GLAUCOMA

Neuroprotection Research, Part One

BY ANNIE STUART, CONTRIBUTING WRITER

Glaucoma research has evolved from a focus on the front of the eye—the so-called plumbing problems—to a focus on similarities with other neurodegenerative diseases, such as Alzheimer's and Parkinson's. No longer defined simply by increased intraocular pressure, glaucoma is now also considered a problem of progressive neurodegeneration.

This new view resulted in part from the realization that the disease cannot always be arrested by IOP-lowering treatment. For about one-third of idiopathic open-angle glaucoma patients, in fact, vision slowly worsens despite the best treatment efforts of ophthalmologists.[1] There is a silver lining here, however. Slow progression opens up the therapeutic window very wide—for years or even decades, according to George A. Cioffi, MD, professor of ophthalmology at Oregon Health & Science University and chairman of the Devers Eye Institute in Portland.

In this two-part series, *EyeNet* takes a look at how notions explored by a new generation of researchers have illuminated the potential of neuroprotection in glaucoma management.

Neuro Disease, Neuro Research

"The concept of neuroprotection for glaucoma has been around for more than a decade," said Robert N. Weinreb, MD, director of the Hamilton Glaucoma Center and professor of ophthalmology at the University of California, San Diego. Neuroprotection is being developed as a therapeutic regimen for slowing, preventing or reversing the death of neurons following an initial insult, said Dr. Weinreb. For most patients, it is likely that it will complement IOP-lowering therapy, not replace it, he said. In some cases, however, neuroprotective agents may also become an alternative for those who can't tolerate IOP-lowering therapy or for whom they have been ineffective.

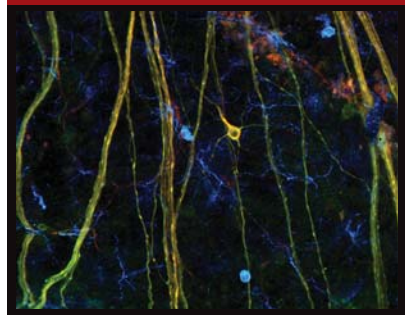
Neuro research. David J. Calkins, PhD, associate professor of ophthalmology and visual science at Vanderbilt University in Nashville, said that making comparisons to other neurodegenerative diseases will allow researchers to better understand the optic nerve's response to glaucoma. "Reasoning through analogy, researchers have been able to identify different components of the disease," said Dr. Calkins.

One injured neuron looks a lot like another. Although glaucoma is not associated with cognitive or motor deficits, it is, at the cellular level, structurally comparable to other neurodegenerative processes. "Nothing is fundamentally different in glaucoma than with other neurodegenerative diseases," said Monica L. Vetter, PhD, professor of neurobiology and anatomy at the University of Utah in Salt Lake City. "The initial triggering events are distinct, and there is clearly a different initial pathology," she said. "But at a certain point, neurons are responding to stress, and other cell populations are recruited, and, in the cross talk between them, I think there are a lot of shared mechanisms during progression."

Work by Drs. Calkins, Vetter and colleagues has thus far supported the notion of shared disease mechanisms among neurological disorders. For example, while identifying changes in genetic expression related to increases in IOP, researchers found that one of the most robust changes occurs in a family of genes associated with inflammation and involved in pathologies of the brain like Alzheimer's.[2]

The eye as a window on the brain. Not only is Dr. Vetter hopeful that glaucoma researchers can learn a lot from diseases such as Alzheimer's and Parkinson's, but she considers glaucoma an attractive reciprocal model for figuring out what's happening temporally and spatially with neural degeneration in other diseases. "In the brain, you have a

Cellular Snapshot



Microglia (stained blue) become activated in the vicinity of damaged axons and RGCs (stained yellow).

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very complex architecture—neurons, axons and synapses—that is not always easily accessible," said Dr. Vetter. "But in the eye, everything is compartmentalized in a way that's not possible in the brain. You have a very distinct neuronal population with highly laminated tissue; the synapses are organized in discrete layers; and you have this beautiful axon bundle that exits the eye and traverses the rest of the brain."

Strategies for Neuroprotection

One disease or several? Dr. Calkins and many other researchers consider glaucoma a multifaceted disease, or a collection of diseases. "Glaucoma can start out in one spatial region and then spread spatially and temporally," added Dr. Vetter.

Dr. Cioffi, in contrast, describes himself "more as a lumper than a splitter. I know there are those who try to split glaucoma and glaucoma's optic neuropathy into a half-dozen different diseases. But my bias is that there is a very characteristic optic neuropathy we know as glaucoma and that has retinal ganglion cell death."

