

Retinal Ganglion Cell Death in Glaucoma: Mechanisms and Neuroprotective Strategies

Markus H. Kuehn, PhD, John H. Fingert, MD, PhD, Young H. Kwon, MD, PhD*

*Department of Ophthalmology and Visual Sciences, University of Iowa Health Care, 200 Hawkins Drive,
Iowa City, IA 52242, USA*

Neuroprotection can be defined as any therapeutic paradigm designed to prevent or delay neuronal cell death and maintain neural function. In glaucoma, progressive death of the retinal ganglion cells (RGCs) leads to optic nerve degeneration and, ultimately, vision loss. The aim of glaucoma therapy is, therefore, to facilitate the survival of RGCs. Currently, glaucoma treatment relies on pharmacologic or surgical reduction of intraocular pressure (IOP). Ample evidence shows that reducing IOP provides effective neuroprotection against the demise of RGCs in glaucoma [1–3].

The development of animal models of glaucoma has allowed investigation into the cellular and molecular mechanisms of this disease. Experimental elevation of IOP animal models of glaucoma induces structural, biochemical, and functional changes that resemble those of the disease in humans, including disorganization and compositional changes in the optic nerve head (ONH), RGC apoptosis, and visual deficits [4–10]. This article describes the cellular mechanisms of RGC death in glaucoma and the emerging neuroprotective strategies that are based on these mechanisms.

Mechanisms of retinal ganglion cell death in glaucoma

Glaucoma represents a group of diseases that share certain clinical characteristics, including exca-

vation of the optic disc and loss of the RGC with resultant visual field loss, with or without elevated IOP. Excavation, or cupping, of the ONH is the clinical hallmark of glaucoma. Although it is unclear whether changes to the ONH are the primary events that precipitates RGC demise or whether glaucomatous RGC death induces events that lead to ONH changes, evidence indicates that increased IOP precipitates distinct compositional and structural changes in the ONH. These include increased synthesis of several extracellular matrix molecules such as tenascin [11,12], matrix metalloproteinases [13,14], NCAM-180, [15] collagen IV, and elastin [16]. Recent in vitro studies have shown that some of the events can be attributed to reactive astrocytes [11,15,16]. Various stimuli can initiate astrocyte activation, including demyelination of adjacent axons, ischemia, mechanical trauma, and increased hydrostatic pressure [17–19]. Reactive astrocytes migrate to the nerve bundles and may form large cavernous spaces through the expression of matrix metalloproteinases [13,14]. It is possible that these changes weaken the architecture of the ONH and facilitate the collapse of the lamina cribrosa beams, eventually leading to injury of the RGC axons that pass through these structures.

Evidence also indicates that apoptosis may be the final common pathway for RGC death in glaucoma. Apoptosis is a programmed cell death pathway designed to remove damaged cells through phagocytosis, and it occurs without eliciting an inflammatory response. The apoptotic process requires the expression of specific genes and can be identified using histochemical and biochemical methods. Apoptotic RGC death has been demonstrated in animal models

* Corresponding author.

E-mail address: young-kwon@uiowa.edu (Y.H. Kwon).

of glaucoma [20,21]. Markers of apoptosis have also been observed in the human glaucomatous retina [22,23]. Several mechanisms that may initiate RGC apoptosis in glaucoma have been proposed (Fig. 1). These include neurotrophic factor deprivation, hypoperfusion/ischemia, glial cell activation, glutamate excitotoxicity, and abnormal immune response. Each of these mechanisms is described in detail below.

Neurotrophic factor deprivation

Axonal transport is vital to the normal functioning of neurons, and retrograde transport of neurotrophic molecules synthesized in the target organ (lateral geniculate body) may be essential for RGC survival [24,25]. In experimental models of glaucoma, elevated IOP blocks the axonal transport at the level of the lamina cribrosa [26–28]. Blocked transport of radioactively labeled protein and cellular organelles at the level of the lamina cribrosa has been observed within hours of IOP elevation in a primate model of glaucoma [29]. Similarly, restoration of normal IOP after several hours allowed axonal transport to resume. This effect on axonal transport precedes

significant ONH disorganization and suggests a direct effect of the elevated IOP on axonal transport. It is likely, however, that ONH disorganization further contributes to blocking axonal transport at later stages of glaucoma.

Brain-derived neurotrophic factor (BDNF) is one of the molecules delivered to the retina by way of retrograde axonal transport. It has been suggested that insufficient BDNF delivery to the retina may contribute to RGC death in glaucoma. In animal models of glaucoma, BDNF delivery to the retina is substantially reduced [30,31]. Injections of BDNF into the vitreous cavity of rats with experimentally elevated IOP increases the number of surviving RGCs compared with untreated eyes [32]. Similar RGC rescue was observed using viral vectors to achieve continuous synthesis of BDNF in the retinas of rats with experimental glaucoma [33]. Partial rescue of RGCs was also reported after the application of ciliary neurotrophic factor in axotomized adult rat eyes [34,35]. These observations support the idea of neurotrophic deprivation as a cause of RGC death. In a recent study of a rat model of glaucoma, however, RGC apoptosis was observed before axonal transport obstruction and alterations in neurotrophin levels [7].

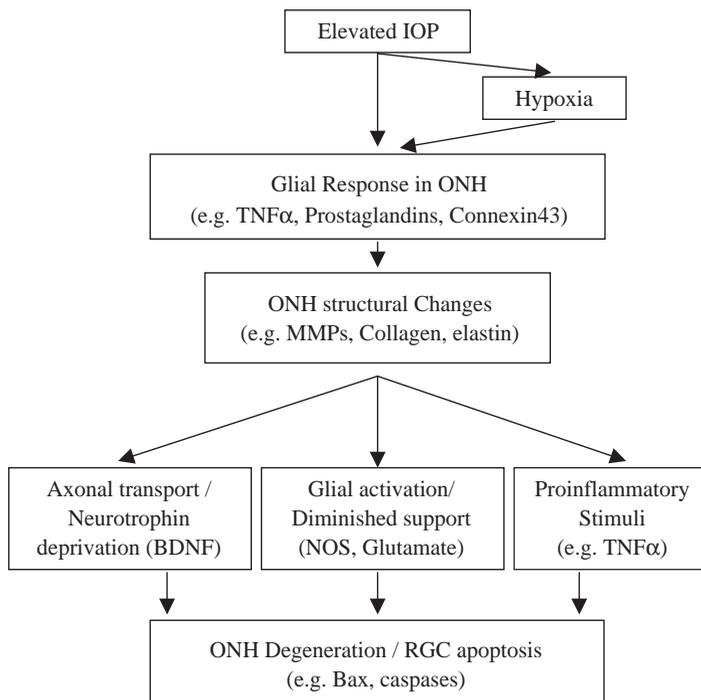


Fig. 1. Proposed mechanism leading to retinal ganglion cell death in glaucoma. MMP, matrix metalloproteinase.

Hypoperfusion/Ischemia of the anterior optic nerve

Glaucomatous optic neuropathy may be a consequence of insufficient blood perfusion to the ONH caused by increased IOP or other vascular risk factors. Blood flow to the anterior optic nerve depends on the perfusion pressure—that is, arterial blood pressure minus IOP—to the optic nerve. Thus, elevated IOP is likely to stress the vascular supply to the optic nerve by causing increased tissue pressure within the optic nerve. Under normal circumstances, autoregulatory mechanisms exist in the ONH vasculature that can maintain normal perfusion pressure, even with moderate IOP elevation. In glaucoma, insufficient autoregulation of optic nerve blood flow may lead to optic nerve ischemia (for a review, see Flammer and colleagues [36]). Although numerous studies suggest impaired blood flow to the optic nerve in glaucoma, accurate blood flow to the ONH *in vivo* has been difficult to measure clinically [37].

