OXIDATIVE STRESS AND DIABETES-ASSOCIATED COMPLICATIONS

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ABSTRACT

Objective: To review the role of oxidative stress in the development of complications of diabetes.

Methods: Evidence that implicates hyperglycemia-derived oxygen free radicals as mediators of diabetes-associated complications is presented and discussed.

Results: Recent studies have indicated that a hyperglycemia-induced overproduction of superoxide seems to be the first and main event in the activation of all pathways involved in the pathogenesis of complications of diabetes. Superoxide overproduction is accompanied by increased generation of nitric oxide and, consequently, formation of the strong oxidant peroxynitrite and by poly(adenosine diphosphate-ribose) polymerase activation, which in turn further activates the pathways involved in the pathogenesis of diabetes-related complications. This process results in acute endothelial dysfunction and activation of inflammation in blood vessels of patients with diabetes, and these factors contribute to the development of complications of diabetes.

Conclusion: In vivo evidence supports the major contribution of hyperglycemia in producing oxidative stress and, ultimately, acute endothelial dysfunction in blood vessels of patients with diabetes. **(Endocr Pract. 2006;12[Suppl 1]:60-62)**

Abbreviations:
ADP = adenosine diphosphate; **eNOS** = endothelial nitric oxide synthase; **GAPDH** = reduced form of glyceraldehyde-3-phosphate dehydrogenase; **iNOS** = inducible nitric oxide synthase; **NO** = nitric oxide

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INTRODUCTION

Hyperglycemia is well recognized as the cause of microvascular complications (1,2) and possibly macrovascular complications (3) of diabetes. Moreover, cardiovascular disease is known to be the leading cause of death in patients with diabetes (4). Evidence suggests that oxidative stress has a key role in the pathogenesis of both microvascular and macrovascular complications of diabetes (5). Therefore, the possibility of reducing oxidative stress in patients with diabetes may be of considerable therapeutic relevance.

MARKERS OF OXIDATIVE STRESS

The direct measurement of free radicals, particularly in vivo, is extremely difficult. Usually, the products of radical damage in the cell—namely, DNA, lipids, and proteins—are considered good markers of oxidative stress (6). DNA damage can be evaluated by measuring 8-hydroxy-2′-deoxyguanosine and its free base 8-hydroxyguanine in blood cells or urine (6). Various studies in patients with diabetes have shown that levels of 8-hydroxy-2′-deoxyguanosine in serum are increased (7,8). Lipid peroxidation has probably been the most extensively investigated process induced by free radicals. Lipid peroxides, derived from polyunsaturated fatty acids, are unstable and decompose to form a complex series of compounds. The most frequently studied have been isoprostanes, prostaglandin-like compounds generated in vivo by the free radical-catalyzed peroxidation of arachidonic acid, independently of cyclooxygenase (6). In patients with diabetes, evidence of increased isoprostane levels in plasma and urine has been reported (9).

The modification of proteins may result in cross-linking, peptide fragmentation, and conversion of one amino acid to another or to a modified residue by oxidation of the amino acid side chain. Recently, a new interesting marker of oxidative damage to protein has been proposed: nitrotyrosine (10). When superoxide and nitric oxide (NO) exist in close proximity, they can spontaneously form peroxynitrite, a powerful oxidant (11). Nitration in the 3-position (ortho) of tyrosine is the major product of the peroxynitrite attack on protein (10). Increased plasma levels of nitrotyrosine in patients with type 2 diabetes have been reported (12,13). Piconi et al (6) and Mercuri et al (10) provided a detailed review of oxidative stress in diabetes and particularly the role of nitrotyrosine.

HYPERGLYCEMIA

Therefore, as reported, the levels of all biomarkers of oxidative stress are modified in patients with diabetes. This finding suggests that diabetes is associated with an overgeneration of free radicals. Substantial data, however, indicate that the main source of oxidative stress in patients with diabetes is hyperglycemia and that the overproduction of free radicals has a major role in the pathogenesis of complications of diabetes.