Glaucoma experts appear to agree, though, that glaucoma processes call forth both destructive and protective components. "There are protective cascades that are inducted in response to glaucomatous injury," said Dr. Calkins. "The nervous system responds to the disease as though it is trying to rescue the cells from death, and so the disease takes time to finish its course."

Block damage or boost repair. Whether glaucoma is one disease or several, there are at least two broad neuroprotective drug-development strategies. One is to try to neutralize the effects of nerve-derived toxic factors; the other would work to boost the body's own repair mechanisms.[3] Dr. Cioffi described some of the neurodynamics behind the two strategies.

Genes that help, genes that hurt. Functions of Bax gene expression are detrimental and promote cell death, while functions of the Bcl gene and nerve growth factors promote survival or enhance repair. "These offer us two approaches to neuroprotection," said Dr. Cioffi. "We can either block cell-death promoters or enhance cell-survival signals. If we decided to enhance survival signals, do we try to turn on the cells' innate protection systems—prompt a cell to make more nerve growth factor—or do we try to provide the end product? We could provide the retinal ganglion cells with a growth factor or other macromolecule via an intravitreal injection or sustained release system and thereby enhance survival."

Robert W. Nickells, PhD, professor of ophthalmology and visual science at the University of Wisconsin in Madison, is focusing on the Bax gene. "We think it is a really important step in preventing apoptosis because it blocks the involvement of mitochondria," he said. "As long as you can keep the mitochondria from becoming involved, you've stopped cell death before the point of no return."

In any case, success requires that researchers understand the basic biology first, said Dr. Vetter, so they know which pathways are involved. "It may require—just as with HIV or cancer treatment—that you've got to hit multiple pathways," she said. "That's manageable if we can come up with a reasonable model."

Dying little deaths. One challenge is that the compartments of the retinal ganglion cell—the axon, synapse, dendrites and cell body—can die independently, said Dr. Nickells.[4] "Each compartment has its own molecular program it can turn on that doesn't require the previous deaths of other compartments," said Dr. Nickells, adding that this may require agents to address the deaths of all these different compartments.

Is a silver bullet then out of the question? It probably is, according to Dr. Calkins, who argued that multiple sequential or simultaneous interventions are a more likely scenario, particularly when the disease is not caught early.

Where Best to Intervene

IOP elevation and the factors promoting disease progression may be very different processes, said Dr. Vetter. She and other researchers are focused on early and middle stages of the disease.

First stop, nerve head. Dr. Cioffi suggested that focusing on early initiating events will prove most productive. "We've gotten better in recent years at being more sensitive to early functional and structural changes—better visual field tests for function and better ways of looking at the optic nerve and nerve fibers structurally—and so we're picking up problems earlier and earlier," said Dr. Cioffi. "I think we'll learn a lot more by looking at models that mimic early disease as opposed to models that mimic late blown-out terrible disease. I believe the initial insult is at the optic nerve head and, therefore, it's more fruitful to go after the axon."

Downstream damage. In addition to the primary insult, however, a secondary cascade of events can cause death of retinal ganglion cells. Transsynaptic degeneration may act like dominoes, toppling connected neurons one after another. That might explain why IOP-lowering medications are sometimes ineffective.[5]

The immune system: hero or hellion? "I think that a lot of recent research has shown that neural inflammation plays a really critical role in how the neurons respond," said Dr. Nickells. A student of Dr. Nickells' has shown how macrophages can stimulate ganglion cells.

"There are resident surveillance macrophage-like cells called microglia in the nervous system," explained Dr. Vetter. "They act very locally in terms of detecting damage and changes in the nervous system. We think that these are playing an important role at that juncture. They may not be the triggering step, but I actually think they do play an important role in this progression."

Dr. Weinreb is a consultant for Alcon, Allergan, Merck and Pfizer. The other experts report no related financial interests.

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GLAUCOMA

Neuroprotection Research, Part Two

BY ANNIE STUART, CONTRIBUTING WRITER

Last month *EyeNet* began a two-part examination of new theories in glaucoma pathogenesis and treatment. This month we look at the specifics of neuroprotective agents, as researchers face many opportunities—and many questions: Will the ideal intervention involve sequential or simultaneous therapies? Will treatments be easy for patients to use? Will they address mechanisms specific to retinal ganglion cells? Will they avoid inflammatory responses?

Challenges of Drug Development

Approved only for lowering intraocular pressure, today's glaucoma medications may also have neuroprotective properties, but none has definitively demonstrated that in humans. Neuroprotection was simply not a part of their initial therapeutic rationale or their subsequent approval by the FDA, said Robert N. Weinreb, MD, director of the Hamilton Glaucoma Center and professor of ophthalmology at the University of California, San Diego. Because each IOP-lowering medication has distinct biological properties, it is difficult to know how or whether they also provide neuroprotection independent of their IOP effects. One such medication, brimonidine, has been under study to answer that question, but results are not yet available.