The hypoperfusion theory of glaucoma is also supported by the epidemiologic association between low perfusion pressure and primary open-angle glaucoma [38]. Vascular factors such as migraine and Raynaud phenomenon have been clinically associated with normal-tension glaucoma. Animal studies have shown that reduction of optic nerve blood flow through exogenous application of the vasoactive peptide endothelin-1 can result in RGC death in the absence of elevated IOP [39,40]. A

recent immunohistochemical study showed that the expression of hypoxia-induced factor 1 α (HIF-1 α) is elevated in the human glaucomatous retina and ONH compared with expression in healthy controls [41]. The biosynthesis of this transcription factor is initiated in response to low-cellular oxygen tension and induces transcription of genes whose functions are related to oxygen delivery and metabolic adaptation hypoxia. Although the precise cellular consequences of chronically compromised ocular blood flow are unclear, studies of other disease models suggest that mild hypoxia and lack of metabolites can induce cellular apoptosis.

Glial cell activation

Glial cell activation may be an important factor contributing to RGC death in glaucoma. Under normal conditions, glial cells support neuronal function through a variety of mechanisms, including removal of extracellular glutamate and synthesis of growth factors and metabolites. The mammalian retina contains three types of glial cells: astrocytes, microglia, and Mueller cells. Retinal glial cells appear to become activated in the glaucoma. Glial fibrillary acidic protein, a class 3 intermediate filament, is a cell-specific marker that distinguishes astrocytes from other glial cells (Fig. 2). It is expressed by retinal astrocytes under normal conditions, but its synthesis

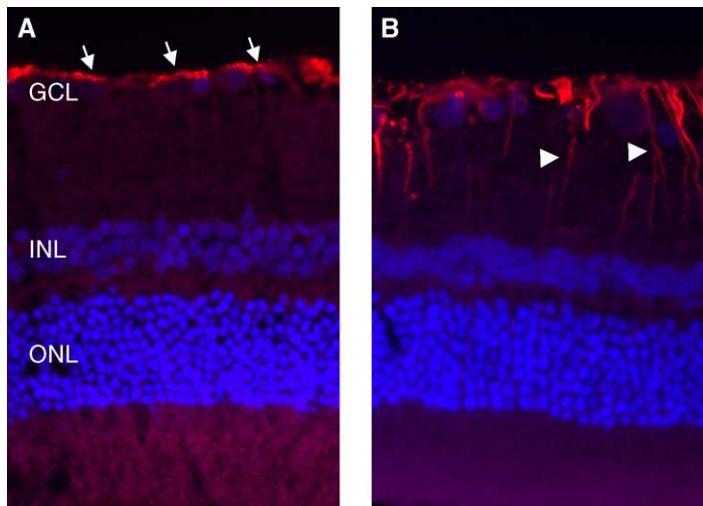


Fig. 2. Immunohistochemical localization of glial fibrillary acidic protein (GFAP) in the normal (A) and glaucomatous (B) rat retina. In the healthy retina, GFAP is restricted to retinal astrocytes (arrow). IOP elevation induces glial cell activation and synthesis of GFAP by Mueller cells (arrowheads). GCL, ganglion cell layer; INL, inner nuclear layer; ONL, outer nuclear layer. (Courtesy of Dr. Chan Y. Kim, University of Iowa, Iowa City, Iowa.)

is significantly elevated in glaucoma by astrocytes and Mueller cells [42]. Increased retinal expression of glial fibrillary acidic protein appears to be an early event in the pathogenesis of glaucoma; in animal models, it can be observed as early as 4 days after the induction of elevated IOP, before widespread RGC apoptosis [43].

It is possible that RGC death caused by glial cell activation results from decreased levels of glial support. For example, glial activation appears to result in reduced biosynthesis of glutamate receptors that may contribute to RGC death [44]. However, evidence is increasing that the activation of retinal glia results in the active synthesis of substances that are harmful to RGCs. Reactive glial cells can exacerbate neuronal damage through the release of cytokines, reactive oxygen species, or nitric oxide. Cultures of mixed retinal glia exposed to elevated hydrostatic pressure secrete tumor necrosis factor alpha (TNF- α) [45]. TNF- α is a proinflammatory cytokine that, when bound to its receptor, can induce apoptosis through a caspase-mediated pathway. An immunohistochemical study of human eyes with and without glaucoma showed that TNF- α is synthesized by retinal glia in glaucoma and that the TNF- α receptor is present on RGCs [46].

Another glial cell-mediated apoptotic stimulus may be the increased production of nitric oxide (NO). NO serves a variety of physiologic functions, including regulation of vascular tone, neurotransmitter release, and synaptic plasticity. Excessive levels of this molecule can lead to cell death in a variety of cell types, including RGCs [47,48]. NO can be generated by three different enzymes: neuronal nitric oxide synthase (NOS1), macrophage- or inducible-NO synthase (NOS2), and endothelial NO synthase (NOS3). In a rat model of glaucoma induced by cauterization of the episcleral veins, increased expression levels for these molecules in the ONH have been reported [49], and total NO is elevated in the retina [50]. In particular, it has been suggested that NOS2 plays an important role in glaucoma [51]. Inhibition of NO production appears to reduce RGC damage in this rat model of glaucoma [52,53]. Increased NOS levels, however, were not detected, and inhibition of NOS did not confer protection to RGCs in a different rat model of glaucoma induced by hypertonic saline injection into the episcleral veins [54,55]. To date, conclusive evidence demonstrating a significant role of elevated NO levels in the pathogenesis of human glaucoma has not been reported, though an immunohistochemical study suggests that increased levels of NOS are present in the human glaucomatous nerve head [56].

Glutamate excitotoxicity

Glutamate is a major excitatory neurotransmitter in the retina. It is released by the presynaptic cells and acts through various postsynaptic receptors, including the *N*-methyl-D-aspartate (NMDA) receptor. If excessive amounts of glutamate are released or if glutamate clearance is insufficient, neuronal death can result in a process known as excitotoxicity. Results obtained from cultured RGCs suggest that these cells are highly vulnerable to glutamate induced excitotoxicity [57]. Elevated levels of extracellular glutamate have been reported in a primate model of glaucoma and in human patients with glaucoma [58]. More recent investigations have failed to confirm these initial findings of elevated glutamate levels both in human patients with [59] and in animal models of glaucoma [60–62]. The role of glutamate excitotoxicity in glaucoma remains unclear. Interestingly, a recent study showed that RGCs, in the presence of neurotrophic factors, are relatively resistant to glutamate excitotoxicity [63], whereas retinal amacrine cells were found to be highly sensitive to elevated glutamate levels. These investigators suggest that the decrease in the number of cells observed *in vivo* in the ganglion cell layer after intravitreal glutamate injections might have resulted from a loss of amacrine cells and from a lack of trophic support for RGCs.

Abnormal immune response

Numerous studies have suggested a role for humoral immune response in the pathogenesis of glaucoma [64,65]. These studies show the presence of autoimmune antibodies directed against retinal antigens in the sera of patients with glaucoma [64,66–69]. Autoantibodies to ONH proteoglycans in the sera of patients with glaucoma have also been reported [70]. Proteoglycans perform various functions, including the formation of a spatial framework to support the optic nerve and blood vessels. An immune response directed against these molecules may weaken the extracellular matrix supporting the lamina cribrosa and may induce or increase optic nerve cupping. In addition, these proteoglycans are present in the cell walls of blood vessels, and their dysfunction may contribute to the development of splinter optic disc hemorrhages or disturbances in blood flow autoregulation in glaucoma.

