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Neuroprotection in Glaucoma: Animal Models and Clinical Trials

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Abstract

Glaucoma is a progressive neurodegenerative disease that frequently results in irreversible blindness. Glaucoma causes death of retinal ganglion cells (RGCs) and their axons in the optic nerve, resulting in visual field deficits and eventual loss of visual acuity. Glaucoma is a complex optic neuropathy, and a successful strategy for its treatment requires not only better management of known risk factors such as elevated intraocular pressure and the development of improved tools for detecting RGC injury but also treatments that address this injury (i.e., neuroprotection). Experimental models of glaucoma provide insight into the cellular and molecular mechanisms of glaucomatous optic neuropathy and aid the development of neuroprotective therapies.

1. RATIONALE FOR NEUROPROTECTION IN GLAUCOMA

1.1. Glaucoma Is an Optic Neuropathy with Key Pathophysiological Changes

Glaucoma is the most common irreversible cause of blindness in the world and the second most common in many developed countries (Quigley 2005). Although initially thought to be simply a disease of elevated intraocular pressure (IOP), it is now recognized as a primary optic neuropathy, with key pathophysiological changes that distinguish it from other optic neuropathies (Ghaffarieh & Levin 2012). These include a specific morphological change of the optic nerve head (optic disc), where so-called cupping or excavation is observed (Burgoyne 2015a). This is different from all other optic neuropathies, where there is disk pallor, with or without excavation (**Figure 1*a–c***). Another differentiating feature of glaucoma relates to the visual field defects, which are typically those associated with loss of groups of retinal ganglion cell (RGC) axons in the nerve fiber layer of the retina (**Figure 1*d*,*e***) (Johnson et al. 2000). These correspond to damage occurring along the border of the disk, which affects nerve fiber bundles. Although this is seen in glaucoma, it can also be seen in other optic nerve diseases, where the damage occurs at the same location as glaucoma (i.e., at the optic nerve head). At the optic-nerve-head level, glaucoma causes disorganization of laminar beams in the connective tissue and deposition of extracellular matrix materials, with appearance

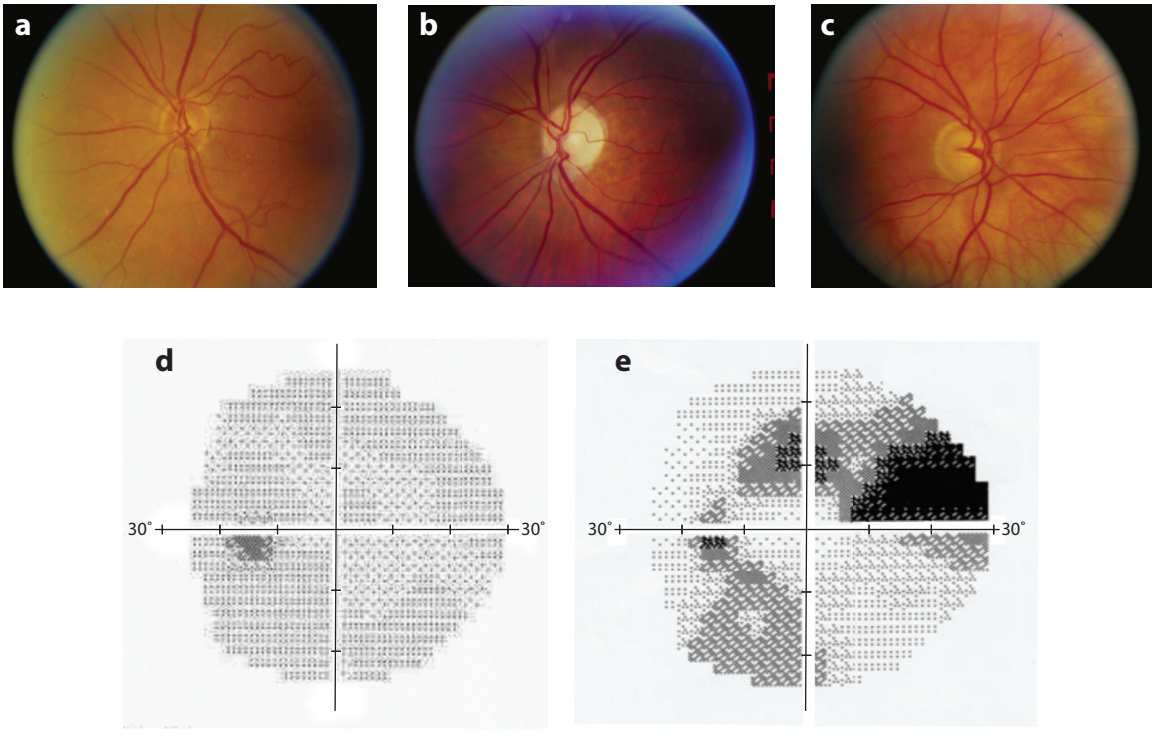

Figure 1

Morphological features of the glaucomatous optic nerve head and loss of visual field. (*a*) Normal optic nerve head. (*b*) Nonglaucomatous optic atrophy, characterized by pallor of the optic disk. (*c*) Glaucomatous optic disk, with typical cupping of the optic nerve head. (*d*) Normal visual field from a healthy eye. The dark area on the left corresponds to the physiological blind spot. (*e*) An abnormal visual field from a patient with glaucoma. The visual defects (*dark areas*) are mostly peripheral early in the disease and can go on to affect central vision with progression.

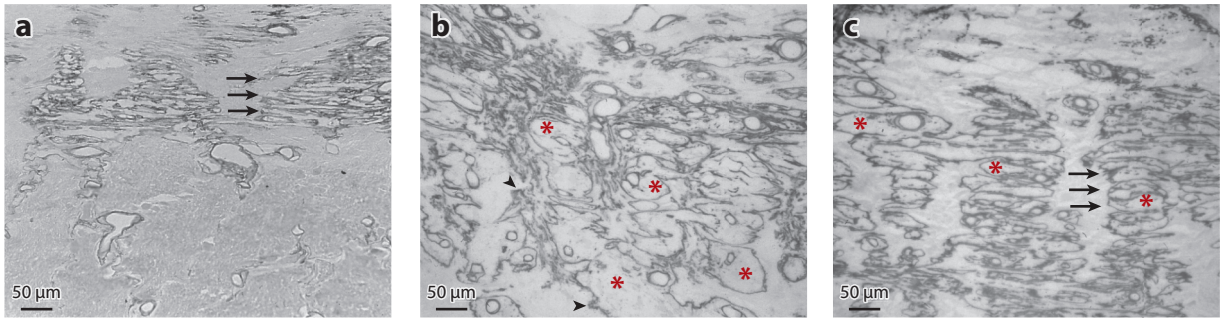

Figure 2

Different patterns of optic-nerve-head structure in nonglaucomatous and glaucomatous optic atrophy. (*a*) Normal pattern of collagen IV in a longitudinal transection of an intact optic nerve head, with organized laminar beams (*arrows*), of a cynomolgus monkey. (*b*) Chronic elevation of intraocular pressure in a glaucomatous eye results in thickening of laminar beam membranes (*arrowheads*) and deposition of extracellular matrix (ECM) materials (*red asterisks*). (*c*) Optic nerve transection also results in increased labeling of membranes and deposition of ECM; however, the laminar beams keep their organization (*arrows*). Figure (adapted) courtesy of John Morrison, MD, and Harry Quigley, MD.