As agents are reviewed for their neuroprotective properties, noted Dr. Weinreb, some criteria must be considered:

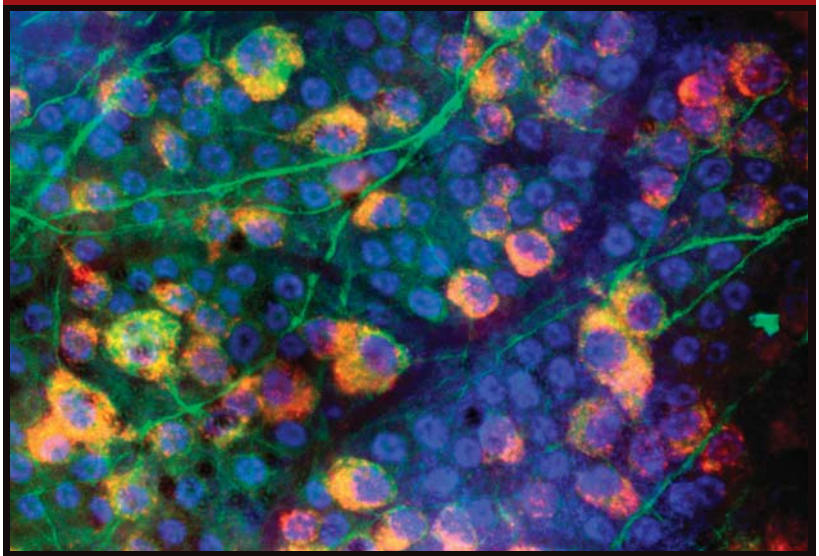
- Does the drug have a specific receptor target in the retinal nerve cells or the optic nerve?
- Does activation of the drug's targets trigger pathways that enhance neuronal survival or decrease neuronal damage in animal models?
- Can it actually reach the retina or optic nerve in pharmacologically effective concentrations?
- Has neuroprotection been demonstrated in an appropriately designed clinical trial—a randomized, controlled study in patients?

A number of agents have been tested in the lab and have demonstrated potential for clinical neuroprotection, said Dr. Weinreb. In cell culture models, many have enhanced the viability of cultured retinal ganglion cells. And in experimental models, a significant number of drugs have demonstrated neuroprotective properties.

Clinical trials. But the last criterion —human trials —is an incredibly expensive hurdle, said George A. Cioffi, MD, professor of ophthalmology at Oregon Health & Science University and chairman of the Devers Eye Institute in Portland. There are two reasons why.

1. Study size and duration. Glaucoma is a very slowly progressing disease. At

The Colors of Research



Retinal ganglion cells in a mouse can be marked red by gamma-synuclein mRNA, and their axons green by the TUJ-1 antibody, which labels beta 3-tubulin. Nuclei of surrounding cells are blue.

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birth, the human eye has approximately one million retinal ganglion cells and optic nerve fibers, and it is estimated that healthy individuals lose fewer than 20 cells per day, said Dr. Weinreb. With glaucoma, that loss multiplies several-fold but still is not always noticeable, he said. Therefore, progressive glaucomatous injury is difficult to detect, and clinical trials must then be lengthy and enroll many patients.

2. Definition of study endpoints. Study goals remain problematic, said Dr. Weinreb, because regulatory agencies equate glaucoma progression with standard visual field loss. Thus far, they have not permitted a surrogate to serve as an endpoint, he said, such as a selective functional test or change in the optic disc or retinal nerve fiber. Dr. Cioffi believes this will change as researchers get better at detecting structural and functional changes. "As we convince regulatory agencies that these are good barometers of disease, we'll be able to test drugs much faster," he said.

Memantine: The first neuroprotector? One glutamate receptor antagonist, memantine, is a good example of these challenges. Approved by the FDA in 2003 to treat Alzheimer's disease, it is the only drug demonstrating glaucoma neuroprotection in primates. In monkeys, it protected against optic nerve fiber loss, neuronal shrinkage within the central visual pathway and visual function loss.[1] These results may be due to its ability to mitigate excitotoxicity and the release of excess glutamate into the extracellular space, which causes ganglion cells to die by apoptosis from secondary damage.[2]

Human trials of memantine for open-angle glaucoma have produced confusing and disappointing results. Progression of disease in studies last year appeared to be lower in patients receiving the higher dose of the drug compared to those receiving a low dose. But in January Allergan announced that final analysis of a memantine phase 3 trial revealed that overall the drug did not perform any better than a placebo.[3]

Despite these inconclusive results, Dr. Cioffi remains optimistic about the memantine trials. "Going into such studies we don't know if we are testing the right drug, but we're going to learn something from each trial, including the memantine trial," he said, pointing to the large number of patients—2,000—who were followed for four years with state-of-the-art structure and function measures. "That's a huge step for us in glaucoma," he added. "It sets the stage and teaches us how to do these things better in the future."