Others have reported that enhanced T-cell activity resulting from immunization with a synthetic polymer (copolymer-1 [COP-1]) confers RGC neuroprotection in animals subjected to optic nerve crush,

glutamate excitotoxicity, and chronically elevated IOP [65,71,72]. COP-1 is a synthetic amino acid polymer originally designed to mimic myelin basic protein (MBP) and to induce experimental autoimmune encephalomyelitis (EAE) [73]. Instead, injection of COP-1 was found to suppress MBP-induced EAE [74]. Protective autoimmunity has been beneficial in other diseases involving secondary degeneration, such as spinal cord contusion and EAE [73,75]. The exact mechanism of T cell-mediated neuroprotection is unknown. It is thought that an immune response to the degeneration of optic nerve results in secondary degeneration of additional RGCs. If T cells accumulate at the site of injury and are presented with specific antigen, however, they appear to secrete numerous neurotrophic factors, including neurotrophins 3, 4, and 5, nerve growth factor, and BDNF [76]. Thus, it may be the presence of these neurotrophins at the site of injury that facilitates the survival of neighboring RGCs. A neuroprotective effect after optic nerve crush or glutamate excitotoxicity was also observed when T cells were activated using low-dosage γ -irradiation [77]. Finally, in a mouse model of hereditary pigmentary glaucoma, neuroprotection was observed after high-dose irradiation and bone marrow transplantation [78]. It is conceivable that T cell-mediated immune response contributed to the observed neural rescue.

Neuroprotection

The therapeutic goal of preventing the death of neural tissue is referred to as neuroprotection. Neuroprotective treatment strategies have been developed for neurologic conditions ranging from traumatic central nervous system injuries to neurodegenerative diseases such as Parkinson's disease. A major goal of glaucoma research has been to develop analogous treatment approaches to prevent the death of ganglion cells of the retina. Risk factors such as elevated IOP, decreased neurotrophin support, glutamate-associated excitotoxicity, hypoperfusion, and vasospasm have been implicated in ganglion cell death in glaucoma. Neuroprotective strategies have focused on mitigating these risk factors associated with RGC loss in glaucoma.

Treatment of elevated intraocular pressure

A strong association exists between elevated IOP and the development and progression of glaucoma. Reduction in IOP halts or slows the progression of

primary open-angle glaucoma and normal-tension glaucoma [1–3].

All standard medical and surgical treatments for glaucoma are designed to lower IOP. A full discussion of these well-established treatment modalities is available elsewhere [79,80].

Hypoperfusion/Vasospasm

Several nonocular features consistent with vasospasm (eg, migraine, Raynaud phenomenon) reportedly occur at higher frequency in patients with normal-tension glaucoma. These observations suggest that patients with normal-tension glaucoma may develop optic neuropathy, at least partly because of vasospasm and decreased perfusion of the optic nerve.

Calcium channel blockers may improve the perfusion of the optic nerve by vasodilation of the cerebral vasculature. This class of agents has been evaluated as potential therapy for normal-tension glaucoma. Prospective clinical studies have assessed the effects of oral calcium channel blockers on the progression of normal-tension glaucoma. Patients treated with calcium channel blockers were noted to experience fewer ONH and visual field changes than those not taking this medication [81–83]. Nimodipine, a calcium channel blocker, has been shown to acutely improve contrast sensitivity in patients with normal-tension glaucoma [84]. These beneficial effects suggest that calcium channel blockers may have a role in the treatment of normal-tension glaucoma. The risk for serious adverse effects, however, such as systemic hypotension, prohibits widespread use of this class of drugs in glaucoma therapy. In fact, nocturnal hypotension secondary to antihypertension medications has been associated with visual field loss in patients with normal-tension glaucoma [85].

Neurotrophic support

The development and maintenance of RGCs is regulated in part by neurotrophins, including BDNF and ciliary neurotrophic factor [34,86–89]. BDNF has been studied intensively and may play an important role in RGC death associated with glaucoma. BDNF produced at the superior colliculus (in rodents) or the lateral geniculate body (in primates) binds to its receptor (TrkB) on the RGC axons and is delivered to the cell bodies by retrograde axoplasmic transport [90,91]. Animal studies suggest the retrograde axoplasmic supply of BDNF is an important factor in the survival of RGCs [31]. Elevated IOP has been shown to reduce BDNF axoplasmic delivery to the RGCs [30], which suggests that optic nerve

damage in patients with glaucoma may be mediated by a decreased supply of BDNF (and potentially other neurotrophins).

It is reasonable to assume that neurotrophin supplementation can effectively treat RGC death associated with reduced optic nerve levels of BDNF. The neuroprotective effects of BDNF on ganglion cells have been tested in several experimental animal models of glaucoma. Intravitreal injection of BDNF has been shown to promote ganglion cell survival after transection of the optic nerve in the rat [92]. Similar effects were observed in a rat experimental model of glaucoma [32].

More recently, transgenic techniques have been used to deliver BDNF to the retina of the rat. Using adenoviral vectors, transgenic expression of BDNF has been shown to temporarily reduce ganglion cell loss after optic nerve transection [93]. In subsequent studies, prolonged expression of BDNF and extended protective effects on ganglion cells has been achieved with adeno-associated viral vectors [33]. As the safety and stability of gene therapy continue to improve, transgenic delivery of neurotrophins may become a therapeutic reality.

Treatment of glutamate-associated excitotoxicity

Glutamate is a central nervous system excitatory neurotransmitter that has a central role in the normal conduction of signals between neurons. However, excessive extracellular levels of glutamate have been shown to cause neuronal cell death in traumatic and ischemic injury to the spinal cord and brain [94]. This cell death pathway is mediated in part by overstimulation of the NMDA subtype of glutamate receptors. When glutamate (and other factors) bind to the NMDA receptor, the receptor opens and allows calcium and sodium to enter the neuron. Pathologic concentrations of glutamate may allow an abnormally high intracellular influx of calcium, which is thought to activate apoptotic pathways of cell death. This mechanism of glutamate-induced toxicity has been termed excitotoxicity.

Elevated extracellular glutamate levels and excitotoxicity have also been implicated in glaucoma pathogenesis [95]. Several types of evidence have been used to support the hypothesis that glutamate-associated excitotoxicity may be involved in glaucomatous optic neuropathy. First, Lucas and coworkers [96] have demonstrated that high levels of exogenous glutamate are toxic to the RGCs in an animal model. Second, high concentrations of endogenous glutamate have been measured in animal models of glaucoma and in the vitreous humor of

a series of patients with glaucoma [97]. Finally, numerous investigations have suggested that glutamate is toxic to the RGCs at physiologically relevant levels [98,99]. Although these initial studies support a role for glutamate-associated excitotoxicity in glaucoma pathogenesis, subsequent investigations have not confirmed key findings. One recent study [59] found no difference between the levels of glutamate in vitreous humor obtained from patients with glaucoma and from controls. Similarly, in two independent investigations of experimental monkey models of glaucoma, no difference in vitreous glutamate levels was identified between eyes with induced glaucoma and control eyes [88,100].

Despite these controversies, drugs that inhibit NMDA-gated channels have been explored for their usefulness in treating glutamate-associated excitotoxicity as potential neuroprotective agents. One NMDA-channel antagonist, memantine, has been approved in the United States for the treatment of dementia associated with Alzheimer disease [100]. Memantine has also been tested as a treatment for potential excitotoxic mechanisms in the pathogenesis of glaucoma. Intravitreal injections of glutamate have been used to create a rat model of excitotoxicity that results in RGC death. Treatment of these rats with intraperitoneal memantine has been shown to provide some protection from the effects of intravitreal glutamate [98]. Memantine has also had favorable effects on the RGC loss that occurs in DBA/2J mice, which have a pigmentary form of glaucoma [101]. More recently, the efficacy and safety of memantine has been examined with an induced model of glaucoma in primates. Chronic elevation of IOP was induced in macaque monkeys by laser cautery of the anterior chamber angle, which resulted in a measurable decrease in visual function (as determined with electroretinography and visual evoked potentials). The elevated IOP also caused changes in the ONH appearance and loss of RGCs (as measured by Heidelberg Retinal Tomography and histopathologic examination). Oral memantine was shown to have a protective effect on both the visual function and the structural damage caused by elevated IOP, though the effects shown on the electroretinogram were not persistent [102,103]. The results of these animal studies have paved the way for a phase 3 trial, which is under way, of memantine in the treatment of glaucoma in humans.