different from that of nonglaucomatous optic neuropathy (**Figure 2**). A final set of differences between glaucoma and other optic neuropathies is that the loss of visual acuity and color vision occurs late in the disease because the earliest affected fibers are those that supply the peripheral retina. Recent studies have shown that fibers from RGCs within the macula are also affected (Hood et al. 2011, Lee et al. 2017, Wang et al. 2015); however, the vast majority of patients with glaucoma have visual acuity and color vision loss late in the disease. Although late loss of central vision is seen in glaucoma, it can also be seen in other anterior optic neuropathies (e.g., papilledema optic neuropathy). Glaucoma shares with other optic neuropathies the pathophysiological feature that damage occurs primarily to axons and not to the cell body (Vrabec & Levin 2007). In other words, it is an axonal damage–mediated disease, shared by all other primary optic neuropathies.

1.2. Current Treatments Used for Glaucoma and Evidence for Their Efficacy

The current treatments used for glaucoma solely target the lowering of intraocular pressure (**Table 1**). High-quality randomized control trials have shown that lowering IOP can decrease the rate of progression of visual-field damage in glaucoma. Analysis of studies such as the Early Manifest Glaucoma Trial (Heijl et al. 2002) and the United Kingdom Glaucoma Treatment Study (Garway-Heath et al. 2013) have also shown that the effects of lowering IOP are beneficial for patients who have elevated pressure, as well as for those with pressures in the "normal" range. In other words, lowering IOP for glaucoma is helpful despite the beginning level of pressure. Finally, lowering IOP in those with high pressure but no evidence of glaucoma is also successful in decreasing the likelihood of conversion to manifest glaucoma (Kass et al. 2002).

1.3. People Go Blind from Glaucoma, Even with Treatment

Unfortunately, people continue to go blind from glaucoma, despite treatment. It is undeniable that many of these patients go blind because of issues regarding compliance with or adherence to therapy, lack of surveillance detecting their disease, inadequate treatment, or adverse effects associated with the treatment, such as infection following filtering surgery to lower the pressure. However, it is also the case that patients can go blind despite highly efficacious lowering of the

Table 1 Intraocular pressure–lowering medications currently used for the treatment of glaucoma

Drug name	Administration	Pharmacological action	Adverse effects
Apraclonidine HCl Brimonidine tartrate	Topical	α_2-Adrenergic agonists[a]	Eye discomfort with redness, itching, and burning; blurred vision; dizziness; drowsiness; tiredness; upset stomach; dry mouth; dry nose; bad taste in mouth
Timolol maleate Betaxolol HCl Levobunolol HCl Metipranolol	Topical	β-Adrenergic antagonists[b]	Blurred vision; burning, stinging, itching and redness of eye; watery eyes; headache; trouble sleeping; dizziness or drowsiness
Brinzolamide Dorzolamide HCl	Topical	Carbonic anhydrase inhibitors[c]	Blurred vision, itching and redness of eye, bitter or sour taste in mouth, dry eyes, headache, sensitivity of eyes to light
Methazolamide Acetazolamide	Oral	Carbonic anhydrase inhibitors	Blurred vision, headache, nausea, loss of appetite, vomiting, diarrhea, frequent urination, dizziness, drowsiness, tiredness, lightheadedness, upset stomach, dry mouth
Pilocarpine HCl Carbachol	Topical	Cholinergic agonists[d]	Irritation, burning, redness, swelling, or stinging of eye; blurred vision and poor vision in dim light; headache; increased tearing; nearsightedness
Travaprost Bimatoprost Tafluprost Latanoprost	Topical	Prostaglandin $F_{2\alpha}$ analogs[e]	Iris discoloration; blurred vision; itching and redness of eye; dry eyes; tearing; eyelid crusting; increase in eyelash number, length, or thickness; darkening of the eyelashes and eyelids; increased sensitivity to light; muscle or joint pain

[a] α_2-Adrenergic agonists activate receptors that are responsible for induction of smooth muscle contraction and vasoconstriction. Topical application of α_2 agonist drugs to the eye lowers intraocular pressure (IOP) by decreasing the production of aqueous through vasoconstriction and reduced blood flow to the ciliary body and by reducing the resistance to aqueous outflow.

[b] β-Adrenergic antagonists decrease IOP by blocking the sympathetic nerve endings of ciliary epithelium in the ciliary body, resulting in reduction of aqueous humor production.

[c] Carbonic anhydrase is an enzyme present in the ciliary epithelial cells and catalyzes conversion of CO_2 and H_2O to HCO_3 and H^+, necessary for producing aqueous humor. Carbonic anhydrase inhibitors reduce IOP by blocking this enzyme and reduce aqueous humor production.

[d] Cholinergic agonists are miotic agents where the muscarinic action causes pupillary constriction. They lower IOP by increasing aqueous humor outflow through the trabecular meshwork.

[e] Prostaglandin analog treatment lowers IOP mainly by facilitating uveoscleral outflow. It has been suggested they do so by inducing the activity of matrix metalloproteinases, resulting in remodeling of the extracellular matrix within the ciliary muscle and surrounding sclera, thereby increasing their permeability.

pressure. In some cases, they do not go blind from the disease but because the adverse effects associated with intensifying treatment limit the amount of pressure lowering that can be achieved. Studies such as those conducted by Oliver et al. (2002) and Hattenhauer et al. (1998) have shown that the rate of blindness from glaucoma despite treatment can approach 20% to 27% for one eye and 9% for both eyes.

