42). Thus, a great deal of supportive evidence is available to suggest that oxidative stress is an important pathway activated by high glucose levels to cause many surrogate markers of diabetic vascular and neurologic pathologies.

**Animal Studies**

Similar to cell-cultured studies, multiple antioxidants have been studied in diabetic and in insulin-resistant animals, mostly rodents, to determine whether antioxidants are effective in preventing or delaying the onset of vascular and neurologic functions (2,26,28,30,33–35,37,38,40,41). Again, favorable results have been reported on early changes of diabetic nephropathy, neuropathy, retinopathy, endothelial dysfunction, and other surrogate markers of atherosclerosis.

α-Lipoic acid, a superoxide scavenger, is needed to regenerate glutathione and oxidized vitamins C and E in animal models of diabetes. Vitamins C and E and α-lipoic acid have been shown to improve nerve conduction velocity and blood flow to the peripheral nerves, retinal leukocyte adhesions, cataract formation, and mesangial expansion, suggesting that it may be effective to treat diabetic complications (33,38,40–44). However, α-lipoic acid was able to normalize other metabolic abnormalities of diabetes such as improved glycemic control. Thus, it is unclear whether the effects of α-lipoic acids are due only to its antioxidant actions or may mediate some of its actions in improving glycemic actions in diabetic animals (37).

**Vitamins C and E**

Vitamins C and E have been characterized in numerous studies using a variety of animal models of diabetes. In general, vitamin C or E either individually or in combination normalized many parameters of oxidative stress such as lipid peroxidation, increasing isoprostanes, plasma malondialdehyde (MDA), and cellular markers of oxidative stress such as NF-kB in diabetic animals (28,33,38,40–45). Besides biochemical changes, many early or functional markers of diabetic retinopathy, nephropathy, neuropathy, and even cardiovascular disease have been reported to be prevented or reversed, including blood flow, nerve conduction velocity, permeability, endothelial dysfunctions, albuminuria, and vascular contractility (40). A few reports have shown that vitamins C and E may even prevent late or pathology changes in retina and peripheral nerves of diabetic animals (28,42). For vitamin E, studies using supra-antioxidant doses have reported to normalize oxidative stress parameters and inhibit hyperglycemia induced DAG/PKC activation and the associated vascular dysfunctions in the retina and renal glomeruli (42,44). The mechanisms of action of high doses of vitamin E to prevent diabetic complications are unclear. Besides the neutralization of superoxide and lipid peroxidation, vitamin E, especially d-α-tocopherol, at 50 μM or higher can activate DAG kinase and decrease DAG level, leading to decrease in PKC activities (42). This unusual effect of d-α-tocopherol has been reported in the retina, renal glomeruli, and macrophages isolated or derived from diabetic animals (42,45) and in other cell types such as aortic smooth muscle cells in response to several other growth factors and may have implications for other diseases such as atherosclerosis (46–49). The results of antioxidant treatment in diabetic animals have been mostly positive, but the end points are usually early changes or potential surrogate markers of vascular and neurologic complications. No significant untoward side effects have been reported in diabetic animals at either low or high doses. Very few of the studies have shown that antioxidant treatment will prevent or delay changes in the pathologies of diabetic microvascular or cardiovascular diseases except early nonproliferative microvascular changes in the retina (28).

**Clinical Studies**

Similar to animal studies, most clinical studies on the effects of antioxidative treatments on diabetic complications have been of short duration, with very few people, and using early surrogate markers. Studies using α-lipoic acid have provided suggestive evidence that it may improve the symptoms of diabetic polyneuropathy, but, again, data from a large controlled study are not yet available (34,50). In addition, α-lipoic acid has an additional property of being able to increase glu-
cose transport in muscle cells, which may be related to its antioxidant properties (37,51). Vitamins C and E have been used individually or in combination in several clinical studies. Similarly, most studies, again, have reported that early markers of complications improved, such as oxidative stress markers in the plasma, urine, and circulating cells (52). Functionally, vitamins C and E at the usual antioxidant doses may improve endothelial dysfunctions and microalbuminuria (43,53). Previously, we reported that at high doses of vitamin E (1800 IU/d) in a placebo-controlled trial, abnormalities of retinal blood flow and renal hyperfiltration can be normalized in patients with type 1 diabetes (33). However, large studies, such as Heart Outcomes Prevention Evaluation, which used 400 IU/d, did not find any benefit in microvascular or cardiovascular events in >3000 patients with diabetes and after several years (54). Two smaller studies, Cambridge Heart Antioxidant Study and Secondary Prevention with Antioxidants of Cardiovascular Disease in End-Stage Renal Disease (SPACE), provided supportive evidences that vitamin E at higher doses of 600 mg/d or greater may be helpful in cardiovascular events (55,56).

**Summary**

In summary, the evidence on the role of oxidative stress on diabetic complications suggests that oxidant production is clearly increased as the result of glucose or FFA metabolism via multiple pathways. It is likely that oxidative stress may accelerate the basic pathogenic process of diabetic complications. However, oxidative stress may not be playing a leading role in the microvascular complications because these complications are not evident in patients with only insulin resistance without diabetes, even though increases in oxidative stress also exist to a similar extent in both. Conversely, the beneficial effects of antioxidants seem to be present in animal models of diabetes. However, supportive evidence that antioxidants can provide beneficial effects on diabetic complications in large clinical trials is lacking. Thus, new and more powerful antioxidants are needed for future studies. Alternatively, if oxidative stress plays only a supporting role in diabetic complications, then antioxidants may be helpful only when paired with other treatment of diabetic complications.

**Acknowledgments**

This study was supported by National Institutes of Health Grants NEY05110 and EY9178. Dr. Keiji Isshiki received a mentor fellowship from the American Diabetes Association.

**References**