Inhibition of nitric oxide synthase

Nitric oxide is a vasoactive molecule that can modulate vascular tone and is also a cytotoxic agent

produced by the immune system. Prior studies have suggested that local production of nitric oxide may have a significant role in the development of multiple neurodegenerative diseases, which has prompted investigations of potential neurotoxic effects of this molecule in glaucoma.

Nitric oxide is produced by the enzyme nitric oxide synthase. NOS-2 is not constitutively produced, and its expression can be induced in many cell types (including neurons, endothelial cells, and astrocytes) by injury or cytokines [104–106]. The induction of NOS-2 expression generates high levels of nitric oxide, [107,108] which have been associated with toxicity to neural tissue [109].

Studies of NOS-2 expression have provided evidence that nitric oxide production may be involved in glaucomatous optic neuropathy. NOS-2 expression in the optic nerve has been shown to correlate with the presence of glaucoma. Histopathologic investigations have demonstrated that NOS-2 is expressed in the ONH of patients with glaucoma but not in control subjects [56]. The expression pattern of NOS-2 in cell culture systems and animal models also support a potential role for nitric oxide in glaucoma pathogenesis. Astrocytes cultured from human optic nerve tissue have been shown to produce NOS-2 in response to elevated atmospheric pressure [110]. Finally, in an experimental rat model of glaucoma generated by cautery of three episcleral vessels, the expression of NOS-2 is induced by elevated IOP [49]. These observations suggest that the production of nitric oxide at the ONH has a role in the pathogenesis of glaucoma. The association between the production of NOS-2 and glaucoma implies that pharmacologic agents that inhibit NOS-2 may have therapeutic value. Two drugs that inhibit NOS-2 have been tested for their ability to treat experimental glaucoma in animal models. Aminoguanidine has been shown to reduce ganglion cell loss in a rat model of induced glaucoma induced by cautery of three episcleral vessels [52]. Similarly, L-N6-(1-iminoethyl)lysine 5-tetrazole amide, which is a prodrug of the NOS-2 inhibitor L-NIL, also prevents ganglion cell loss in the same rat model [53]. However, a recent study showed the inhibition of NOS did not confer protection for RGCs in a different rat model of glaucoma induced by hypertonic saline injection into the episcleral veins [54,55]. A clinical trial of aminoguanidine for the treatment of diabetic nephropathy is under way, [111] and L-N6-(1-iminoethyl)lysine 5-tetrazole amide has been safely used in human clinical trials for other conditions [112]. The efficacy of these or other inhibitors of NOS-2 in treating glaucoma has not yet been assessed.

Radiation and bone marrow transplantation

Epidemiologic and animal studies have provided evidence that exposure to radiation may confer protection from glaucomatous optic neuropathy. In one study of atomic bomb survivors, those who were exposed to radiation had a lower incidence of glaucoma [113]. The effect of gamma radiation on RGC death has been explored using experimental rat models (optic nerve crush and NMDA toxicity). Radiation was shown to provide only minimal beneficial effects on RGC death in these model systems [77]. More recently, the potential effects of high-dose gamma radiation on glaucoma have been investigated with studies of DBA/2J mice, which exhibit a pigmented form of glaucoma caused by mutations in the *Gpnmb* and *Tyrp1* genes [114]. In addition to classic signs of glaucoma (optic neuropathy and elevated IOP), DBA/2J mice also have defects in the normally immunosuppressive environment of the eye [115]. High-dose gamma radiation and bone marrow transplantation were later shown to mitigate the glaucoma phenotype in DBA/2J mice, suggesting that cell-mediated immunity makes a contribution to the development of glaucoma in these animals [78]. These findings suggest that interventions targeted to the cell-mediated immune system may have a role in glaucoma therapy.

Immunologic vaccine

Animal studies of optic nerve injury and glutamate toxicity have suggested that regulation of the inflammatory response is important to promote injury repair and to minimize secondary nerve damage. One element of the immune response to injury is the localization of T lymphocytes to damaged neural tissue [116]. A subset of these T lymphocytes, which have receptors specific to proteins of the myelin sheath, such as MBP, have been shown to have protective effects on ganglion cell death in mouse models of optic nerve injury [117]. Similarly, direct immunization with proteins of the myelin sheath has also been shown to have neuroprotective effects in other animal model systems [118]. These experiments suggest that a vaccine based on myelin sheath antigens might have therapeutic value for treating optic nerve damage and possibly glaucoma. However, the potential benefits of these interventions were greatly overshadowed by significant untoward side effects. MBP immunization and T cells specific for MBP induce a severe paralytic condition known as EAE, which has some similarities to multiple sclerosis.

A vaccine, known as COP-1, was developed based on a synthetic peptide with similarities to MBP. COP-1 has neuroprotective effects in animal models of optic nerve damage and does not induce EAE. Vaccination with COP-1 has been studied as potential therapy for multiple sclerosis in numerous animal models and in human trials, and it appears to have clinical benefit and an excellent safety profile [119]. COP-1 immunization has also been explored as a glaucoma therapy and has been shown to reduce ganglion cell death in rat models of optic nerve damage, including optic nerve crush and laser-induced ocular hypertension [65,71]. The results of these studies suggest that a COP-1 vaccine might have a role in glaucoma therapy.

Antiapoptotic therapy

Most of the disease processes thought to cause ganglion cell death in glaucoma converge into a common pathway involving apoptosis of RGCs. Apoptosis is tightly regulated by complex interactions among many proapoptotic and antiapoptotic factors [120,121]. The expression level of many apoptotic factors can be modulated to promote ganglion cell survival in optic nerve transection animal models. Overexpression of the antiapoptotic factor bcl-2 in transgenic mice has been shown to decrease RGC loss after optic nerve transection [122]. Similarly, intravitreal injection of inhibitors of proapoptotic caspases also reduces ganglion cell death in a mouse optic nerve transection model [123]. Down-regulation of proapoptotic factors (c-Jun and Apaf-1) using short interfering RNAs has been shown to reduce RGC death in the rat after optic nerve transection [124]. Finally, intravitreal injection of Bax-inhibiting peptide has been shown to limit RGC death in a rat optic nerve transection model [125]. Modifications in the regulation of apoptosis clearly promote RGC survival after optic nerve transection. Future investigations of the effects of these antiapoptotic interventions in animal models of optic nerve disease that more closely reflect glaucoma are needed to prove their therapeutic usefulness.

β -2 Adrenergic agonist

The selective α -2-adrenergic class of topical medications (including apraclonidine and brimonidine) are effective IOP-lowering drugs that have a major role in glaucoma treatment. These drugs lower IOP primarily by decreasing the production of aqueous humor [80]. In addition to its established usefulness in reducing IOP, brimonidine has been

investigated for IOP-independent neuroprotective activity.

The mechanism of proposed brimonidine neuroprotection is unclear. In one study of ischemic injury to the retina in a rat model, brimonidine was shown to lower glutamate concentrations in the vitreous humor. Based on these results, it has been suggested that brimonidine might be neuroprotective because it prevents glutamate-associated excitotoxicity [126]. Alternatively, it has been suggested that brimonidine may directly inhibit apoptotic pathways [127].

Studies using rat models of ocular disease have investigated the effects of brimonidine on RGC death. The effects of continuously administered subcutaneous brimonidine on ganglion cell loss were tested in a rat model of glaucoma in which IOP was elevated by laser photocoagulation of episcleral and limbal veins. Brimonidine administered subcutaneously did not cause a measured decrease in IOP but was found to reduce RGC death [128]. In other studies using a rat model of transient ischemic retinal injury, ganglion cell death appeared to be reduced by pretreatment with topical brimonidine [129–131]. In general it has been challenging to isolate the known beneficial effects of brimonidine on IOP from any other potential neuroprotective effects.

