The causes of glaucoma are complex and so are its treatment regimens. It is almost as if glaucoma were several separate diseases. Controlling intraocular pressure (IOP) in the anterior segment is a prime consideration, but the pathologic endpoint of glaucoma is the death of the retinal ganglion cell in the posterior segment. The disease itself is probably best called an optic neuropathy, one of many diseases that can affect neuronal cells. However, it is unique as to the cell affected (the ganglion cell) and the endpoints of the disease process. Biologically and physically, glaucoma is characterized by changes in the optic disc, optic nerve, and brain and by ganglion cell death. Functionally, the changes lead to visual impairment, as best exemplified by a decrease in visual field. Thus, developing methods of preserving ganglion cell function and lifespan are the ultimate goals in maintaining vision in glaucoma. Functionally, the changes lead to visual impairment, as best exemplified by a decrease in visual field. Thus, developing methods of preserving ganglion cell function and lifespan are the ultimate goals in maintaining vision in glaucoma. Functionally, the changes lead to visual impairment, as best exemplified by a decrease in visual field. Thus, developing methods of preserving ganglion cell function and lifespan are the ultimate goals in maintaining vision in glaucoma.

One way to achieve preservation is through neuroprotection—the use of neuron-survival (neurotrophic) agents.

In a rational approach to disease control, one should first ask: What are the stages of glaucoma pathology where neurotrophic agents might be effective? Probably not at the stage of an initial insult or injury such as with a genetic mutation, increased IOP, and so on. Intervention can come relatively early, though, maybe at the first signs of physical and structural changes in the disc region and optic nerve, or at least at the first signs of vision loss. These changes would be rooted in biochemical alterations secondary to the primary insult but still early enough to slow, halt, or even reverse the disease process. A prime therapeutic target would be the process of cell death of the ganglion cells; similarly, a secondary target would be the induced degeneration to neighboring ganglion cells through a “bystander” effect. An important but difficult area would be controlling gliosis and the changes that occur in glial activation. However, the effects of neurotrophic agents will be different at different stages of the disease, with obviously no effect after most or all the ganglion cells are dead.

Major Biochemical Pathways of Damage and Opportunities for Treatment

One of the leading theories as to a cause of glaucoma is loss of neurotrophin action on ganglion cells. This loss would most likely be due to the effect of increased IOP in blockage of retrograde axonal transport from the lateral geniculate area of the brain. A second pathway could involve toxic damage (excitotoxicity) to the ganglion cells due to abnormal increases in glutamate, nitric oxide (NO), among others. A third important pathway leading to cell damage is an oxidative pathway. Oxidative damage can occur in almost any cell area (e.g., the nucleus, mitochondrion, or cell membrane) and increases with aging. A fourth common pathway of cell death is thought to be through apoptosis—programmed cell death. In fact, most of the damage pathways activate this route to cell death. Finally, extensive damage can be done through glial cell activation and gliosis.

These pathways are not mutually exclusive. Treatments that address any or all of these paradigms should theoretically be effective in prolonging ganglion cell life; in most cases, a neuron-survival factor could be effective in prolonging ganglion cell life and possibly even in improving vision. The following are summaries of research in these five areas of interest in relation to the use of neurotrophic agents with possible future directions for therapeutic applications.

1. Neurotrophin Supplementation

Ganglion cells appear to be tonically dependent on neurotrophic agents for maintenance of cellular homeostasis.1 For example, obstructed axonal transport of brain-derived neurotrophic factor (BDNF) has been reported in animal models of glaucoma—with increased ganglion cell survival after BDNF administration. BDNF receptors (TrkB) are found on ganglion cell axons. Ciliary neurotrophic factor is also effective in preserving ganglion cells for an extended period when supplied by lentivirus gene therapy. However, ganglion cell apoptosis has been reported to precede blockage of axonal transport and changes in neurotrophic levels. An unresolved question arises: How much damage is actually done by neurotrophin deprivation, and if significant, can it be effectively reversed?