1.4. Neuroprotection Could Help Prevent Visual Loss

Given that blindness occurs from glaucoma and that even pressure lowering is in some cases insufficient, it is of great interest to develop therapies that are independent of pressure lowering. Neuroprotection is the term used for therapies that are independent of IOP lowering and that

protect the RGC, the neuron that dies in glaucoma (Almasieh et al. 2012, Chang & Goldberg 2012, Danesh-Meyer 2011, Levin 2016). The rationale for neuroprotection is that the optic neuropathy of glaucoma involves axonal injury to the RGC, the cell that projects its axons to target areas within the brain and carries information for visual function. The presumption is that by maintaining the health of the RGC despite injury at the optic nerve head to its axon, visual function and structure can be preserved (Almasieh et al. 2012).

2. THE LOST IN TRANSLATION PROBLEM

In the past two decades, there have been many animal studies demonstrating the ability of various neuroprotective treatments to preserve RGCs and their function in animal models of glaucoma, including drugs (Chen et al. 2015, Foxton et al. 2013, Kimura et al. 2017, Salt et al. 2014, Stankowska et al. 2015, Van de Velde et al. 2015, White et al. 2015), immunotherapy (Russo et al. 2016, Von Thun Und Hohenstein-Blaul et al. 2016), preconditioning (Zhu et al. 2012), and other methods (Crowston et al. 2017). For the most part, these animal studies have been performed in rodents, but a variety of other animals have been utilized, including nonhuman primates. These have been recently reviewed (Almasieh et al. 2012, Danesh-Meyer 2011).

2.1. Previous Clinical Trials of Neuroprotection

The apparent success in animal models of neuroprotection for glaucoma has not translated to clinical trials. The most well-known translational failure is the Memantine in Patients with Chronic Glaucoma study (**https://clinicaltrials.gov/ct2/show/NCT00168350**), which was funded and overseen by Allergan, a pharmaceutical company. The trial studied more than 2,000 patients at multiple sites around the globe, using visual function measures as an outcome. Although there is little published on the details of the study and the final results have not been published, except for being printed in press releases, we know that the study failed to meet its primary endpoint in two different parallel studies that were performed. Allergan published some of the preclinical nonhuman primate and rat data that they used to support going into clinical trials, although the primate data were relatively weak (Hare et al. 2004a,b; WoldeMussie et al. 2002).

Another example of a clinical trial of neuroprotection glaucoma was the Low-Pressure Study of Glaucoma Treatment, which randomized subjects between brimonidine and timolol (Krupin et al. 2011). Brimonidine had been shown in multiple animal studies to be neuroprotective for RGCs and was the basis for performing this clinical study (Wheeler & WoldeMussie 2001, Yoles et al. 1999). All the subjects had pressures in the "normal" range but otherwise had glaucoma. The study decreased visual-field progression in the group that received brimonidine. However, issues with respect to the large number of dropouts in the group receiving brimonidine, low amounts of IOP reduction, and other concerns made it less clear that the clinical trial proved neuroprotective efficacy (Cordeiro & Levin 2011).

2.2. Common Features of Translational Failure

There are multiple neuroprotection clinical trials in neurology and neurosurgery that have failed to prove efficacy, and this has been reviewed elsewhere (Danesh-Meyer & Levin 2009, Hill 2007, Savitz 2007). The specific issues with respect to failure to demonstrate efficacy of neuroprotection in pivotal trials in stroke led to the Stroke Academic-Industry Roundtable (STAIR) recommendations, which focus on how preclinical research is carried out, how proofs of concept in clinical trials are designed, and other factors (Albers et al. 2001; Feuerstein et al. 2008; Fisher et al. 2007, 2009).

In general, there are multiple features common to translational failures, which have been reviewed (Levin & Danesh-Meyer 2010) and for which potential solutions have been suggested (Ergorul & Levin 2013). Often, a simple reason for failure is that the preclinical research is not well performed, for a variety of reasons. Studies are not always masked, the disease being modeled is not mimicked by the animal disease, or the outcome measures are unrelated to the clinical outcome seen in patients. Another clear-cut reason for translational failure is that the clinical trials themselves are poorly carried out or failed to account for the variability associated with a disease such as glaucoma. Another reason is that the stage of visual loss in a clinical trial is often very different from that being studied in the preclinical model. Rodents, for example, have life spans of up to two years, and, therefore, a human patient with long-standing glaucoma progresses over a much longer time than a rodent. The biomechanical forces in a rodent are very different from those in a human. Use of nonhuman primates theoretically should decrease the rate of translational failure, but because of the ethical and financial concerns related to doing large, nonhuman primate studies, these are less commonly used.

A more complex type of translational failure relates to a different set of issues. The first is the concept of chaos theory, in that some animal models are highly sensitive to small differences in design. For example, a study of the use of the inducible nitric oxide synthase inhibitor 2–aminoguanidine in rat glaucoma showed different results, depending on exactly how the IOP was raised in the rodent (Neufeld et al. 1999, Pang et al. 2005). If a small difference in the animal model leads to a great difference in the outcome, then it is apparent that translation to a human is even more of a jump and can lead to unpredictability. Another, more complex reason for translational failure is the spread of variability when research studies transition from animals to humans. The use of inbred animals, of the same age, gender, weight, stage of disease, and schedule of drug administration will decrease variability. In contrast, human subjects in clinical trials have a variety of ages, stages of disease, compliance with the use of the therapy, and performance on visual outcome measures, and drugs will have different pharmacokinetic pharmacodynamic profiles. It is, therefore, not surprising that this increased variability may make it far harder to detect differences in clinical trials, compared to preclinical studies.

2.3. What Can Be Done to Improve Translation?

There are several steps that can be taken to improve translation from laboratory results to clinical trials in glaucoma neuroprotection, many of which apply to translational research in general (Ergorul & Levin 2013). The problem of chaotic behavior in animal models can be addressed on the basis of theory of using ensembles of models, similar to what is done in weather prediction. Specifically, preclinical support should include more than one animal model, using different methods of inducing glaucoma, and different species of different ages. This will improve the likelihood that an observed neuroprotective effect of a therapy is likely to be translated to the clinic.

Procedural steps, such as randomization of trials within the laboratory, strict masking, and performing pivotal preclinical studies, are also useful. Pivotal laboratory studies are those where one commits the go/no-go decision on the basis of the results of a prespecified number and type of experiments. If the experiments do not achieve a significant result, one stops the development within the preclinical process rather than continuing to attempt to get positive results by doing more and more experiments. Although there is a possibility of a premature ending by chance, the pivotal experiment method avoids the possibility of a false-positive result based on multiple experiments reaching significance purely based on multiplicity. This approach is one that can be used only for hypothesis testing; exploratory and mechanistic studies necessarily are open-ended.

