A New Clue to Glaucoma Pathogenesis

Junjian Z. Chen, PhD, Fred F. Kadlubar, PhD

Oxidative stress is an inevitable consequence of aerobic life. Oxidative damage to deoxyribonucleic acid (DNA) and other biomolecules accumulates with age and has been implicated in age-related degenerative diseases and cancer (1). Reactive oxygen species, including superoxide anion, hydrogen peroxide (H_2O_2), hydroxyl radical, and singlet oxygen, are generated as by-products of aerobic metabolism, as well as by exposure to various natural and synthetic toxicants and ultraviolet/ionizing radiation. Reactive oxygen species can attack DNA to form cytotoxic and mutagenic lesions. The most frequently measured oxidized DNA product, 8-oxo-deoxyguanosine (or 8-OH-dG), may be formed by the direct attack of highly reactive hydroxyl radicals on the guanine residues in DNA. Oxygen radicals may also initiate autocatalytic lipid peroxidation, giving rise to additional reactive species, such as lipid epoxides, lipid hydroperoxides, lipid alkoxyl and peroxyl radicals, and aldehydes. Besides oxidative damage, reactive oxygen species can act as secondary messengers to induce the expression of a variety of transcriptional factors involved in stress responses and pathological processes. Fortunately, mammalian cells have several levels of antioxidant defense against constant infusion of reactive oxygen species by maintaining a pro-oxidant/antioxidant balance. The glutathione redox system appears to be essential in antioxidant metabolic processes because of its ability to scavenge free radicals and its role in the removal of detoxified oxidation product (2). Perturbation of the pro-oxidant/antioxidant balance caused by either biological or genetic variances can lead to increased oxidative damage.

Glaucoma is the second leading cause of blindness in the world after cataracts. Elevated intraocular pressure due to reduction in aqueous outflow facility is a major causal effect (3). The eye's outflow system consists of a series of endothelial cell–lined structures in the angle of the anterior chamber, which include the trabecular meshwork, Schlemm canal, collecting channels, and episcleral venous system. Several lines of evidence suggest that chronic oxidative stress is important in glaucoma pathogenesis, most notably, its age-dependent clinical onset, constant exposure of the trabecular meshwork to a sublethal concentration of H_2O_2 in the aqueous humor, and altered cellular and molecular responses of trabecular meshwork cells to H_2O_2 exposure in vitro (4). Nevertheless, there is not yet substantial proof of a molecular or genetic basis of glaucoma.

In this issue of the *Journal*, however, Izzotti et al. provide convincing evidence linking oxidative DNA damage in a small but critical tissue structure in the outflow system to glaucoma (5). They observed a more than threefold increase in the amount of 8-OH-dG in the trabecular meshwork tissue of glaucoma patients. The increased oxidative DNA damage correlated further with clinical parameters, such as intraocular pressure indexes and visual field loss. The analysis of 8-OH-dG is technically challenging because of the small tissue size of the trabecular meshwork and the extra steps used in minimizing potential artifacts. The authors also identified an association of the Mu-class glutathione S-transferase (*GSTM1)* null allele with primary open-angle glaucoma, suggesting a possible genetic predisposition of defective *GSTM1* detoxification to the disease, although the sample size was relatively small.

The findings by Izzotti et al. provide not only a basis for the role of oxidative stress in glaucoma pathogenesis, but also a new perspective in understanding its molecular mechanisms. Detection of 8-OH-dG in diseased trabecular meshwork tissues suggests ongoing oxidative DNA damage in glaucoma. How oxidative DNA damage could contribute to the disease process warrants further investigation, but considering the unique cellular composition of the trabecular meshwork, it is remarkable to detect such high levels of oxidative DNA damage in a tiny region rich in extracellular matrix.

To the human eye, the trabecular meshwork is made up of collagen beams covered by endothelial-like cells. The space between the beams is filled with extracellular matrix where the aqueous humor filters through. The region of maximal resistance to aqueous humor outflow resides at the peripheral juxtacanalicular trabecular meshwork, which connects the trabecular meshwork to the Schlemm canal. It is thus conceivable that accumulation of oxidative DNA damage in the cellular component of the trabecular meshwork could directly affect the regulation of the extracellular matrix structure and associated intraocular pressure, leading to clinical onset of glaucoma. Because trabecular meshwork cells have a very low replication rate, oxidative DNA damage is less likely to manifest its effects through somatic mutagenesis in the nuclear genome, but through mitochondrial decay and alteration in apoptosis. Mitochondrial decay due to mtDNA damage is common in a variety of degenerative diseases and aging, and may be involved in glaucoma pathogenesis. Conversely, oxidative DNA damage may be

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From the Division of Molecular Epidemiology, National Center for Toxicological Research, Jefferson, Arkansas.

Requests for reprints should be addressed to Junjian Z. Chen, PhD, or Fred F. Kadlubar, PhD, Division of Molecular Epidemiology, National Center for Toxicological Research, 3900 National Center for Toxicological Research Road, Jefferson, Arkansas 72079, or fkadlubar@nctr.fda.gov.

a constant source of stress stimuli in trabecular meshwork cells, thus indirectly contributing to glaucoma pathogenesis through signal pathways. For example, an endothelial leukocyte adhesion molecule–1 (ELAM-1) protein, a key component of biological structures involved in fluid containment and transport, was ubiquitously expressed in the trabecular meshwork cells of glaucoma patients with diverse etiology, but not in those of patients with normal eyes (6). The differential expression of ELAM-1 appears to be activated through the interleukin 1/nuclear factor-κB (NF-κB) pathway, which is implicated in stress responses to oxidative insult (7) and extracellular matrix regulation in the human trabecular meshwork (8). It is likely that increased oxidative damage may be a major source of stress stimuli in the trabecular meshwork cell that acts through the NF-κB pathway, causing disease over time, especially in aging eyes.

To counter oxidative stress, there are many antioxidant defenses in the aqueous humor and the trabecular meshwork cell. As Izzotti et al. indicate, ascorbic acid is present in high concentrations in the aqueous humor, where it functions as both a pro-oxidant and antioxidant agent and provides a constant source of H_2O_2. In addition, other abundant low molecular weight antioxidants (e.g., glutathione, tyrosine) and antioxidant enzymes (e.g., superoxide dismutase, catalase, glutathione peroxidase) collectively provide a first line of defense against superoxide and H_2O_2 (9). The glutathione S-transferases are structurally highly diverse enzymes that not only contribute towards protection from cancer (10), but also represent a second line of defense against the highly toxic spectrum of substances produced by reactive oxygen species–mediated reactions (2). However, the pro-oxidant/antioxidant balance changes with age because levels of superoxide dismutase and catalase decline in aging eyes, leading to diseases such as cataractogenesis and, probably, glaucoma. The age-dependent shift in the pro-oxidant/antioxidant balance can become more pronounced because of the genetic predisposition of defective genes involved in the detoxification of oxidants and electrophiles. A GSTM class protein, one of the retinal antigens targeted by the serum antibodies, has been detected in glaucoma patients with increased titers, suggesting its role in tissue stress or damage responses (11). Moreover, the overrepresentation of the *GSTM1* null genotype in patients who have primary open-angle glaucoma reinforces the importance of glutathione S-transferases in the detoxification of oxidative damage in the eyes. Thus, lack of the *GSTM1* allele appears to affect a person's response to oxidative stress, leading to glaucoma, especially when the first line of antioxidant defense weakens with age. In light of this finding, it seems likely that other genetic polymorphisms in genes involved in antioxidant defenses and DNA damage repair await identification as genetic factors that predispose to an increased risk of glaucoma.

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