Work on the usefulness of neurotrophins in glaucoma has lagged behind the study of their efficacy in other retinal diseases. In fact, work on retinitis pigmentosa (RP) leads the way in defining ocular neuron-survival agents. In 1990, LaVail and his group (Faktorovich et al.2) first showed that basic fibroblast growth factor, a natural growth factor, could delay degeneration of a retinal neuron (the photoreceptor cell) in an animal model of RP. Since then, more than 30 natural factors in the brain, retina, and other tissues have been found that inhibit photoreceptor cell death. These were originally called growth factors but now are more appropriately termed neurotrophic factors or neuron-survival agents. Most of these have several biological functions including growth regulation, but all are inhibitors of apoptosis—albeit with different levels of neuroprotection. The paradigm used by LaVail and coworkers is one of constant-light–induced damage to photoreceptor neurons, but it is probable that most of these are candidate neuron-survival agents in glaucoma as well. One of the classic neurotrophic factors studied by LaVail et al., BDNF, has indeed been shown to be effective in a rat glaucoma model by Martin and coworkers3 using AAV-BDNF transfection. Another interesting possible therapeutic agent in glaucoma would be pigment epithelium-derived factor (PEDF). Zhou et al.4 found that...
transfected PEDF is protective of ganglion cells in the DBA/2J glaucoma mouse model. Reduced levels of tumor necrosis factor (TNF), IL-18, and glial fibrillary acidic protein were found after PEDF treatment, and it was postulated that PEDF has an anti-inflammatory effect in preserving ganglion cells. Similarly, Ishibashi and coworkers (Miyazaki et al.) treated normal rats with SIV-hPEDF, and 2 weeks later, subjected the retinas to transient ocular hypertension stress or NMDA injection. They subsequently found that the number of ganglion cells was higher in treated eyes, and the ERG improved. Because of other properties of the PEDF molecule, it has also been suggested that it could be useful in neovascular glaucoma and that it may protect from gliosis (see below). Also effective as a neurotrophic agent is brimodine, an IOP-lowering agent. Calkins and coworkers (Lambert et al.15) have recently reported it to be a ganglion cell neuroprotectant “relevant not only at the cell body but throughout the entire optic projection.” Similarly, DiPolo et al. (Almasieh et al.) have suggested that muscarinic receptors are good therapeutic targets in glaucoma, with galantamine activating M1 and M4 muscarinic ACh receptors. Interestingly, Bai et al.2 have reported that α2-macroglobulin could be a target in ganglion cell neuroprotection, since it is upregulated in an animal model of glaucoma and is neurotoxic “by inhibiting the neuroprotective activity of NGF via TrkA receptors.”

Based on the research on photoreceptor degeneration regarding its relative safety and efficacy in preclinical testing, NeurotechUSA (Lincoln, RI) has mounted clinical trials of ciliary neurotrophic factor (CNTF) in patients with RP and dry age-related macular degeneration (AMD). A technique called encapsulated cell technology delivers CNTF to the retina. In this system, a small capsule is placed inside the eye of the patient. Within the capsule are special cells that overproduce CNTF. The CNTF leaves the capsule and enters the retina, where it helps protect the sick photoreceptor cells.

Future Directions. Most neurotrophic agents have been tested on photoreceptor degenerations and similar neuropathies. A systematic search for the efficacy and safety of all these agents must be performed in preclinical glaucoma models. Proven neurotrophic agents such as CNTF and BDNF should be taken to clinical trial in glaucoma. Drug delivery systems such as encapsulated cell technology should be considered for use in glaucoma, as they are available and have been proven to be effective.

2. Neurotoxic Damage

Several substances can lead to neurodegeneration. Among them are increases in glutamate, calcium, and nitric oxide and other free radicals.