3. TARGETS FOR NEUROPROTECTION

3.1. Somata

Even before translational failure issues, there is the critical question of where the site of injury is and what is being protected. RGC somas are located in the retina. They connect to other neurons, receiving visual signals from bipolar cells via their dendrites and sending visual information to the brain by way of their axons, which form the optic nerve (**Figure 3**). Many studies of neuroprotection focus on soma survival (Kuehn et al. 2005, Levin 2001, Levin & Peeples 2008). Typically, soma survival is assessed by retrograde labeling or immunologically identifying RGCs and quantifying their numbers in an animal model of glaucoma after treatment with a neuroprotective agent (Almasieh et al. 2011, WoldeMussie et al. 2001). Soma neuroprotection is necessary but not sufficient to predict functional neuroprotection in subsequent human studies because the soma may survive, but if the axon is damaged or lost, then connectivity between the retina and rest of the central nervous system is impossible (Ghaffarieh & Levin 2012, Levin 2005, Levin & Peeples 2008).

An example of this important concept was the demonstration that the NMDA antagonist memantine blocked RGC excitotoxicity (Sucher et al. 1997). As mentioned earlier, axons of the RGCs are the primary site of injury in glaucoma, and this is followed by soma loss (Guo et al. 2005, Howell et al. 2007). If the soma is neuroprotected, then the loss of the axon will still result in a failure to preserve visual function, which can explain why memantine may have failed to be efficacious in two phase 3 clinical trials.

3.2. Axons

Loss of RGC axons occurs in glaucoma by multiple mechanisms (Fortune et al. 2016, Howell et al. 2013), making axonal protection the critical target for therapy. If a therapy protects axons against injury, then it is likely that the subsequent secondary effects from the soma may be avoided (Almasieh et al. 2010). If the axon itself can stay structurally and functionally active despite the pathophysiology of glaucoma, then this is most likely to translate to a human trial. In general, axonal protection is less studied, with much focus on either ischemic injury to the optic nerve (Mozaffarieh et al. 2008, Zhu et al. 2012) or correlates of the Wallerian-degeneration slow mutation (Beirowski et al. 2008, Zhu et al. 2013), which results in a slowed axonal degeneration after injury (Lorber et al. 2012). This area has been extensively reviewed (Howell et al. 2013, Nickells et al. 2012).

3.3. Dendrites

RGCs make many contacts with bipolar and horizontal cells through their dendrites, and thus dendritic changes would have a significant effect on visual function (Masland 2012). Several groups have demonstrated changes in the dendritic tree after axonal injury and, in particular, in animal models of glaucoma (Feng et al. 2013, Jakobs et al. 2005, Shou et al. 2003, Williams et al. 2013). Dendritic connectivity between the RGC and bipolar cells or amacrine cells is necessary for transmission of visual information transduced by photoreceptors to RGCs because they are the output neurons of the retina (Wässle 2004). Much less is known about what is necessary to preserve dendrites in the presence of axonal injury (Morquette & Di Polo 2008). Recent studies have started to explore the role of dendritic connectivity in glaucoma, as shown by the finding that suppression of the immune response with inhibitors of the complement cascade in a mouse glaucoma model prevents dendritic and synaptic loss (Williams et al. 2016). Mechanisms that maintain synaptic and dendritic contacts of RGCs such as the Rho family of GTPases, the mammalian target of rapamycin

