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Reactive Oxygen Species and Diabetic Nephropathy

HI BAHL LEE,* HUNJOO HA,* and GEORGE L. KING†
**Hyonam Kidney Laboratory, Soon Chun Hyang University, Seoul, Korea; and †Joslin Diabetes Center, Harvard Medical School, Boston, Massachusetts.*

Diabetes is the leading cause of end-stage renal disease worldwide. Intense glycemic and BP control and the use of angiotensin-converting enzyme inhibitors and/or angiotensin II receptor blockers delay but may not prevent or stop the onset and progression of diabetic vascular complications, including nephropathy. The prevalence of diabetes is predicted to rise from 6% to 10% worldwide in the next decade, and more people will experience diabetic complications unless new and more effective therapeutic and preventive measures become available.

Hyperglycemia induces vascular injury through complex overlapping pathways: formation of advanced glycation end products (AGE), activation of protein kinase C (PKC), and generation of reactive oxygen species (ROS), among others. Growing evidence suggests that ROS may play an important role in the initiation and progression of diabetic nephropathy. The effect of antioxidant therapy is well documented in cell and animal studies, although convincing evidence for clinical efficacy is still lacking.

At the Fourth Hyonam Kidney Laboratory International Diabetes Symposium entitled "Reactive Oxygen Species and Diabetic Nephropathy" held in Seoul, Korea, January 18 to 19, 2003, the expanding role of ROS in the pathogenesis of diabetic renal injury was discussed in depth. Rhee *et al.* provided evidence that hydrogen peroxide (H_2O_2) is an intracellular messenger. H_2O_2 is produced in response to receptor stimulation and propagate its signal by oxidizing cysteines in the active sites of protein tyrosine phosphatase. The function of various proteins including protein kinases and transcription factors can be altered through oxidation of their H_2O_2-sensitive cysteine residues. Intracellular concentration of H_2O_2 is regulated by highly controlled processes involved in the production and elimination. Peroxiredoxins, whose activity is regulated by protein phosphorylation, are a novel family of peroxidases that efficiently eliminate intracellular H_2O_2.

Kuroki *et al.* discussed controversies that exist in the role of oxidative stress in the pathogenesis of diabetic complications. The observation that classical changes of diabetic retina and glomeruli are rarely observed in patients with insulin resistance but without diabetes, despite increased superoxide production, together with the lack of solid evidence that antioxidant therapy is clinically effective suggested to them that oxidative stress may play a supportive but not the lead or initiating role in diabetic microvascular disease.

Brownlee, however, suggested in his presentation (manuscript not submitted) that hyperglycemia-induced overproduction of superoxide by mitochondrial electron transfer chains is the single driving force of the major molecular mechanisms implicated in the glucose-mediated vascular damage: increased polyol and hexosamine biosynthetic pathways, increased AGE formation, and activation of PKC isoforms. Independent of superoxide production from the mitochondria, the role of NADPH oxidase as a source of ROS generation in nonphagocytic cells including vascular smooth muscle and endothelial cells, renal glomerular mesangial and tubular epithelial cells, and fibroblasts has recently been the subject of interest. Li and Shah reviewed the current understanding of the nonphagocyte NADPH oxidase at both structural and biochemical levels and the possible role in diabetic nephropathy. Inoguchi *et al.* demonstrated that PKC is actively involved in high glucose– and free fatty acid–induced activation of NADPH oxidase. High glucose–, free fatty acid–, and phorbol ester–induced ROS generation was effectively inhibited by diphenylene iodonium and PKC inhibitors, and high glucose–induced activation of Rac 1, a component of NADPH oxidase, was effectively inhibited by PKC inhibitors. Chung *et al.* used a genetic approach and confirmed the contribution of polyol pathway to diabetes-induced oxidative stress. Transgenic mice overexpressing aldose reductase showed an increase in malondialdehyde and a decrease in glutathione (GSH) in lenses when they were rendered hyperglycemic, and sorbitol dehydrogenase–deficient mutation in these mice partially normalized malondialdehyde and GSH levels. Diabetic aldose reductase null mice showed no change in nerve GSH levels, suggesting that the polyol pathway plays a role in increased oxidative stress in diabetic lenses and nerves. Pfeilschifter *et al.* showed that nitric oxide is another source of free radical and acts as a signaling molecule by itself and by interaction with ROS. Nitric oxide regulates the redox state of the cells, cellular signal transduction, and expression of genes related to renal injury transcriptionally, posttranscriptionally, and posttranslationally.