Glutamate and Excitotoxicity. Glutamate is the primary excitatory neurotransmitter in the central nervous system. Receptor (e.g., NMDA) binding of glutamate leads to Ca\(^{2+}\) and Na\(^+\) entry into the neuron. Too much glutamate, however, can allow too much Ca\(^{2+}\) to enter the neuron, activating apoptosis. Normally, the enzyme glutamine synthetase in Müller cells sops up excess glutamate; but it can be overwhelmed.

Reports on excitotoxicity and glaucoma comprise a mixed bag of results. The toxic effect of t-glutamate on ganglion cells has long been reported in animal models. In 1996, Dreyer et al.10 created great interest in reporting increased glutamate in the vitreous bodies of patients with glaucoma and in dog and monkey glaucoma models. However, Carter-Dawson et al.11 as well as others could not replicate these findings in humans or in a study of a monkey glaucoma model. In a similar vein, Honkanen et al.12 saw no increase in vitreous samples obtained from human glaucoma patients. In fact, others have given evidence of the invulnerability of retinal ganglion cells to NMDA excitotoxicity and that the amacrine cell may be the primary site of sensitivity and damage.

Despite this, several drugs that inhibit NMDA-gated channels and thus could function as excitotoxicity blockers have been studied. For example, memantine, an NMDA channel blocker, was shown to have a protective effect in mouse and monkey glaucoma models. Because of this, Allergan (Irvine, CA) conducted two clinical trials (phase 3) on memantine. Unfortunately, the second trial did not replicate the results of the first, and the results showed that patients receiving a high dose of memantine did no better than those on placebo. Yet Weinreb and his group (Yucel et al.13) reported that memantine protects neurons of the lateral geniculate from shrinkage in an experimental glaucoma monkey model.

A decade ago, there was a flurry of activity in examining similar neuroprotectives in neurodegenerates such as stroke, epilepsy, and Parkinson’s disease as well as in glaucoma. Eliprodil (ifenprodil), for example, is a noncompetitive antagonist that binds to the NMDA receptor and can block voltage-gated Ca\(^{2+}\) channels. Early animal results looked promising, and the drug was patented by Alcon (Fort Worth, TX) for use in glaucoma. Wehrwein et al.14 reported that both acetylcholine and nicotine protect against glutamate excitotoxicity in isolated retinal ganglion cells (RGCs); Brandt et al.15 found that calcium preconditioning is neuroprotective. Recently, bis(7)-tacrine, an ACh and NMDA receptor inhibitor, has been shown to protect against glutamate-induced ganglion cell apoptosis in cultured rat neonatal ganglion cells.16

Future Directions. Even though the damaging effect of glutamate makes much theoretical sense, data are mixed as to glutamate’s role in glaucoma. More basic work is needed.

Calcium Channel Blockers. Elevated glutamate leads to an increased inflow of Ca\(^{2+}\) into ganglion cells, which in excess can lead to neuron damage. It is logical then to investigate calcium channel blockers as to neuroprotection in glaucoma, although there are at least six types of voltage-dependent calcium channels. Flunarizine (a T-type blocker) can protect retinal neurons against retinal ischemia, whereas nimodipine (an L-type blocker) does not protect against glutamate-induced damage in brain neurons. No difference in glaucoma progression has been found in patients who use systemic calcium channel blockers for other purposes. Negative side effects have been seen, for example, in cardiac patients who use beta blockers. Ca\(^{2+}\)-dependent intracellular mechanisms related to glaucoma were recently reviewed by Crish and Calkins.17

Future Directions. Research in calcium channel blockers is another mixed bag of results and possibilities. There is a good theoretical basis for their use, but there are problems in practical use. We have to go back to the preclinical drawing board for more study.

NO and Other Free Radicals. With an increase in calcium, there can be an increase in free radicals such as NO. Without a free electron, NO\(^+\) can downregulate the NMDA receptor and actually be neuroprotective. As a free radical, though, NO can be apoptotic, disrupting mitochondrial function, degrading DNA, and so forth. NO\(^+\) can also react with superoxide to form the toxic peroxynitrite molecule.