Ginkgo biloba extracts

Extracts from the leaves of the ginkgo biloba tree (including flavinoid glycosides and terpenes) have been sold as a dietary supplement for a range of potential beneficial effects, including improved memory. Ginkgo biloba has also been investigated as a drug to treat a wide variety of medical conditions, including vascular insufficiency and Alzheimer disease [132]. Although, many of the claims of the beneficial effects of ginkgo biloba have not been supported by rigorous scientific studies, evidence indicates that ginkgo biloba be useful for treating select diseases. For example, findings from one clinical trial have suggested that ginkgo biloba may be useful for treating dementia associated with Alzheimer disease [133,134]. The mechanism of action of the active components of ginkgo biloba is unknown. Studies have suggested, however, that ginkgo biloba influences several important biologic processes, including intracellular signaling and neutralizing reactive oxygen species [135].

Ginkgo biloba has been tested for potential neuroprotective activity using a rat model of chronic glaucoma. In this model, a moderate elevation of IOP is generated by cautery of episcleral and scleral vessels. Rats treated with ginkgo biloba were found

to have reduced RGC loss compared with control animals despite their having similar IOPs [136].

Ginkgo biloba extracts have also been evaluated as a possible therapy for patients with glaucoma. In one prospective clinical trial, ginkgo biloba extracts were found to have a beneficial effect on preexisting visual field defects in patients with normal tension glaucoma. After 4 weeks of treatment with ginkgo biloba, patients experienced significant improvement in visual field testing [137]. Although the basis of ginkgo biloba's effects on the visual field are unknown and possibly are related to improved cognitive function, the results of this study are encouraging and suggest that further studies are warranted.

Challenges ahead

Successful clinical application of one or more neuroprotective strategies outlined above depends on several factors: (1) the strategy has to have a rational scientific basis; (2) the neuroprotective agent must be delivered safely and efficiently to the site of damage; and (3) the efficacy and safety profile of the neuroprotective agent must be demonstrated in a randomized prospective clinical trial. For a chronic, slowly progressive disease such as glaucoma, proving clinical efficacy remains a challenge because it may take many years to detect significant benefit. Nonetheless, the goal of clinically significant optic nerve protection in glaucoma seems within reach.

Acknowledgments

This work was funded in part by Research to Prevent Blindness, New York, NY. Authors have no financial interest in the subject matter or in the materials discussed in this article.

References

- [1] Collaborative Normal-Tension Glaucoma Study Group. The effectiveness of intraocular pressure reduction in the treatment of normal-tension glaucoma: Collaborative Normal-Tension Glaucoma Study Group. *Am J Ophthalmol* 1998;126:498–505.
- [2] Collaborative Normal-Tension Glaucoma Study Group. Comparison of glaucomatous progression between untreated patients with normal tension glaucoma and patients with therapeutically reduced intraocular pressure: Collaborative Normal-Tension Glaucoma Study Group. *Am J Ophthalmol* 1998;126:487–97.
- [3] Hejil A, Leske MC, Bengtsson B, et al. Reduction of intraocular pressure and glaucoma progression: results from the early manifest glaucoma trial. *Arch Ophthalmol* 2002;120:1268–79.
- [4] Quigley HA, Addicks EM. Regional differences in the structure of the lamina cribrosa and their relation to glaucomatous optic nerve damage. *Arch Ophthalmol* 1981;99:137–43.
- [5] Quigley HA, Addicks EM, Green WR. Optic nerve damage in human glaucoma, III: quantitative correlation of nerve fiber loss and visual field defect in glaucoma, ischemic neuropathy, papilledema, and toxic neuropathy. *Arch Ophthalmol* 1982;100:135–46.
- [6] Hernandez MR. The optic nerve head in glaucoma: role of astrocytes in tissue remodeling. *Prog Retin Eye Res* 2000;19:297–321.
- [7] Johnson EC, Deppmeier LM, Wentzien SK, et al. Chronology of optic nerve head and retinal responses to elevated intraocular pressure. *Invest Ophthalmol Vis Sci* 2000;41:431–42.
- [8] Grozdanic SD, Kwon YH, Sakaguchi DS, et al. Functional evaluation of retina and optic nerve in the rat model of chronic ocular hypertension. *Exp Eye Res* 2004;79:75–83.
- [9] Grozdanic SD, Betts DM, Sakaguchi DS, et al. Laser-induced mouse model of chronic ocular hypertension. *Invest Ophthalmol Vis Sci* 2003;44:4337–46.
- [10] Harwerth RS, Crawford ML, Frishman LJ, et al. Visual field defects and neural losses from experimental glaucoma. *Prog Retin Eye Res* 2002;21:91–125.
- [11] Pena JD, Varela HJ, Ricard CS, et al. Enhanced tenascin expression associated with reactive astrocytes in human optic nerve heads with primary open angle glaucoma. *Exp Eye Res* 1999;68:29–40.
- [12] Ricard CS, Pena JD, Hernandez MR. Differential expression of neural cell adhesion molecule isoforms in normal and glaucomatous human optic nerve heads. *Brain Res Mol Brain Res* 1999;74(1–2):69–82.
- [13] Yan X, Tezel G, Wax MB, et al. Matrix metalloproteinases and tumor necrosis factor alpha in glaucomatous optic nerve head. *Arch Ophthalmol* 2000;118:666–73.
- [14] Agapova OA, Kaufman PL, Lucarelli MJ, et al. Differential expression of matrix metalloproteinases in monkey eyes with experimental glaucoma or optic nerve transection. *Brain Res* 2003;967(1–2):132–43.
- [15] Ricard CS, Kobayashi S, Pena JD, et al. Selective expression of neural cell adhesion molecule (NCAM)-180 in optic nerve head astrocytes exposed to elevated hydrostatic pressure in vitro. *Brain Res Mol Brain Res* 2000;81(1–2):62–79.
- [16] Hernandez MR, Pena JD. The optic nerve head in glaucomatous optic neuropathy. *Arch Ophthalmol* 1997;115:389–95.
- [17] Eddleston M, Mucke L. Molecular profile of reactive astrocytes—implications for their role in neurologic disease. *Neuroscience* 1993;54:15–36.