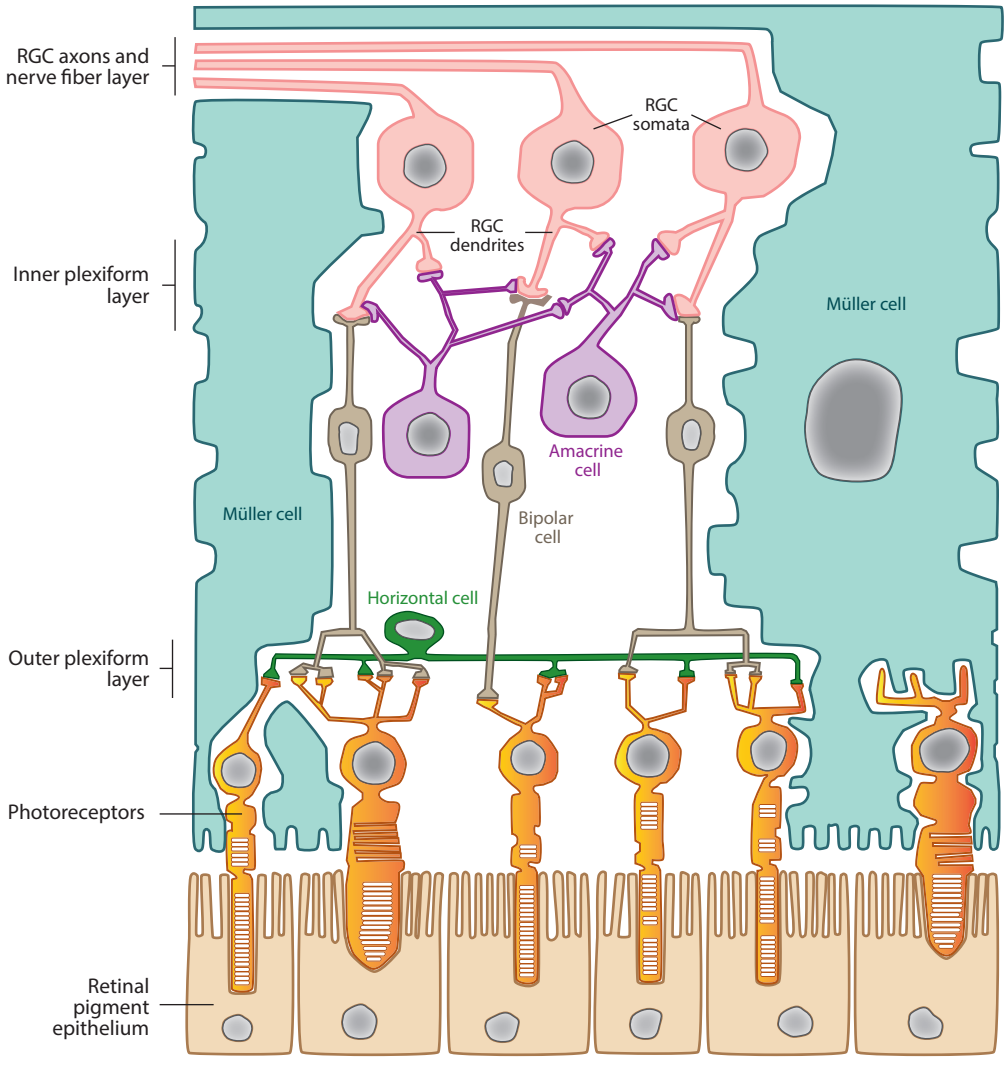

Figure 3

Schematic of the vertebrate retina demonstrating the principal cell types involved in visual signaling. Light-sensitive photoreceptors are adjacent to the retinal pigment epithelium and form the outermost neuronal cells of the retina. They transform light stimuli into electrical signaling. Photoreceptors make synaptic contact with bipolar cells in the outer plexiform layer, which in turn make synaptic contacts with retinal ganglion cell (RGC) dendrites in the inner plexiform layer. RGC axons in the retinal nerve fiber layer converge at the optic nerve head to exit the eye and form the optic nerve, which conducts visual signals to the brain. Müller cells are the principal glial cells in the retina and radially stretch across the thickness of the retina to support retinal neurons. Horizontal cells are retinal interneurons, primarily making synaptic contact with photoreceptors in the outer plexiform layer and forming lateral inhibitory feedback circuits. Amacrine cells are retinal interneurons that make inhibitory synaptic contacts with RGC dendrites and bipolar cells in the inner plexiform layer.

(mTOR) kinase, and other methods are compelling targets for studies of neuroprotection in glaucoma (Agostinone & Di Polo 2015, Binley et al. 2016).

3.4. Connectivity

Protecting the connectivity between the RGC and its targets in the brain is essential to maintaining visual function in disease. Work by several laboratories, including those of Yücel and Gupta (Yücel et al. 2006), have demonstrated changes in the lateral geniculate nucleus neurons in glaucoma. Presumably, this is largely secondary to the optic neuropathy of glaucoma, the primary site of injury (Danesh-Meyer & Levin 2015). Work from the Calkins laboratory in an experimental glaucoma model has demonstrated changes along the most distal parts of the optic tracks at the superior colliculus (Crish & Calkins 2015), again showing the importance of distal central nervous system connections in the pathophysiology of glaucoma. It is unclear whether protection of the optic nerve itself will also prevent those secondary changes within the efferent neurons of the RGCs or whether both need to be protected separately (Dekeyster et al. 2015).

3.5. Glia

The last few years have demonstrated an increased interest in the role of glia, particularly astrocytes, in the pathophysiology of glaucoma. There have been two meetings of the Lasker/International Retinal Research Foundation's Initiative for Innovation in Vision Science on the role of glia in glaucoma (Dowling 2011). The reason for this interest is that changes in the optic nerve head in glaucoma reflect not just the loss of axons of RGCs but also reactive changes in astrocytes and the closely related lamina cribrosa cells (Tovar-Vidales et al. 2016), which have similarities and differences compared to other astrocytes in the central nervous system. Studies of the effect of stretch and other biomechanical injuries on optic-nerve-head glia are highly relevant to understanding the development of disease in glaucoma (Paula et al. 2016, Rogers et al. 2012, Wallace & O'Brien 2016). There are a variety of special mechanistic features of optic-nerve-head astrocytes that may explain some of the pathophysiology of glaucoma (Davis et al. 2014, Nguyen et al. 2011). It is possible that work in this area may help develop therapies for preventing changes in astrocytes at the optic nerve head that might otherwise lead to progression of glaucoma via effects on axons (Ju et al. 2015).

Another relevant type of glia is found in the retina (Vecino et al. 2016), particularly Müller cells. By releasing antioxidant agents and growth factors and transporting neurotransmitters, Müller glia play a significant role in protecting RGCs (Vecino et al. 2016). Müller cells are also thought to participate in NMDA-induced excitotoxic death of RGCs (Lebrun-Julien et al. 2009). Protection of retinal or optic-nerve-head astrocytes may be a potential alternative or additive therapy to neuroprotection (Kaja et al. 2015, Wang et al. 2014).

3.6. Inflammation

Inflammation is not a common clinical feature of glaucoma. However, retinal glia can release proinflammatory mediators that can facilitate RGC death, given the findings that intraocular application of drugs such as ibudilast (a cAMP phosphodiesterase inhibitor) (Cueva Vargas et al. 2016) or candesartan (an angiotensin II type 1 receptor antagonist) (Semba et al. 2014) significantly improved RGC survival in glaucoma by suppressing Müller-cell activation.

Other evidence suggests an autoimmune component in pathology of the glaucoma on the basis of the presence of autoantibodies against heat shock proteins and other retinal and optic nerve

proteins (Tezel & Wax 2004, Von Thun Und Hohenstein-Blaul et al. 2016, Wax & Tezel 2009). An increased level of proinflammatory cytokines (Gramlich et al. 2013b) can lead to neurodegeneration. Conversely, there is evidence of neuroprotection by autoantibodies in glaucoma (Gramlich et al. 2013a). These observations suggest that a successful treatment strategy for glaucoma should incorporate the modulation of glial-cell immune function.

3.7. Retinal Ganglion Cell Types

There are multiple types of RGCs, each with different physiologies, anatomies, functions, and/or response to injury (Della Santina & Ou 2017, Ou et al. 2016, Sanes & Masland 2015, Stone 2013). One of the most interesting is the intrinsically photosensitive RGCs, which contain melanopsin that enables them to be photoreceptive (Berson et al. 2002, Liao et al. 2016, Provencio et al. 2000). This is also relevant to the use of pupillometry as an objective measure of neuroprotection, discussed in Section 8.2. It is possible that protection of one type of RGC may be easier to achieve than another, and if the former is associated with visual function to a greater degree than the latter, then this may provide a strategy for developing RGC-subtype-specific therapies (Vidal-Sanz et al. 2015).

3.8. Amacrine Cells

It is possible that protection of other cell types within the retina besides the RGC may be necessary to maintain visual function. This is particularly important in regard to bipolar and amacrine cells because they make extensive connections with RGC dendritic arbors (Masland 2012). As mentioned earlier, there has been substantial evidence for the effects of glaucoma on loss of RGC dendritic connectivity, implying a need to extend neuroprotective efforts to that area (Agostinone & Di Polo 2015). For example, it was recently shown that glaucoma also leads to amacrine cell death, particularly the subtype that forms gap junctions with RGCs (Akopian et al. 2016).