In light of the effect of free radicals, NO\(^+\)-inhibitors have been tested in preclinical animal models of glaucoma. Neufeld et al.18 have been the strongest proponents of the theory that NO contributes to neurotoxicity in the optic nerve head of patients with primary open-angle glaucoma (POAG). They proposed that increased NO\(^-1\) and induction of NO\(^-2\) in astrocytes of the lamina cribrosa in POAG leads to levels of NO that could be neurotoxic. Based on these findings, they reported that inhibition of NO\(^-2\) by aminoxyguanidine is neuroprotective of ganglion cells in a rat model of glaucoma. They also reported...
that NO as well as endothelin-1 (but not glutamate) was higher in aqueous and vitreous humors in eyes of dogs with a spontaneous form of glaucoma. However, Pang et al. reported that, in a rat model of increased IOP, "optic neuropathy was not associated with a significant change in the expression of NOS-2 in the retina, ONH, or optic nerve." Also, aminoguanidine treatment did not affect the development of pressure-induced optic neuropathy. Again, results on modification of NO concentrations are a mixed bag, with great theoretical appeal but too little definitive therapeutic work in the area.

It must be remembered that NO has many functions. For example, it is an important messenger involved in vasodilation and contractility and thus in aqueous humor dynamics. In the anterior segment, NOS has been reported in the surgically extracted trabecular meshwork of glaucoma patients. An increase in NO has been interpreted as causing vasodilation, with a resultant decrease in IOP. Both NO and ET-1 are increased in the aqueous humor of POAG and in chronic closed-angle glaucoma and were recently postulated as being helpful in controlling IOP. Last year, an interesting new compound was synthesized comprising latanoprost and an NO-donating moiety (NCX 125, BOL-305259-X). It successfully lowered IOP in rabbit, dog, and primate models of glaucoma and is reportedly entering clinical trial.

Future Directions. NO has many diverse effects on the eye, and so care must be taken in its use. Agents that precisely control its concentration may yet find a place in glaucoma therapy—but in the anterior and posterior segments. Hope lies in compounds such as latanoprost and its hybrid, NO-donating cousin.

3. Antioxidants

In general, an imbalance between pro- and antioxidants can lead to cellular damage. Thus, in many types of pathologies, oxidation has been reported to be part of the damage pathway, with antioxidants helpful in at least slowing the course of cellular damage. In animals, ganglion cell death is reported to be inhibited by antioxidants in a glaucoma mutant of optineurin. In humans, a recent study reports the finding that oxidative DNA damage and total antioxidant status decrease "in the serum and aqueous humor of glaucoma patients." However, a large, prospective epidemiologic study showed little association between antioxidant consumption and the risk of POAG. Despite these disparate findings, recent studies have demonstrated cellular dysfunction involving oxidative stress in lamina cribrosa cells from glaucoma patients. The effects include disruption of mitochondrial function and also impaired calcium extrusion. These studies and other evidence indicate that oxidative damage can result in (or at least accompany) significant ganglion cell damage and lead to cell dysfunction and death.

It is clear that antioxidants can slow disease progression in some neuropathologies, including some patients with midstage dry AMD. This effect was proven in the Age-Related Eye Disease Study (AREDS) clinical trials with a selected subset of antioxidative agents. Ongoing trials with antioxidants such as lutein and zeaxanthin, testing is also being conducted with omega-3 long chain polyunsaturated fatty acids (LC-PUFAs) in the hope that they act as antioxidants and thus protect photoreceptor cells. In the Women’s Antioxidant and Vitamin B Study, epidemiology data from a very large group of women at risk of cardiovascular disease indicate that daily supplementation with B vitamins reduces the risk of AMD. It is probable that all these antioxidants would be effective in slowing ganglion cell death.

Future Directions. There is no doubt that antioxidants can be effective in delaying many of the neuropathologies, especially those that are age related. Questions remain, however: Will any of these definitively delay glaucoma? If so, what are the best agents or combinations of agents?