- [18] Ridet JL, Malhotra SK, Privat A, et al. Reactive astrocytes: cellular and molecular cues to biological function. *Trends Neurosci* 1997;20:570–7.
- [19] Hernandez MR, Pena JD, Selvidge JA, et al. Hydrostatic pressure stimulates synthesis of elastin in cultured optic nerve head astrocytes. *Glia* 2000;32:122–36.
- [20] Quigley HA, Nickells RW, Kerrigan LA, et al. Retinal ganglion cell death in experimental glaucoma and after axotomy occurs by apoptosis. *Invest Ophthalmol Vis Sci* 1995;36:774–86.
- [21] Garcia-Valenzuela E, Shareef S, Walsh J, et al. Programmed cell death of retinal ganglion cells during experimental glaucoma. *Exp Eye Res* 1995;61:33–44.
- [22] Kerrigan LA, Zack DJ, Quigley HA, et al. TUNEL-positive ganglion cells in human primary open-angle glaucoma. *Arch Ophthalmol* 1997;115:1031–5.
- [23] Okisaka S, Murakami A, Mizukawa A, et al. Apoptosis in retinal ganglion cell decrease in human glaucomatous eyes. *Jpn J Ophthalmol* 1997;41:84–8.
- [24] Pearson HE, Stoffer DJ. Retinal ganglion cell degeneration following loss of postsynaptic target neurons in the dorsal lateral geniculate nucleus of the adult cat. *Exp Neurol* 1992;116:163–71.
- [25] Schulz M, Raju T, Ralston G, et al. A retinal ganglion cell neurotrophic factor purified from the superior colliculus. *J Neurochem* 1990;55:832–41.
- [26] Hayreh SS, March W, Anderson DR. Pathogenesis of block of rapid orthograde axonal transport by elevated intraocular pressure. *Exp Eye Res* 1979;28:515–23.
- [27] Gaasterland D, Tanishima T, Kuwabara T. Axoplasmic flow during chronic experimental glaucoma, I: light and electron microscopic studies of the monkey optic nervehead during development of glaucomatous cupping. *Invest Ophthalmol Vis Sci* 1978;17:838–46.
- [28] Anderson DR, Hendrickson A. Effect of intraocular pressure on rapid axoplasmic transport in monkey optic nerve. *Invest Ophthalmol* 1974;13:771–83.
- [29] Quigley H, Anderson DR. The dynamics and location of axonal transport blockade by acute intraocular pressure elevation in primate optic nerve. *Invest Ophthalmol* 1976;15:606–16.
- [30] Quigley HA, McKinnon SJ, Zack DJ, et al. Retrograde axonal transport of bdnf in retinal ganglion cells is blocked by acute iop elevation in rats. *Invest Ophthalmol Vis Sci* 2000;41:3460–6.
- [31] Pease ME, McKinnon SJ, Quigley HA, et al. Obstructed axonal transport of BDNF and its receptor TRKB in experimental glaucoma. *Invest Ophthalmol Vis Sci* 2000;41:764–74.
- [32] Ko ML, Hu DN, Ritch R, et al. Patterns of retinal ganglion cell survival after brain-derived neurotrophic factor administration in hypertensive eyes of rats. *Neurosci Lett* 2001;305:139–42.
- [33] Martin KR, Quigley HA, Zack DJ, et al. Gene therapy with brain-derived neurotrophic factor as a protection: retinal ganglion cells in a rat glaucoma model. *Invest Ophthalmol Vis Sci* 2003;44:4357–65.
- [34] Ji JZ, Elyaman W, Yip HK, et al. CNTF promotes survival of retinal ganglion cells after induction of ocular hypertension in rats: the possible involvement of STAT3 pathway. *Eur J Neurosci* 2004;19:265–72.
- [35] van Adel BA, Kostic C, Deglon N, et al. Delivery of ciliary neurotrophic factor via lentiviral-mediated transfer protects axotomized retinal ganglion cells for an extended period of time. *Hum Gene Ther* 2003;14:103–15.
- [36] Flammer J, Orgul S, Costa VP, et al. The impact of ocular blood flow in glaucoma. *Prog Retin Eye Res* 2002;21:359–93.
- [37] Hayreh SS. The blood supply of the optic nerve head and the evaluation of it—myth and reality. *Prog Retin Eye Res* 2001;20:563–93.
- [38] Tielsch JM, Katz J, Sommer A, et al. Hypertension, perfusion pressure, and primary open-angle glaucoma: a population-based assessment. *Arch Ophthalmol* 1995;113:216–21.
- [39] Chauhan BC, LeVatte TL, Jollimore CA, et al. Model of endothelin-1-induced chronic optic neuropathy in rat. *Invest Ophthalmol Vis Sci* 2004;45:144–52.
- [40] Orgul S, Cioffi G, Bacon D, et al. An endothelin-1-induced model of chronic optic nerve ischemia in rhesus monkeys. *J Glaucoma* 1996;5:135–8.
- [41] Tezel G, Wax MB. Hypoxia-inducible factor 1alpha in the glaucomatous retina and optic nerve head. *Arch Ophthalmol* 2004;122:1348–56.
- [42] Wang X, Tay SS, Ng YK. An immunohistochemical study of neuronal and glial cell reactions in retinae of rats with experimental glaucoma. *Exp Brain Res* 2000;132:476–84.
- [43] Woldemussie E, Wijono M, Ruiz G. Müller cell response to laser-induced increase in intraocular pressure in rats. *Glia* 2004;47:109–19.
- [44] Martin KR, Levkovitch-Verbin H, Valenta D, et al. Retinal glutamate transporter changes in experimental glaucoma and after optic nerve transection in the rat. *Invest Ophthalmol Vis Sci* 2002;43:2236–43.
- [45] Tezel G, Wax MB. Increased production of tumor necrosis factor-alpha by glial cells exposed to simulated ischemia or elevated hydrostatic pressure induces apoptosis in cocultured retinal ganglion cells. *J Neurosci* 2000;20:8693–700.
- [46] Tezel G, Li LY, Patil RV, et al. TNF-alpha and TNF-alpha receptor-1 in the retina of normal and glaucomatous eyes. *Invest Ophthalmol Vis Sci* 2001;42:1787–94.
- [47] Morgan J, Caprioli J, Koseki Y. Nitric oxide mediates excitotoxic and anoxic damage in rat retinal ganglion cells cocultured with astroglia. *Arch Ophthalmol* 1999;117:1524–9.
- [48] Dawson VL, Dawson TM. Nitric oxide actions in neurochemistry. *Neurochem Int* 1996;29:97–110.
- [49] Shareef S, Sawada A, Neufeld AH. Isoforms of nitric oxide synthase in the optic nerves of rat eyes with