New Theories, New Targets

In the past few years, researchers have begun to formulate new theories about the role of neurodegeneration in glaucoma. Some of the most exciting work has focused on three ideas, best encapsulated as axonal degeneration, gliosis and pressure injury.

1. Axonal degeneration. A precursor to eventual vision loss, changes begin to occur months or even years before retinal nerve cells die. Retinal ganglion cells (RGCs) sample the microenvironment of the brain via the axon. However, the transport machinery used to bring nourishment from the brain to the RGCs becomes dysfunctional—long before the cells die—as first shown by the lab of Donald J. Zack, MD, PhD, at Johns Hopkins University.[4] The axon also allows the retina to communicate with the brain. So if the axon becomes damaged, the retina cannot send visual information to the brain, even if the RGC is still alive, explained Monica L. Vetter, PhD, professor of neurobiology and anatomy at the University of Utah in Salt Lake City.

Researchers have also discovered that deficits in transport have early, middle and late-stage components. "This allows researchers to break down the progression and home in on particular models involved in each stage," said David J. Calkins, PhD, associate professor of ophthalmology and visual science at Vanderbilt University in Nashville.

The drug minocycline has been shown to improve retrograde transport and morphology of the optic nerve. If delivered at stages well before evidence of disease, it can suppress activation of microglia, said Dr. Vetter. This shows that axonal changes are coupled with activation of microglia. "We wanted to capture this window before microglia get activated and cranky," she said, acknowledging that the picture is considerably more complex than this, likely involving many cellular players.

2. Gliosis. Dr. Calkins added that recent research continues to unearth the multiple roles of glial cells. Thought of in the past as only support cells for the neurons, glial cells have begun to earn greater respect in recent years. For example, researchers have found that, in response to pressure at the optic head, glial cells release proteins that may be toxic to neurons. In fact, changes in glial cells are the earliest known event in the progression of glaucoma, happening well before vision loss occurs. This makes them another potential therapeutic target.[4]

Two other main models exist for how glial changes affect RGCs, said Robert W. Nickells, PhD, professor of ophthalmology and visual science at the University of Wisconsin in Madison. "Cells may pump out neuropeptides that affect vascular flow, creating a kind of microischemic event at the optic nerve head," he said. "Or the cells themselves may become stressed, losing their ability to accommodate the axons they're surrounding."

In any event, some of the first changes that can be seen in the level of gene expression or morphology are glial changes, said Dr. Vetter. In early stages of the mouse model, she said, there are changes in gene expression. There is clear optic nerve pathology, but the retinal ganglion cells are still there until late in the game. Studies by the lab of Philip J. Horner, MD, at the University of Washington in Seattle, document that the ganglion cells persist for a long time, detectable by the expression of a general neuronal marker.[5] "There is still the potential for rescue because the cells are simply quiescent or atrophied—not gone," said Dr. Vetter. In addition to other factors, loss of neurotrophic support may eventually contribute to cell body death. "But apoptosis is not the driving force for quite a while in this disease," she added.

3. Pressure injury. Designed to sense pressure, a family of molecules called

mechanicoreceptors is located throughout the brain and retina. These molecules might allow cells in the retina and optic nerve to respond directly to ocular pressure. "This could be similar to how neurons in the spinal cord respond to pressure from walking, sitting and keeping balance," said Dr. Calkins. Pressure injury may overload the cells with calcium, which can cause a direct degenerative cascade, he said. Researchers suggest that the molecules sensing pressure might be the factor translating pressure into neuronal damage. If so, blocking the pressure-sensitive calcium channel might restore contact with the brain.

Dr. Vetter and other researchers express excitement about these and other theories concerning glaucoma and neuroprotection. "We do feel that we've learned enough to have some candidate pathways to target, and we're hopeful that in the next couple of years we'll be able to figure out—along with a number of other excellent labs—which ones are responding."

Dr. Weinreb is a consultant for Alcon, Allergan, Merck and Pfizer. The other experts report no related financial interests.

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Glaucoma Research Foundation

The valuable work of three of the researchers interviewed for this two-part story, Drs. Vetter, Calkins and Horner, as well as researcher Nicholas Marsh-Armstrong, PhD, who contributed the images, is sponsored by the Glaucoma Research Foundation. For more information, visit www.glaucoma.org.