Recent studies have demonstrated that a hyperglycemia-induced process of overproduction of superoxide by the mitochondrial electron-transport chain appears to be the initial and key event in the activation of all other pathways involved in the pathogenesis of diabetes-related complications (14). These include increased polyol pathway flux, increased formation of advanced glycation end products, activation of protein kinase C and nuclear factor-κβ, and increased hexosamine pathway flux. Hyperglycemia also promotes, through the activation of nuclear factor-κβ, an increased expression of inducible nitric oxide synthase (iNOS), accompanied by increased generation of NO, and an overactivity of the reduced form of nicotinamide adenine dinucleotide phosphate—which, in turn, overgenerates superoxide (15,16).

FACTORS LEADING TO ENDOTHELIAL DYSFUNCTION

Superoxide overproduction, when accompanied by increased generation of NO, favors the formation of the strong oxidant peroxynitrite (11), which in turn avidly oxidizes tetrahydrobiopterin, an iNOS and endothelial nitric oxide synthase (eNOS) cofactor, into dihydrobiopterin (16). Under conditions of tetrahydrobiopterin deficiency, iNOS and eNOS are in an uncoupled state, which means that electrons flowing from the iNOS and eNOS reductase domain to the oxygenase domain are diverted to molecular oxygen rather than to L-arginine. The result is production of superoxide rather than NO (16,17). This process leads to the formation of peroxynitrite. Furthermore, peroxynitrite causes DNA damage, which is an obligatory stimulus for the activation of the nuclear enzyme poly(adenosine diphosphate [ADP]-ribose) polymerase (15). Poly(ADP-ribosyl)ation of the reduced form of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) by poly(ADP-ribose) polymerase, which is activated by DNA strand breaks produced by mitochondrial superoxide overproduction, in turn inhibits GAPDH activity (18). GAPDH inhibition seems to be the final mechanism through which the pathways involved in the development of complications of diabetes are activated (18). The process concludes in acute endothelial dysfunction in blood vessels of patients with diabetes, which also convincingly contributes to the development of diabetes-associated complications (15). In humans and animals, evidence suggests that these pathways are actively working and that, in patients with diabetes, hyperglycemia is directly related to this phenomenon.

As previously described, the production of peroxynitrite can be indirectly inferred by the presence of nitrotyrosine (10), and increased plasma levels of nitrotyrosine in patients with type 2 diabetes have been reported (12,13). Nitrotyrosine formation has been detected in the arterial walls of monkeys during hyperglycemia (19), in the plasma of healthy subjects during hyperglycemic clamp (20), and in patients with diabetes after consumption of a meal, when postprandial hyperglycemia leads to an increased nitrotyrosine plasma concentration (21). Hyperglycemia is also accompanied by deposition of nitrotyrosine in the perfused working hearts from rats, and it is related to the unbalanced production of NO and superoxide, through iNOS overexpression (22). Moreover, nitrotyrosine formation is followed by the development of endothelial dysfunction in both healthy subjects (20) and patients with diabetes (23) as well as in the coronary vasculature of perfused hearts of rats (22). Therefore, these collective data strongly support the correlation between peroxynitrite and endothelial dysfunction.

The toxic action of nitrotyrosine, and thus of peroxynitrite, is also supported by the evidence that increased apoptosis of myocytes, endothelial cells, and fibroblasts in heart biopsy specimens from patients with diabetes (24), in hearts from rats with streptozocin-induced diabetes (25), and in perfused working hearts from rats during hyperglycemia (22) is selectively associated with levels of nitrotyrosine found in those cells. Finally, investigators have recently shown that increased plasma levels of nitrotyrosine are strong and independent predictors of cardiovascular disease (13).

CONCLUSION

Many findings suggest that oxidative stress is actively involved in the pathogenesis of diabetes-associated complications. Studies exploring the possible beneficial effects of antioxidant compounds will better elucidate the clinical effect of these basic findings.

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