Lee *et al.* reviewed ROS-regulated signaling pathways leading to extracellular matrix (ECM) deposition in diabetic kidney. ROS generated by high glucose levels activate signal transduction cascade (PKC, MAPK, and JAK/STAT) and transcription factors (NF-κB, AP-1, and Sp1) and upregulate TGF-β1 and fibronectin in renal cells, and antioxidants effectively inhibit high glucose– and H_2O_2-induced activation. Ha

Correspondence: Dr. Hi Bahl Lee, Hyonam Kidney Laboratory, Soon Chun Hyang University, 657 Hannam-dong, Yongsan-ku, Seoul 140-743, Korea; Phone: +82-2-709-9171; Fax: +82-2-792-5812; E-mail: hblee@hkl.ac.kr

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and Lee provided evidence that, in addition to upregulation of ECM synthesis, ROS play an important role in ECM degradation and epithelial-mesenchymal transition in tubular epithelial cells leading to glomerular mesangial and tubulointerstitial expansion. Koya *et al.* demonstrated that dichlorofluorescein-sensitive ROS are increased in the glomeruli isolated from streptozotocin-diabetic rats, providing a direct evidence of increased ROS in diabetic glomeruli.

AGE are known to have a wide range of chemical, cellular, and tissue effects implicated in the development and progression of diabetic nephropathy. AGE generate ROS directly or through receptors for AGE, whereas ROS, in turn, promote formation of AGE. Forbes *et al.* reviewed the potential pathogenic role of AGE in diabetic nephropathy. The effect of angiotensin-converting enzyme inhibitor on AGE accumulation in diabetes may be through inhibition of ROS generation. Sakurai *et al.* demonstrated that overexpression of receptor for AGE (RAGE) exaggerates nephropathy and retinopathy of diabetic mice, which are prevented by inhibition of AGE formation. Endogenous secretory RAGE (esRAGE), a novel splice variant coding for a soluble RAGE protein, is able to capture AGE ligands and neutralize AGE actions on endothelial cells and exist in human circulation. ELISA for esRAGE was developed by the authors, who are now screening for esRAGE in subjects who have diabetes with and without complications. Iacobini *et al.* showed that galectin-3, another AGE receptor, is weakly expressed in endothelium and glomerular mesangium but upregulated under diabetes. Galectin-3 deficiency accelerates diabetic glomerulopathy as evidenced by more pronounced proteinuria, mesangial expansion, and matrix gene expression in association with more renal/glomerular AGE accumulation, suggesting that galectin-3, unlike RAGE, may confer protection against AGE-induced renal injury.

Genomic approaches based on PCR or microarray technology are new powerful tools for dissecting the molecular basis of a disease process. Susztak *et al.* discussed conceptual, practical, statistical, and logistical considerations for the use of microarrays and introduced their ongoing project analyzing different diabetic mouse models. The results will help to define patterns of gene expression that will aid in diagnosis as well as define susceptibility loci that may lead to the identification of individuals who are at risk. Connolly *et al.* identified a cluster of genes not previously associated with diabetic nephropathy: connective tissue growth factor, gremlin, and actin cytoskeleton regulatory binding proteins using suppression subtraction hybridization. Yoneda *et al.* summarized gene expression profiles in bronchial epithelial cells in response to smoke and H_2O_2 using high-density DNA microarray nylon membrane. Three different phases in gene expression were observed: the immediate event (not transient but persistent activation), followed by induction of various stress proteins and ubiquitin, and the later induction of genes involved in reducing oxidative stress by metabolizing the cellular levels of ROS. Eaton *et al.* induced protein S-thiolation in the kidney by ischemia-reperfusion. Labeling with biotin-cysteine allowed detection and identification of several proteins oxidized. Oxidation of protein thiols may contribute to cellular dysfunction during oxidative stress, provide a protective mechanism that guards against terminal protein thiol oxidation, or may constitute an important signaling event in the transduction of adaptive or protective pathways. However, the effect of protein S-thiolation remains to be elucidated.

It is hoped that this symposium will stimulate scientists to generate fresh perspectives that can resolve the various controversial roles of ROS in the pathogenesis of diabetic nephropathy. Hopefully, these new ideas will translate into therapeutics that can prevent or reverse diabetic renal damage in near future.

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