4. ANIMAL MODELS OF GLAUCOMA

Rodents have been the mainstay of animal models of glaucoma for neuroprotection studies. However, recent years have demonstrated interest in either new models or new species for models. Given that elevated IOP is a major risk factor for the development of glaucoma, many animal models of glaucoma were developed around the concept that increasing the IOP in the animal eye would mimic the conditions in patients with glaucoma, even though it more accurately reproduces ocular hypertension. To accomplish IOP elevation, many models take advantage of our knowledge about the anatomical structures of the eye, in particular the elements involved in circulation of the aqueous humor. Aqueous is a clear fluid produced by the ciliary body that provides nutrition for the lens and removes metabolic waste. It flows into the anterior chamber through the pupil and, after filling the anterior chamber, drains through the area between the cornea and iris known as the iridocorneal angle (**Figure 4**). The aqueous flows through a sieve-like structure called the trabecular meshwork and then into Schlemm's canal. The aqueous is then collected into the limbal venous plexus via collector channels and finally flows into the episcleral veins to enter the blood circulation. Some aqueous drains through the uveoscleral pathway, passing through spaces in the ciliary muscle, sclera, and the connective tissue. Below, we review animal models of glaucoma in which blockade of the aqueous humor drainage system is induced by different techniques, or occurs spontaneously, to achieve IOP elevation. In all but the genetic models, glaucoma is usually

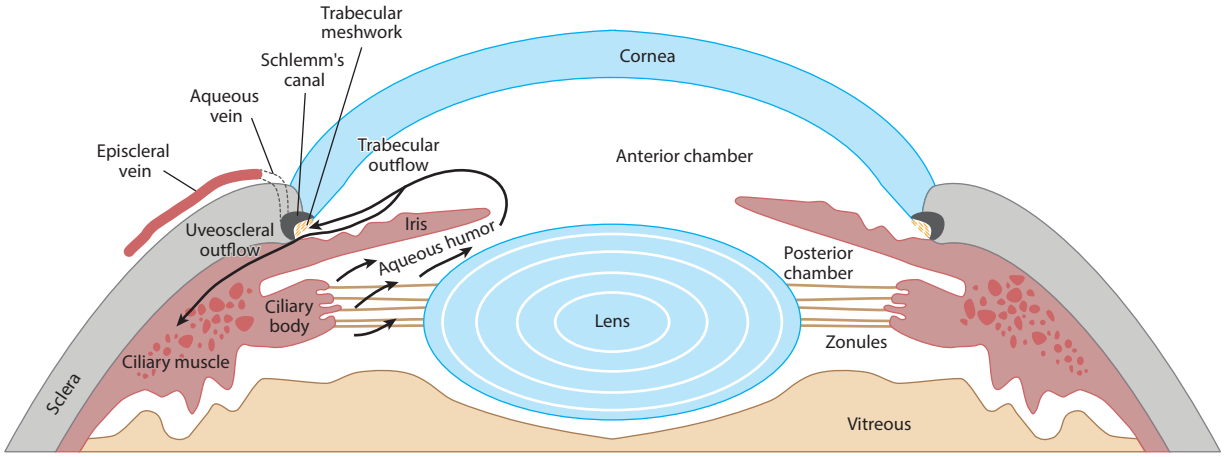

Figure 4

Schematic of the iridocorneal angle anatomy and aqueous humor circulation and drainage. Aqueous humor is produced by the ciliary body, entering the anterior chamber via the pupil, circulating in the anterior chamber, and then flowing toward the iridocorneal angle where it leaves the eye. There are two pathways for aqueous drainage. Trabecular outflow drains most aqueous through the sieve-like trabecular meshwork, located circumferentially at the angle between the cornea and iris. The aqueous then enters Schlemm's canal and is collected by aqueous veins to join the systemic circulation via episcleral veins. Uveoscleral outflow drains the aqueous through the extracellular space of muscle bundles within the ciliary body, then exits through the sclera to be collected by lymphatic vessels.

induced only in one eye of the animal, with the contralateral eye used as a control. This also avoids ethical issues with causing bilateral blindness in animals.

Several animal models of glaucoma that are based on increased IOP are summarized in **Table 2**. Here, we briefly review the most common models and methods for increasing IOP.

4.1. Rat Glaucoma Models

There are several experimental models of glaucoma in rats that reproduce several characteristics of primary open-angle glaucoma. The lamina cribrosa of the rat is a glial lamina somewhat dissimilar to that of primates or pigs.

4.1.1. Hypertonic saline ocular hypertension model. The Morrison model of rat ocular hypertension (OHT) is induced by retrograde injection of hypertonic saline solution into an episcleral vein, causing sclerotic damage to the trabecular meshwork cells and thereby disrupting the trabecular network's structure, resulting in gradual reduction of aqueous outflow (Morrison et al. 1997). The procedure results in gradual elevation of IOP in the operated eye, leading to optic-nerve-head cupping and degeneration of RGC axons in the optic nerve (Johnson et al. 1996, Morrison et al. 1997). Brown Norway rats are used for the Morrison model because their docile nature allows easy handling and daily IOP measurements without the need for general anesthetics, providing accurate IOP documentation. This is important because general anesthetic drugs can significantly decrease IOP and cause major variability in measured IOP values during the course of experiments (Jia et al. 2000). The equipment used in measurement of IOP is Tono-Pen and, more recently, TonoLab, although both instruments are capable of measuring IOP. However, Tono-Pen can more readily detect the smallest significant IOP elevations (Morrison et al. 2009). To avoid the circadian rhythm of IOP, the Brown Norway rats in the Morrison model are housed

Table 2 Commonly used animal models of glaucoma (all with elevated intraocular pressure)

