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Forum Editorial

Redox-Based Mechanisms in Diabetes

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ALTHOUGH THE PATHOGENETIC MECHANISMS underlying the development of the chronic complications of diabetes remain poorly understood, in recent years a consensus has emerged, implicating a key role for increased oxidative/nitrosative stress. Controversy now centers upon the precise metabolic pathways responsible for the generation of oxidative damage, as well as the specific downstream targets involved. Increasingly, there is recognition that hyperglycemia and/or insulin deficiency may precipitate an almost unique metabolic insult, being capable of simultaneously stimulating the production of various free radical species, while downregulating the host's natural antioxidant defense systems.

Before considering the precise redox mechanisms involved, is there irrefutable evidence for increased oxidative/nitrosative stress in diabetes? In experimental diabetes, evidence of increased oxidative (5) and nitrosative (29) stress and impaired antioxidant defense enzymes (24) has been reported in complications-prone tissues of diabetes, including the peripheral nerve (5, 24), the eye (10), the kidney (2), and the vasculature (17). In subjects with diabetes, of course, the evidence for increased oxidative stress is more indirect and somewhat controversial. Antioxidant buffering capacity appears to be reduced in type 1 (23, 27) and type 2 diabetes (6). In concert, plasma protein carbonyls (26), plasma nitrotyrosine (7), nitric oxide (NO) degradation products (16), and urine and plasma F-2 isoprostanes (18, 23) are increased and circulating taurine (11) and paraoxonase (4) are reduced. Moreover, the extent of these abnormalities appears to correlate with the severity of the complications.

But what of the mechanisms leading to such a vulnerable state, which targets are involved, and could increased oxidative stress play a role in the development of diabetes itself?

Recent studies have, once again, focused attention on the role of the sorbitol pathway in the generation of oxidative stress in diabetes. Sorbitol pathway-related superoxide generation, nitrosative stress, oxidation of the $NADP^+$/NADPH and reduction of NAD^+/NADH redox couples, depletion of intracellular antioxidants, fructose formation, and activation of mitogen-activated protein kinases (MAPKs) and poly(ADP-ribose) polymerase (PARP) have all been implicated as critical pathogenetic pathways. However, some reports, in contrast, suggest that the sorbitol pathway may actually play a role in the detoxification of lipid peroxidation products, or that its activation may be a consequence, rather than a cause, of the increased production of superoxide anions or PARP activation [reviewed in this issue (21)].

The formation of advanced glycosylation end products (AGEs) from nonenzymatic glycation and their interaction with an up-regulated receptor for AGE (RAGE) have also been critically implicated in the development of glucose-mediated oxidative stress, particularly within diabetic vasculature (8). Binding of AGE to RAGE results in activation of a cascade of signaling pathways, including MAPKs, cdc42/rac, and JAK/STAT, and nuclear translocation of the proinflammatory signal transducer nuclear factor-κB. More recently, the identification of additional ligands for RAGE has broadened the spectrum of the potential deleterious effects of its activation in diabetes. Coupled with glucose-enhanced oxidation of the major protein of the low-density lipoprotein particle, apolipoprotein B-100 [described in this issue (13)], and PARP activation, activation of RAGE may contribute to the accelerated rates of atherosclerosis in diabetes.

Additionally, in diabetes, other mechanisms have also been implicated in the development of oxidative stress, which include overproduction of superoxide by the mitochondrial electron transport chain (20), which in turn inhibits glyceraldehyde-3-phosphate dehydrogenase (GAPDH) activity, leading to a cascade of detrimental downstream consequences (9), including activation of protein kinase C, hexosamine flux, and AGE formation. Oxidative/nitrosative

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stress can activate PARP (12) (which also inhibits GAPDH), thereby impairing critical energy-producing pathways leading to the development of endothelial dysfunction (25) and chronic diabetic complications [reviewed in this issue (22)]. In turn, activation of PARP and MAPKs can lead to upregulation of cyclooxygenase-2 (COX-2), inducible NO synthase, cell adhesion molecules such as ICAM-1, and various inflammatory mediators, all of which have been implicated in the development of cardiovascular disease. Interestingly, the studies of Kellogg and Pop-Busui reported herein (14) directly implicate activation of the COX-2 pathway in the development of oxidative stress in experimental diabetic neuropathy, potentially implicating a vicious cycle. The critical loci of COX-2 activation and oxidative damage have been proposed to be the vasculature (leading to perfusion deficits) in the peripheral nerve. However, data presented by Wang and colleagues in this issue also demonstrate a potential susceptibility of the Schwann cell to oxidative stress and apoptosis, which may also play an important role in the development of neuropathy via a nonvascular mechanism (30).

Oxidative stress and apoptosis have also been implicated in the pathogenesis of diabetic retinopathy. Considerable recent interest has focused on the glucose-induced mechanisms involved, which appear to differ among cell types. For example, this issue contrasts the responses of three different retinal cell lines to hyperglycemia, which highlights the heterogeneous responses involved (15). Retinal endothelial cells, for example, demonstrate impaired antioxidant defense, increased oxidative stress, and mitochondrially mediated apoptosis in response to high glucose (15). In contrast, retinal pericytes appear to be less susceptible to glucose-induced oxidative stress, because antioxidant defense systems are not down-regulated and levels of lipid peroxidation products are unchanged (1). Finally, in retinal pigment epithelial cells, our laboratory has shown that high expression of the aldose reductase gene appears to impair antioxidant defense in response to high glucose, in part via down-regulation of active taurine transport (19).

Finally, how does our better understanding of the mechanisms underlying the development and consequences of increased oxidative stress translate to improved outcomes for patients with diabetes? Firstly, increased mitochondrial reactive oxygen species production has been shown to activate cellular stress-sensitive pathways, resulting in impaired insulin action, which may contribute to the development of insulin resistance (3) (a key factor in the development of type 2 diabetes). Therefore, perhaps attenuating oxidative stress prior to the development of diabetes may contribute to an effective preventative strategy. Secondly, diabetes and its complications are increasingly viewed as vascular disorders, and thus the exciting therapeutic potential for PARP and AGE/RAGE inhibition are currently being explored. Thirdly, the utilization of antioxidants to prevent or reverse diabetic complications would seem to be a logical approach, but in practice has proved disappointing. Perhaps the relative lack of efficacy reflects an ineffective therapeutic strategy, typically utilizing single agents or several agents with similar pharmacologic profiles. Vincent *et al.* (28) in this issue explore the ability of a triple antioxidant approach to prevent dorsal root ganglia neuron oxidative stress and cell death. The answer to the question as to whether the *in vitro* neuronal protection achieved by this combination of therapy can be translated to subjects with type 1 diabetes and early neuropathy will have to wait until completion of the ongoing clinical trial.

ABBREVIATIONS

AGE, advanced glycosylation end product; COX-2, cyclooxygenase-2; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; MAPK, mitogen-activated protein kinase; NO, nitric oxide; PARP, poly(ADP-ribose) polymerase; RAGE, receptor for AGE.

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