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Commentary

Nitric-oxide synthase and neurodegeneration/neuroprotection

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Glaucoma is an optic neuropathy in which the retinal ganglion cells and their axons, which package and transmit visual impulses from the photoreceptors and associated retinal interneurons to the brain, die individually or in small groups, typically over many years (Figs. 1 and 2) (1). The resulting functional deficit is the second leading cause of irreversible visual loss in the U.S., and the most common cause among African-Americans. In its most prevalent form, the condition is strongly dependent upon age, race, and family history. Approximately 3–5% of White Americans, 10% of African-Americans, and 20% of Afro-Caribbeans over the age of 70 years have the disease (2–5). As lifespan and the proportion of the population comprising the aged increase, so will the number and proportion afflicted.

Intraocular pressure (IOP) plays a causal, albeit not necessarily exclusive, role in most cases of glaucomatous visual loss; the higher the IOP the greater the risk (4, 6). There is controversy about whether the primary insult occurs at the level of the axon or the cell body, and the pathophysiology of pressure-induced glaucomatous optic neuropathy is unclear and fiercely debated (7–11). Leading candidate theories include obstruction of axoplasmic flow within the retinal ganglion cell axons at the lamina cribrosa (the connective tissue plate at the back of the eye where the axons coalesce to form the optic nerve "head" or "disk," from whence they continue as the optic nerve through the orbit and into the brain); compromise of the optic nerve microcirculation at the level of the lamina; and alterations in the laminar glial cells and connective tissues. Secondary insults (e.g., excitotoxicity caused by glutamate or glycine released from injured neurons, growth factor deprivation, oxidative damage caused by overproduction of reactive oxygen species) may contribute. Whatever the initial injury site and the initial and subsequent mechanisms (Table 1 lists various possibilities), the end stage of the process is death of the retinal ganglion cell by means of the triggering of an apoptosis program (12, 13), akin to that which eliminates 50% of the retinal ganglion cells during normal developmental organization of the visual pathway (9).

All present glaucoma therapy is directed at lowering IOP (6). However, assaulting or bypassing the anterior eye tissues, which produce and drain the fluid aqueous humor, and thereby determine and regulate IOP, completely neglects the retinal ganglion cells and their axons, whose dysfunction and death are directly responsible for the visual loss. Only recently has knowledge of the mechanisms of neuronal death and its prevention, delay, or even reversal after a variety of insults reached the point where we can seriously entertain the possibility of glaucoma therapy directed at the retinal ganglion cell bodies or axons themselves. Studies in cultured neurons (including retinal ganglion cells) and *in vivo* models of mechanical (crush, transection), ischemic, and pharmacologic (e.g., intraocular glutamate injection) optic nerve or brain injury have suggested various strategies for neuroprotection, neurorescue, and neuroregeneration (14–21) (Table 2). However, none of these strategies had been tested, much less shown effective, in a glaucomatous animal model.

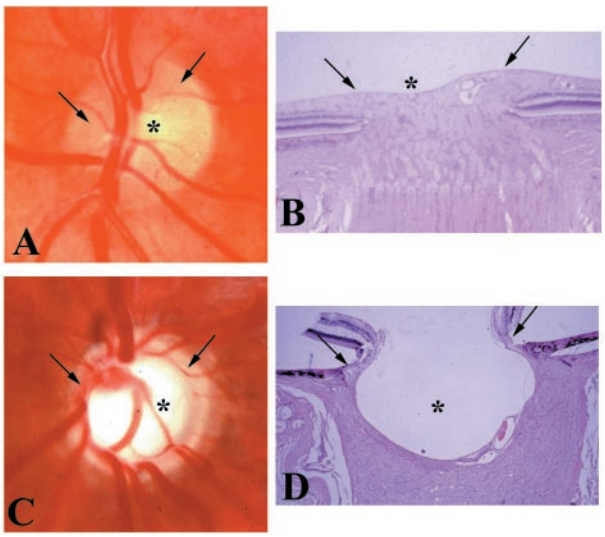

FIG. 1. Human optic nerve head. (*A* and *C*) *In vivo* photograph. (*B* and *D*) Postmortem photomicrograph. (Hematoxylin and eosin stain; ×20.) (*A* and *B*) Normal. (*C* and *D*) Advanced glaucoma. Note the small, shallow central physiologic "cup," (asterisk) and the full neuroretinal rim (arrow) (which comprises approximately 10^6 axons) in the normal nerve head. Compare with the deep, wide central excavation, the nasal displacement of the blood vessels, and the narrowing of the neuroretinal rim, indicating loss of most optic nerve fibers and their retinal ganglion cells, in the glaucomatous eye.