Paradigm for induction of glaucoma	Species	Pros	Cons
Injection of hypertonic saline into episcleral veins	Rat	Reproducible and reliable model Ease of IOP measurements in Brown Norway rats Detectable ONH cupping	Requires high amount of skill to perform the procedure Limited availability of transgenic rats
Cauterization of episcleral veins	Rat	Easy to perform Detectable ONH cupping	Potential for early IOP spike Variability in the pattern and amount of RGC death
Laser photocoagulation of trabecular meshwork or limbus	Rat Mouse Rabbit Primates	Adaptable to multiple species High success rate of IOP elevation Possibility of genetic manipulation in mice	Retinal edema and hemorrhage may occur Variability of IOP magnitude and decline of IOP to baseline with time Primates: high cost, limited availability, and difficulties in working with animals Rabbits: difficulties associated with housing and working with animals
Injection of microbeads into anterior chamber	Rat Mouse Primates	Adaptable to multiple species Relatively simple procedure Possibility of genetic manipulation in mice	Repeated injection of beads often required to reach and maintain desirable IOP levels High variability in IOP magnitude Primates: high cost, limited availability, and difficulties in working with animals
Genetic models of glaucoma	Mouse Dog Cat Zebrafish	Spontaneous nature of glaucoma development, helpful for developmental studies Helpful for genetic studies Zebrafish: transparency of egg and embryos for imaging	Variability in the onset and progression among individual animals Confounding by the mechanism inducing the elevated IOP (e.g., pigmentary glaucoma in DBA/2J mice) Difficulties associated with housing and working with large animals or fish

Abbreviations: IOP, intraocular pressure; ONH, optic nerve head; RGC, retinal ganglion cell.

under continuous dim light (Moore et al. 1996, Morrison et al. 2005). Regardless, it is strongly recommended that IOP be measured at the same time each day. The Morrison model provides a reproducible and reliable model of experimental glaucoma, suitable for neuroprotection studies because the progressive loss of RGCs and their function has been well documented (Almasieh et al. 2010).

4.1.2. Microbead injection model. Another method for reducing aqueous outflow to elevate IOP based on obstruction of the trabecular meshwork is by injecting latex (Urcola et al. 2006) or polystyrene microbeads into the anterior chamber of rats (Sappington et al. 2010) and mice (Chen et al. 2011). Microbeads diffuse in the aqueous humor and move with its flow, migrating to cluster at the iridocorneal angle. This results in obstruction of outflow through the trabecular meshwork and a consequent increase in IOP (Sappington et al. 2010). Usually, it is necessary to repeat the injection of beads to reach and maintain a desirable IOP elevation. A newer version of this method uses magnetic beads, which after being injected into the anterior chamber are attracted to the iridocorneal angle by the use of a small handheld magnet. This method not only

facilitates occlusion of the trabecular meshwork but also quickly clears the beads from the pupillary zone, resulting in improved fundoscopy (Samsel et al. 2011).

4.1.3. Episcleral vein cauterization. Aqueous humor flows from limbal venous plexus into the episcleral veins and, from there, into the venous circulation. A method to reduce aqueous outflow is to cauterize a few of the episcleral veins with an ophthalmic cautery and thereby increase IOP (Shareef et al. 1995). A disadvantage is that there can be disruption of choroidal outflow if the vortex veins are cauterized.

4.1.4. Laser photocoagulation. In the laser photocoagulation model, light from a diode or similar laser is concentrated on the circumference of the trabecular meshwork, burning a series of spots and thereby reducing the aqueous outflow and causing OHT in rats (Levkovitch-Verbin et al. 2002).

4.2. Mouse Glaucoma Models

The wide availability of transgenic mice provides a significant advantage to studying mechanisms of glaucoma with this species, because it allows study of the role of genetic influences in the development of glaucoma. However, the mouse optic nerve head's lamina cribrosa is even less similar to the human's than the rat's (May & Lutjen-Drecoll 2002), making it somewhat problematic for modeling the biomechanics of axon injury. Nonetheless, the elevated IOP in this model leads to significant loss of RGC axons (Mabuchi et al. 2003). As mentioned previously, the Tono-Pen and TonoLab are the instruments used in measurement of IOP in experimental glaucoma. Because of the small size of the mouse eye, the TonoLab is the more appropriate instrument for IOP measurements in mice (Pease et al. 2011).

4.2.1. Laser photocoagulation. A model of primary angle-closure glaucoma (PACG) has been adapted for mice by inducing the closure of the anterior chamber angle with laser photocoagulation (Aihara et al. 2003). A diode laser is used to fuse the iris root to the peripheral cornea by focusing the laser beam on the corneal limbus to burn several spots. This results in reduction of aqueous outflow and increased IOP. Compared to the rat, the smaller eye of the mouse can cause difficulty in laser placement, sometimes resulting in complications.

4.2.2. Microbead injection model. Similar to injection into the rat, injection of microbeads into the mouse anterior chamber can be used to induce glaucoma in the eye (Sappington et al. 2010). Using magnetic microbeads increases the efficiency of this method for mice.

4.2.3. Spontaneous mouse glaucoma. A hereditary mutation in the DBA/2J mouse gradually results in a pigment dispersion–like syndrome, with increased IOP over several months, that eventually reaches a high enough level to model glaucoma (John et al. 1998). Adhesion of the iris to the cornea (anterior synechiae) results from chronic inflammation. A major advantage of this model is the ability to cross DBA/2J mice with other strains and thereby precisely dissect genetic pathways. A problem with this model is the variability in the onset and progression of glaucoma among individual animals, necessitating large groups of animals for each set of experiments.

4.3. Nonhuman Primate Glaucoma Models

Nonhuman primate models best mimic human subjects and therefore are theoretically most predictive for extrapolation to subsequent clinical trials. However, nonhuman primates such as

cynomolgus or macaque monkeys are large, making their use in preclinical models difficult. There has been much recent interest in using the marmoset, which has an average height of 185 mm and weight of 256 grams, yet has an eye structure similar to other primates (Shimazawa et al. 2013). Overall, a nonhuman primate model of glaucoma provides other advantages, such as a greater opportunity for studying the central effects of disease, including responses in central visual centers, but these are offset by high costs, variability (compared to inbred animals), and difficulty in working with this highly intelligent species.

4.3.1. Laser photocoagulation. The most commonly performed experimental primate model of glaucoma is conducted by laser photocoagulation of the trabecular meshwork. Rhesus or cynomolgus monkeys undergo laser photocoagulation—similar to what was described for rats—to burn spots on the circumference of the trabecular meshwork, leading to development of OHT and the morphological and functional changes associated with glaucoma (Burgoyne 2015b, Harwerth et al. 1999, Wang et al. 1998).

4.3.2. Microbead injection model. The injection of sterile latex microspheres into the anterior chamber to create OHT was first performed on monkey eyes and subsequently used on other species (Weber & Zelenak 2001). It is less commonly used, compared to the laser photocoagulation model.