- chronic moderately elevated intraocular pressure. *Invest Ophthalmol Vis Sci* 1999;40:2884–91.
- [50] Siu AW, Leung MC, To CH, et al. Total retinal nitric oxide production is increased in intraocular pressure-elevated rats. *Exp Eye Res* 2002;75:401–6.
- [51] Liu B, Neufeld AH. Expression of nitric oxide synthase-2 (NOS-2) in reactive astrocytes of the human glaucomatous optic nerve head. *Glia* 2000;30:178–86.
- [52] Neufeld AH, Sawada A, Becker B. Inhibition of nitric-oxide synthase 2 by aminoguanidine provides neuroprotection of retinal ganglion cells in a rat model of chronic glaucoma. *Proc Natl Acad Sci U S A* 1999;96:9944–8.
- [53] Neufeld AH, Das S, Vora S, et al. A prodrug of a selective inhibitor of inducible nitric oxide synthase is neuroprotective in the rat model of glaucoma. *J Glaucoma* 2002;11:221–5.
- [54] Morrison JC, Johnson EC, Cepurna W, et al. Understanding mechanisms of pressure-induced optic nerve damage. *Prog Retin Eye Res* 2005;24:217–40.
- [55] Pang IH, Johnson EC, Jia L, et al. Evaluation of inducible nitric oxide synthase in glaucomatous optic neuropathy and pressure-induced optic nerve damage. *Invest Ophthalmol Vis Sci* 2005;46:1313–21.
- [56] Neufeld AH, Hernandez MR, Gonzalez M. Nitric oxide synthase in the human glaucomatous optic nerve head. *Arch Ophthalmol* 1997;115:497–503.
- [57] Caprioli J, Kitano S, Morgan JE. Hyperthermia and hypoxia increase tolerance of retinal ganglion cells to anoxia and excitotoxicity. *Invest Ophthalmol Vis Sci* 1996;37:2376–81.
- [58] Dreyer EB. A proposed role for excitotoxicity in glaucoma. *J Glaucoma* 1998;7:62–7.
- [59] Honkanen RA, Baruah S, Zimmerman MB, et al. Vitreous amino acid concentrations in patients with glaucoma undergoing vitrectomy. *Arch Ophthalmol* 2003;121:183–8.
- [60] Levkovitch-Verbin H, Martin KR, Quigley HA, et al. Measurement of amino acid levels in the vitreous humor of rats after chronic intraocular pressure elevation or optic nerve transection. *J Glaucoma* 2002;11:396–405.
- [61] Wamsley S, Gabelt BT, Dahl DB, et al. Vitreous glutamate concentration and axon loss in monkeys with experimental glaucoma. *Arch Ophthalmol* 2005;123:64–70.
- [62] Carter-Dawson L, Crawford ML, Harwerth RS, et al. Vitreal glutamate concentration in monkeys with experimental glaucoma. *Invest Ophthalmol Vis Sci* 2002;43:2633–7.
- [63] Ullian EM, Barkis WB, Chen S, et al. Invulnerability of retinal ganglion cells to NMDA excitotoxicity. *Mol Cell Neurosci* 2004;26:544–57.
- [64] Wax MB. Is there a role for the immune system in glaucomatous optic neuropathy? *Curr Opin Ophthalmol* 2000;11:145–50.
- [65] Schori H, Kipnis J, Yoles E, et al. Vaccination for protection of retinal ganglion cells against death from glutamate cytotoxicity and ocular hypertension: implications for glaucoma. *Proc Natl Acad Sci U S A* 2001;98:3398–403.
- [66] Maruyama I, Nakazawa M, Ohguro H. Autoimmune mechanisms in molecular pathology of glaucomatous optic neuropathy. *Nippon Ganka Gakkai Zasshi* 2001;105:205–12 [in Japanese].
- [67] Yang J, Tezel G, Patil RV, et al. Serum autoantibody against glutathione S-transferase in patients with glaucoma. *Invest Ophthalmol Vis Sci* 2001;42:1273–6.
- [68] Wax MB, Yang J, Tezel G. Serum autoantibodies in patients with glaucoma. *J Glaucoma* 2001;10(suppl 1):S22–4.
- [69] Grus FH, Joachim SC, Hoffmann EM, et al. Complex autoantibody repertoires in patients with glaucoma. *Mol Vis* 2004;10:132–7.
- [70] Tezel G, Edward DP, Wax MB. Serum autoantibodies to optic nerve head glycosaminoglycans in patients with glaucoma. *Arch Ophthalmol* 1999;117:917–24.
- [71] Kipnis J, Yoles E, Porat Z, et al. T cell immunity to copolymer 1 confers neuroprotection on the damaged optic nerve: possible therapy for optic neuropathies. *Proc Natl Acad Sci U S A* 2000;97:7446–51.
- [72] Schori H, Yoles E, Wheeler LA, et al. Immune-related mechanisms participating in resistance and susceptibility to glutamate toxicity. *Eur J Neurosci* 2002;16:557–64.
- [73] Teitelbaum D, Fridkis-Hareli M, Arnon R, et al. Copolymer 1 inhibits chronic relapsing experimental allergic encephalomyelitis induced by proteolipid protein (PLP) peptides in mice and interferes with PLP-specific T cell responses. *J Neuroimmunol* 1996;64:209–17.
- [74] Teitelbaum D, Meshorer A, Hirshfeld T, et al. Suppression of experimental allergic encephalomyelitis by a synthetic polypeptide. *Eur J Immunol* 1971;1:242–8.
- [75] Hauben E, Butovsky O, Nevo U, et al. Passive or active immunization with myelin basic protein promotes recovery from spinal cord contusion. *J Neurosci* 2000;20:6421–30.
- [76] Schwartz M. Vaccination for glaucoma: dream or reality? *Brain Res Bull* 2004;62:481–4.
- [77] Kipnis J, Avidan H, Markovich Y, et al. Low-dose gamma-irradiation promotes survival of injured neurons in the central nervous system via homeostasis-driven proliferation of T cells. *Eur J Neurosci* 2004;19:1191–8.
- [78] Anderson MG, Libby RT, Gould DB, et al. High-dose radiation with bone marrow transfer prevents neurodegeneration in an inherited glaucoma. *Proc Natl Acad Sci U S A* 2005;102:4566–71.
- [79] Kwon YH, Caprioli J. Primary open angle glaucoma. In: Tasman W, Jaeger EA, editors. *Duane's clinical ophthalmology*. Philadelphia: Lippincott Williams & Wilkins; 1999. p. 1–30.
- [80] Alward WL. Medical management of glaucoma. *N Engl J Med* 1998;339:1298–307.

- [81] Kitazawa Y, Shirai H, Go FJ. The effect of Ca^{2+} -antagonist on visual field in low-tension glaucoma. *Graefes Arch Clin Exp Ophthalmol* 1989; 227:408–12.
- [82] Netland PA, Chaturvedi N, Dreyer EB. Calcium channel blockers in the management of low-tension and open-angle glaucoma. *Am J Ophthalmol* 1993; 115:608–13.
- [83] Sawada A, Kitazawa Y, Yamamoto T, et al. Prevention of visual field defect progression with brovimine in eyes with normal-tension glaucoma. *Ophthalmology* 1996;103:283–8.
- [84] Bose S, Piltz JR, Breton ME. Nimodipine, a centrally active calcium antagonist, exerts a beneficial effect on contrast sensitivity in patients with normal-tension glaucoma and in control subjects. *Ophthalmology* 1995;102:1236–41.
- [85] Hayreh SS, Zimmerman MB, Podhajsky P, et al. Nocturnal arterial hypotension and its role in optic nerve head and ocular ischemic disorders. *Am J Ophthalmol* 1994;117:603–24.
- [86] Weibel D, Kreutzberg GW, Schwab ME. Brain-derived neurotrophic factor (BDNF) prevents lesion-induced axonal die-back in young rat optic nerve. *Brain Res* 1995;679:249–54.
- [87] Thanos S, Bahr M, Barde YA, Vanselow J. Survival and axonal elongation of adult rat retinal ganglion cells. *Eur J Neurosci* 1989;1:19–26.
- [88] Meyer-Franke A, Kaplan MR, Pfrieger FW, et al. Characterization of the signaling interactions that promote the survival and growth of developing retinal ganglion cells in culture. *Neuron* 1995;15:805–19.
- [89] Johnson JE, Barde YA, Schwab M, et al. Brain-derived neurotrophic factor supports the survival of cultured rat retinal ganglion cells. *J Neurosci* 1986;6: 3031–8.
- [90] DiStefano PS, Friedman B, Radziejewski C, et al. The neurotrophins BDNF, NT-3, and NGF display distinct patterns of retrograde axonal transport in peripheral and central neurons. *Neuron* 1992;8:983–93.
- [91] von Bartheld CS, Williams R, Lefcort F, et al. Retrograde transport of neurotrophins from the eye to the brain in chick embryos: roles of the P75NTR and TRKB receptors. *J Neurosci* 1996;16: 2995–3008.
- [92] Mansour-Robaey S, Clarke DB, Wang YC, et al. Effects of ocular injury and administration of brain-derived neurotrophic factor on survival and regrowth of axotomized retinal ganglion cells. *Proc Natl Acad Sci U S A* 1994;91:1632–6.
- [93] Di Polo A, Aigner LJ, Dunn RJ, et al. Prolonged delivery of brain-derived neurotrophic factor by adenovirus-infected Müller cells temporarily rescues injured retinal ganglion cells. *Proc Natl Acad Sci U S A* 1998;95:3978–83.
- [94] Choi DW. Glutamate neurotoxicity and diseases of the nervous system. *Neuron* 1988;1:623–34.
- [95] Vorwerk CK, Gorla MS, Dreyer EB. An experimental basis for implicating excitotoxicity in glaucomatous optic neuropathy. *Surv Ophthalmol* 1999;43(Suppl 1): S142–50.
- [96] Lucas DR, Newhouse JP. The toxic effect of sodium l-glutamate on the inner layers of the retina. *AMA Arch Ophthalmol* 1957;58:193–201.
- [97] Dreyer EB, Zurakowski D, Schumer RA, et al. Elevated glutamate levels in the vitreous body of humans and monkeys with glaucoma. *Arch Ophthalmol* 1996;114:299–305.
- [98] Vorwerk CK, Lipton SA, Zurakowski D, et al. Chronic low-dose glutamate is toxic to retinal ganglion cells: toxicity blocked by memantine. *Invest Ophthalmol Vis Sci* 1996;37:1618–24.
- [99] Vorwerk CK, Naskar R, Schuettauf F, et al. Depression of retinal glutamate transporter function leads to elevated intravitreal glutamate levels and ganglion cell death. *Invest Ophthalmol Vis Sci* 2000;41: 3615–21.
- [100] Reisberg B, Doody R, Stoffler A, et al. Memantine in moderate-to-severe Alzheimer's disease. *N Engl J Med* 2003;348:1333–41.
- [101] Schuettauf F, Quinto K, Naskar R, et al. Effects of anti-glaucoma medications on ganglion cell survival: the dba/2j mouse model. *Vision Res* 2002;42: 2333–7.
- [102] Hare WA, WoldeMussie E, Lai RK, et al. Efficacy and safety of memantine treatment for reduction of changes associated with experimental glaucoma in monkey, I: functional measures. *Invest Ophthalmol Vis Sci* 2004;45:2625–39.
- [103] Hare WA, WoldeMussie E, Weinreb RN, et al. Efficacy and safety of memantine treatment for reduction of changes associated with experimental glaucoma in monkey, II: structural measures. *Invest Ophthalmol Vis Sci* 2004;45:2640–51.
- [104] Nathan C, Xie QW. Nitric oxide synthases: roles, tolls, and controls. *Cell* 1994;78:915–8.
- [105] Wallace MN, Bisland SK. NADPH-diaphorase activity in activated astrocytes represents inducible nitric oxide synthase. *Neuroscience* 1994;59:905–19.
- [106] Yun HY, Dawson VL, Dawson TM. Neurobiology of nitric oxide. *Crit Rev Neurobiol* 1996;10:291–316.
- [107] Chao CC, Hu S, Molitor TW, et al. Activated microglia mediate neuronal cell injury via a nitric oxide mechanism. *J Immunol* 1992;149:2736–41.
- [108] Dawson VL, Dawson TM, Bartley DA, et al. Mechanisms of nitric oxide-mediated neurotoxicity in primary brain cultures. *J Neurosci* 1993;13: 2651–61.
- [109] Dawson VL, Brahmabhatt HP, Mong JA, et al. Expression of inducible nitric oxide synthase causes delayed neurotoxicity in primary mixed neuronal-glial cortical cultures. *Neuropharmacology* 1994;33: 1425–30.
- [110] Liu B, Neufeld AH. Nitric oxide synthase-2 in human optic nerve head astrocytes induced by elevated pressure in vitro. *Arch Ophthalmol* 2001;119:240–5.
- [111] Freedman B, Wuerth J, Cartwright K, et al. Design and baseline characteristics for the aminoguanidine