4. Apoptosis

Apoptosis is a multistep pathway by which cells “commit suicide”—in particular, sick or damaged cells. Quigley et al. first showed that ganglion cells die by apoptosis in two animal models of experimental glaucoma. In 1997, McKinnon stated that “retinal cells die by the mechanism of apoptosis.” In 1999, Nickells wrote that “the mechanism of ganglion cell apoptosis is poorly understood.” More than a decade later, this is still true. A large amount of evidence links glaucoma to apoptosis. For example, Huang et al. showed in a rat model that increased IOP led to activation of protein phosphatase calcineurin and ganglion cell apoptosis via dephosphorylation of BAD (a proapoptotic CL-2 family member) and increased cytoplasmic cytochrome c. Importantly, FK506 (a calcineurin inhibitor) reduces BAD dephosphorylation and cytochrome c release while increasing optic nerve and ganglion cell survival. Interestingly, there is evidence that mature RGCs have a greater susceptibility to apoptosis than younger cells because of an age-related decrease in the activation of some survival pathways involving IAPs (inhibitors of apoptosis) and TRAFs (TNF receptor–associated factors). Finally, there is evidence of caspase and calpain involvement in ganglion cell death. Caspases are the signature enzyme system in apoptosis. Calpain inhibition protects ganglion cells in several preclinical paradigms that promote cell death.

There are many agents that inhibit different steps of the apoptotic pathway. Many of these agents mentioned above have been shown to be useful in dry AMD and in RP. In glaucoma, efficacious agents includes (1) caspase inhibitors such as nirodil, which inhibits caspase-3; (2) calpeptin, a calpain-specific inhibitor that protects ganglion cells against Ca2+ influx; (3) x-linked inhibitor of apoptosis protein gene therapy, which is neuroprotective of ganglion cells in retinal ischemia; (4) tafuroprost, which promotes viability in RGC-5 cells when stressed with glutamate; the α-1 receptor (chaperone), which Navlyutov et al. have shown is neuroprotective of RGCs against optic nerve crush; and (6) an inhibitor of c-Jun N-terminal kinase, which has been reported to be protective of ganglion cells, although Quigley et al. recently demonstrated a lack of neuroprotection in experimental glaucoma in c-Jun N-terminal kinase knockout mice.

Future Directions. A wide range of agents demonstrate antiapoptotic activity in many disease processes and are therapeutic candidates in glaucoma. Few have been thoroughly studied; this lack of research should be addressed in model systems. Because apoptosis is far down the biochemical road of cell damage, use of classic apoptotic inhibitors may be less effective in restoring vision than other agents (e.g., antioxidants).

5. Gliosis

Gial cell activation and proliferation are seen in most neuronal degenerations and could be a significant contributing factor in
glaucomatous ganglion cell death. In the DBA/2J mouse model, reactive, nonproliferative glia has been reported to predominate.\(^6\) Others have shown that astrocyte reactivity is an early event in the DBA/2J mouse and that this plays a role in axon loss in glaucoma. Similarly, microglia activation has been pos-
tulated to play a role in the loss of function and degeneration of the optic nerve. Pathologically, glial fibrillary acidic protein is upregulated in Müller cells and astrocytes as an early event in animal models with elevated IOP. Activated glial cells release abnormal levels of cytokines (e.g., TNF\(^a\)), as well as reactive oxygen species and NO, which can harm ganglion cells.

Based on these and similar findings, some leads to therapy have been published. For example, in a kaicin acid glaucoma model, the glial toxins \(\alpha\)-aminoadipic acid and neurostatin decrease reactive gliosis.\(^7\) This then reduces protease levels and ganglion cell apoptosis. Bosco et al.\(^8\) have reported that minocycline treatment reduces retinal microglia activation and improves optic nerve integrity in the DBA/2J mouse model. Inhibition of the STAT3 pathway in the retina has been proposed as a therapeutic measure that is neuroprotective of ganglion cells in glaucoma. As mentioned, PEDF is a potent neurotrophic protein in glaucoma. It has other interesting characteristics that include tumor-differentiating and antiangiogenic properties. It has also been shown to stop glial cell proliferation. In mixed cultures from rat brain containing mostly cerebellar granule cells and some glial elements, it inhibits glial proliferation. Thus, although not tested on retinal glial elements, PEDF may be used as both a neuroprotective\(^4\) and gliastatic agent in glaucoma. Future Directions. The early damaging effect(s) of glosi-
s in glaucoma must be better characterized. Pathways of glial cell activation should be better understood, as well as how therapeu-
tic agents such as minocycline and PEDF could be used therapeutically.