The paper by Neufeld, Sawada, and Becker in this issue of the *Proceedings* (22), demonstrating protection of the optic nerve/retinal ganglion cells from the neurotoxic effects of chronically elevated IOP in a live animal model, by inhibition of the inducible isoform of nitric-oxide synthase (NOS-2), is the first to do so, and will likely be considered classic in years to come. The authors had previously demonstrated the presence of NOS-2 in astrocytes in the optic nerve head and lamina cribrosa of glaucomatous human eyes and chronically hyper-

Table 1. Site and pathophysiology of glaucomatous optic neuropathy

Where is the primary site of injury?
Intraocular retinal ganglion cell axon
Laminar retinal ganglion cell axon
Retinal ganglion cell body
What is the primary mechanism and subsequent sequence of injury?
Obstructed axoplasmic flow
Oxidative/free radical insult
Starvation/growth factor deprivation
Excitotoxicity
Nitric oxide (NO) toxicity
Apoptosis (retinal ganglion cell suicide/programmed cell death)

The companion to this Commentary begins on page 9944.

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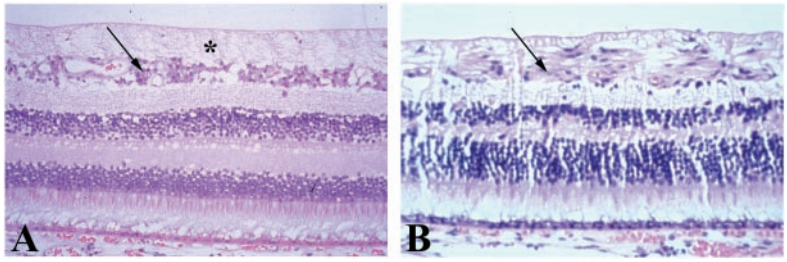

FIG. 2. Human retina, postmortem photomicrograph. (Hematoxylin and eosin stain; ×40.) (*A*) Normal. (*B*) Advanced glaucoma. Note loss of retinal ganglion cells (arrow in *A*) and retinal nerve fiber layer (axons of retinal ganglion cells; asterisk in *A*) and their replacement by glial tissue (arrow in *B*) in the glaucomatous retina.

tensive rat eyes, and its absence in normotensive eyes in these species. They postulated that excessive NO produced and released by these reactive astrocytes led to death and loss of retinal ganglion cell axons, and then the ganglion cells themselves. However, it was not clear whether the increased NOS-2 activity and consequently increased NO production *caused* the neuronal damage by one of the mechanisms described above, or whether it was *consequent*, or perhaps even *unrelated*, to the neuronal damage. By demonstrating that chronic oral administration of aminoguanidine, a relatively specific inhibitor of NOS-2, prevents excavation and axonal degeneration in the optic disk, and loss of retinal ganglion cells, in rats with iatrogenically elevated IOP, they have both validated NO toxicity as an important pathophysiological component of IOP-induced glaucomatous optic neuropathy and identified a potential therapeutic strategy. Furthermore, the significance of their findings may go far beyond glaucoma, with broad pathophysiologic and therapeutic implications for neurodegenerative and neurovascular diseases in general. The roads are of course long and winding from identifying IOP-inducible NO production and consequent optic neurotoxicity to understanding the complete pathophysiological sequence of events: from rat to human; from glaucoma to diseases such as Parkinson's, Alzheimer's, multiple sclerosis, amyotrophic lateral sclerosis, or stroke; and from pathophysiology to therapy. Nonetheless, "the journey of a thousand miles begins with a single step."

Table 2. Potential strategies for preventing retinal ganglion cell death and restoring normal function

Protection of undamaged but at-risk retinal ganglion cells and axons from noxious stimuli released by proximate damaged tissue and/or prevention of initiation of the apoptosis program
N-Methyl-D-aspartate (NMDA)/other excitatory amino acid antagonists (block excitotoxicity)
Anti-oxidants/free radical scavengers (decrease levels of toxic oxygen radicals)
NO synthase inhibitors (block formation of reactive peroxynitrite from NO and superoxide)
Neurotrophins/growth factors
Ca^{2+} channel blockers (prevent entry of toxic levels of calcium into the cell body or axon)
Rescue of marginally damaged retinal ganglion cells and axons
Lazaroids/21-aminosteroids (block lipid peroxidation)
Up-regulation of anti-death genes (bcl-2, bcl-x_L)
Anti-oxidants/free radical scavengers
Ca^{2+} channel blockers
NO synthase inhibitors
Neurotrophins/growth factors
Regeneration/regrowth/replacement of axons
Spanner neural grafts
Growth factors
Transglutaminases/interleukin-2 dimerizers/ oligodendrocytotoxins
Neuroimmunomodulation—appropriately activated macrophages, T cells
Gene therapy (e.g., using viral vectors) vs. small molecule (drug) therapy to modulate the various pathways listed
Difficult and risks of gene and drug delivery to the posterior ocular segment
Duration and specificity of gene expression and drug effects whether delivery is local or systemic

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