- clinical trial in overt type 2 diabetic nephropathy (action II). *Control Clin Trials* 1999;20:493–510.
- [112] Hansel TT, Kharitonov SA, Donnelly LE, et al. A selective inhibitor of inducible nitric oxide synthase inhibits exhaled breath nitric oxide in healthy volunteers and asthmatics. *FASEB J* 2003;17:1298–300.
- [113] Yamada M, Wong FL, Fujiwara S, et al. Noncancer disease incidence in atomic bomb survivors, 1958–1998. *Radiat Res* 2004;161:622–32.
- [114] Anderson MG, Smith RS, Hawes NL, et al. Mutations in genes encoding melanosomal proteins cause pigmentary glaucoma in DBA/2J mice. *Nat Genet* 2002;30:81–5.
- [115] Mo JS, Anderson MG, Gregory M, et al. By altering ocular immune privilege, bone marrow-derived cells pathogenically contribute to DBA/2j pigmentary glaucoma. *J Exp Med* 2003;197:1335–44.
- [116] Hirschberg DL, Moalem G, He J, et al. Accumulation of passively transferred primed t cells independently of their antigen specificity following central nervous system trauma. *J Neuroimmunol* 1998;89:88–96.
- [117] Moalem G, Leibowitz-Amit R, Yoles E, et al. Autoimmune T cells protect neurons from secondary degeneration after central nervous system axotomy. *Nat Med* 1999;5:49–55.
- [118] Fisher J, Levkovitch-Verbin H, Schori H, et al. Vaccination for neuroprotection in the mouse optic nerve: implications for optic neuropathies. *J Neurosci* 2001;21:136–42.
- [119] Bornstein MB, Miller A, Slagle S, et al. A pilot trial of cop 1 in exacerbating-remitting multiple sclerosis. *N Engl J Med* 1987;317:408–14.
- [120] Adams JM, Cory S. The bcl-2 protein family: arbiters of cell survival. *Science* 1998;281:1322–6.
- [121] Nickells RW. Retinal ganglion cell death in glaucoma: the how, the why, and the maybe. *J Glaucoma* 1996;5:345–56.
- [122] Bonfanti L, Strettoi E, Chierzi S, et al. Protection of retinal ganglion cells from natural and axotomy-induced cell death in neonatal transgenic mice overexpressing bcl-2. *J Neurosci* 1996;16:4186–94.
- [123] Chaudhary P, Ahmed F, Quebada P, et al. Caspase inhibitors block the retinal ganglion cell death following optic nerve transection. *Brain Res Mol Brain Res* 1999;67:36–45.
- [124] Lingor P, Koeberle P, Kugler S, et al. Down-regulation of apoptosis mediators by RNAi inhibits axotomy-induced retinal ganglion cell death in vivo. *Brain* 2005;128(part 3):550–8.
- [125] Qin Q, Patil K, Sharma SC. The role of Bax-inhibiting peptide in retinal ganglion cell apoptosis after optic nerve transection. *Neurosci Lett* 2004;372:17–21.
- [126] Donello JE, Padillo EU, Webster ML, et al. Alpha(2)-adrenoceptor agonists inhibit vitreal glutamate and aspartate accumulation and preserve retinal function after transient ischemia. *J Pharmacol Exp Ther* 2001;296:216–23.
- [127] Wheeler L, WoldeMussie E, Lai R. Role of alpha-2 agonists in neuroprotection. *Surv Ophthalmol* 2003;48(Suppl 1):S47–51.
- [128] WoldeMussie E, Ruiz G, Wijono M, et al. Neuroprotection of retinal ganglion cells by brimonidine in rats with laser-induced chronic ocular hypertension. *Invest Ophthalmol Vis Sci* 2001;42:2849–55.
- [129] Lafuente MP, Villegas-Perez MP, Sobrado-Calvo P, et al. Neuroprotective effects of alpha(2)-selective adrenergic agonists against ischemia-induced retinal ganglion cell death. *Invest Ophthalmol Vis Sci* 2001;42:2074–84.
- [130] Lafuente MP, Villegas-Perez MP, Mayor S, et al. Neuroprotective effects of brimonidine against transient ischemia-induced retinal ganglion cell death: a dose response in vivo study. *Exp Eye Res* 2002;74:181–9.
- [131] Aviles-Trigueros M, Mayor-Torroglosa S, Garcia-Aviles A, et al. Transient ischemia of the retina results in massive degeneration of the retinotectal projection: long-term neuroprotection with brimonidine. *Exp Neurol* 2003;184:767–77.
- [132] Kleijnen J, Knipschild P. Ginkgo biloba for cerebral insufficiency. *Br J Clin Pharmacol* 1992;34:352–8.
- [133] Kleijnen J, Knipschild P. Ginkgo biloba. *Lancet* 1992;340:1136–9.
- [134] Le Bars PL, Katz MM, Berman N, et al. A placebo-controlled, double-blind, randomized trial of an extract of ginkgo biloba for dementia: North American EGB Study Group. *JAMA* 1997;278:1327–32.
- [135] Marcocci L, Packer L, Droy-Lefaix MT, et al. Antioxidant action of ginkgo biloba extract EGB 761. *Methods Enzymol* 1994;234:462–75.
- [136] Hirooka K, Tokuda M, Miyamoto O, et al. The ginkgo biloba extract (EGB 761) provides a neuroprotective effect on retinal ganglion cells in a rat model of chronic glaucoma. *Curr Eye Res* 2004;28:153–7.
- [137] Quaranta L, Bettelli S, Uva MG, et al. Effect of ginkgo biloba extract on preexisting visual field damage in normal tension glaucoma. *Ophthalmology* 2003;110:359–62.