**NEUROTROPHINS: POTENTIAL LIMITATIONS**

Although it seems clear that most of the neurotrophic agents discussed above (both the classic neurotrophic agents such as BDNF and CNTF and the other agents that act as apoptosis or gliosis inhibitors) could be helpful in glaucoma, there are limitations to such therapy; notably, these agents only delay cell death and are not actually cures. They have a short half-life when given systemically or topically or injected into the eye. Some have adverse side effects. Neurotrophin administration may even downregulate the neurotrophic factor receptors in the target tissue. Most are growth factors with multiple physi-
ological effects (e.g., some are potent angiogenic agents).

Treatment must be repeated throughout a patient’s life, mak-
ing compliance an issue. Drug delivery to the posterior seg-
mens of the eye (the retina) is a problem. Most routes currently available are invasive and involve injection into the eyeball, generating safety concerns. Finally, viable ganglion cells have to be present, limiting the use of the neurotrophic agent in advanced glaucoma.

**SUMMARY: KEY NEEDS AND OPPORTUNITIES**

We need a better understanding of the underlying cause(s) of glaucoma—that is, more basic research. Specifically, are there other particular points of intervention in the damaging bio-
chemical pathways that would be useful to target? Can genetics (i.e., new knowledge of specific mutated genes and protein pathways) point the way to new sites of therapeutic interven-
tion? Further studies on genome-wide scans\(^9\) and gene expres-
sion\(^10\) and proteomic studies\(^11\) can all be helpful in pinpoint-
ing therapeutic targets in glaucoma. Fingert et al.\(^12\) have used single-nucleotide polymorphisms in assessing POAG and ste-
roid responders. Howell et al.\(^13\) have used molecular clustering (genome-wide expression profiling) to target early molecular events in glaucoma. We also need a better understanding of animal models of glaucoma, both as to how they differ from or faithfully represent the human condition. We have to under-
stand that, because these models differ in some aspects of their pathologies from the human disease, they may give false read-
ings (positive or negative) as to the efficacy of a particular neurotrophic agent in the human. A recent review of mouse models has been published by McKinnon et al.\(^14\)

There should be more preclinical testing of neurotrophic and antioxidative agents that have already been shown to pro-
tect other neurons such as photoreceptor cells. Of interest, it has even been suggested that eradication of *Helicobacter pylori* could act as a neuroprotectant in glaucoma.\(^15\) From all this, we must move to clinical testing of agents for which there is already proof of principle as to effectiveness and safety in preclinical testing. We should apply new methods of drug delivery to the delivery of therapeutic agents to the retina in glaucoma. Several of these methods, some of which are non-or minimally invasive, are currently under investigation in other retinal neuropathies; these include systemic and topical modes of delivery as well as unorthodox delivery modes such as via stem cells. Gene therapy delivery is also an excellent prospect for long-term delivery of a therapeutic agent. The use of mes-
enchymal stem cells bioengineered to overproduce BDNF has been shown to protect ganglion cells in a rat eye model of chronic hypertension.\(^46\) New drug delivery systems for ocular delivery for glaucoma were recently reviewed by Lavik et al.\(^47\) An excellent comprehensive review on the molecular basis of RGC death and neuroprotection was recently published.\(^48\)

Finally, more attention should be paid to prevention. For example, would early use of antioxidant therapy delay or even halt onset in those with a family history of glaucoma, or significantly slow or halt progression if the disease process could be caught at an early stage?

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