4.4. Glaucoma Models in Other Mammals

Many breeds of dogs are prone to naturally develop glaucoma (Reinstein et al. 2009). The high prevalence of glaucoma among some purebred dogs indicates the likelihood of a genetic basis for this increased frequency (Gelatt & MacKay 2004). A well-known model of hereditary PACG occurs in basset hounds. It is characterized by the narrowing and collapse of the iridocorneal angle, and usually leads to angle closure with high IOP in the dogs by 20 months of age (Grozdanic et al. 2010). Glaucoma has been studied with success in cats (McLellan & Miller 2011), rabbits (Galassi et al. 2006), and pigs (Ruiz-Ederra et al. 2005).

4.5. Nonmammalian Glaucoma Models

Although their anatomy is very different than that of the human, nonmammalian models such as zebrafish can be used for glaucoma studies. Despite this difference, which makes translational research less directly relevant, the advantage with these animals is they facilitate sophisticated genetic studies, including knocking in, knocking down, and overexpressing various genes (Chhetri et al. 2014).

4.6. Glaucoma Models Without Elevated Intraocular Pressure

Models where the IOP is not elevated are highly relevant. As mentioned previously, glaucoma is not necessarily a disease of elevated IOP but, rather, a disease where lowering IOP decreases the rate of progression. Using models of optic neuropathies is relevant to understanding how axonal injury can be prevented or ameliorated. Such models include transection or crush of the optic nerve; ischemic optic neuropathy, which can be done in a rat (Bernstein et al. 2003), mouse (Song et al. 2003), or monkey (Cioffi 2005); and optic neuritis (similar to experimental autoimmune encephalomyelitis) (Shindler et al. 2008).

5. DRUG DELIVERY

5.1. Routes of Drug Delivery

Achieving adequate levels of drug at the target is necessary for a therapy to be useful. Although topically applied (eye-drop), IOP-reducing drugs have been proven effective for decreasing progression, there is also the problem of patient adherence (Sleath et al. 2011). Systemic administration can provide a more reliable delivery route, but the major hurdle for systemic delivery of neuroprotective drugs with large molecules is the blood–brain barrier. Intravitreal administration by injecting the drug into the vitreous has the benefit of rapid delivery of high levels of medication to the posterior eye. Repeated injections over a long period have the potential of causing endophthalmitis, retinal detachment, or other complications. However, recent experience in patients with neovascular macular degeneration treated with repeated injections of drugs affecting vascular endothelial growth factor signaling has shown very low risk of injection-related complications (Merani & Hunyor 2015).

To overcome the limitations of these methods, efforts have been made to improve existing methods for more effective drug delivery systems. For example, to improve the efficiency of topical delivery methods, drugs have been packed in liposomes (Mishra et al. 2011) or in soaked hydrogel contact lenses, used for slow release of IOP-lowering drugs (Schultz et al. 2009).

5.2. Sustained-Release Delivery

Glaucoma is a chronic disease, and therapy needs to be given for months to years, making daily administration of a drug less convenient. There has thus been a great deal of interest in sustained-release formulations of neuroprotective drugs (Rathod et al. 2017, Zhao et al. 2017). Use of surgical implants for transscleral drug delivery is an efficient way to achieve sustained and long-term release of neuroprotective drugs to the retina (Kompella et al. 2010). Periocular routes, such as subconjunctival, subtenon, retrobulbar, and peribulbar routes, take advantage of scleral permeability (Raghava et al. 2004). This method has been used for sustained delivery of human ciliary neurotrophic factor (CNTF) in experimental retinal degeneration (Thanos et al. 2004). The same method was used for sustained delivery of CNTF for six months in a phase 1 human trial of retinal degeneration (Sieving et al. 2006). Many of these drug formulations utilize copolymers such as biodegradable poly(lactic-*co*-glycolic acid) (Makadia & Siegel 2011) or silicon dioxide (Hou et al. 2016) to achieve sustained release of drugs.

6. TRANSLATIONAL ASPECT OF ANIMAL MODELS

There is surprisingly little evidence for how well translation occurs in neuroprotection. There are large numbers of preclinical studies of neuroprotection but few well-designed and adequately powered clinical studies. Most clinical studies have had small numbers of patients, often with study designs that are poorly able to prove or disprove neuroprotection. Two examples of clinical trials with sufficient numbers of patients are the memantine and brimonidine studies, described in detail above. It is also difficult to interpret how the quality of translation occurred in these studies. The published preclinical data for memantine are sparse, with most of the published data based on in vitro RGC culture studies. For the Memantine in Patients with Glaucoma study, virtually no data about the clinical studies are available because the sponsor for the clinical studies did not release them. For brimonidine, which was supported by a wealth of published preclinical studies, there were issues with the clinical trial design.

Conversely, there are surprisingly fewer data on the effects of IOP reduction on RGC or optic nerve loss in experimental glaucoma. Given that the clinical data strongly support a role for IOP lowering in decreasing glaucoma progression, the lack of preclinical studies in this area could be addressed.

7. DESIGN OF CLINICAL TRIALS

7.1. Proof-of-Concept Studies

In an ideal world, a neuroprotective therapy would be tested in patients with glaucoma, assessing the effect of the therapy on progression rate. However, such trials are usually lengthy because of the relatively slow progression over time, and there is great interest in using other optic neuropathies as proof of concept for neuroprotection. This section briefly discusses some of the optic neuropathies that have been considered or used for proof-of-concept studies.

Optic neuritis is an inflammatory optic neuropathy, most frequently associated with the development of multiple sclerosis. Subjects with an acute attack of optic neuritis lose visual acuity, color vision, and visual field, with the latter most commonly being central loss. The visual loss is usually associated with a pain in eye movements. Visual acuity in optic neuritis usually returns after a few weeks, as does the visual field. Contrast sensitivity also recovers, although not to the same degree as does visual acuity.

The advantage of using optic neuritis for proof-of-concept studies of neuroprotection is that it is a common disease, especially in areas away from the equator, and the diagnosis is rarely in question. Optic neuritis is also associated with loss of the nerve fiber layer after the attack, which occurs despite recovery of vision. This structural endpoint is useful for proof-of-concept studies, although at present this is less relevant to regulatory agencies. The major disadvantage of using optic neuritis is that there is significant visual recovery in most patients. Subjects may therefore be hesitant to enroll if the therapy involves a drug with potential for severe adverse effects or a route of administration that patients dislike (e.g., intravitreal injections), because the hoped-for benefit is less apparent.

Nonarteritic anterior ischemic optic neuropathy (NAION) is an ischemic event of the anterior optic nerve, which usually occurs in older individuals. It is typically painless, associated with swelling of the optic nerve head, affects visual acuity and the visual field to varying degrees, and, unlike optic neuritis, does not usually recover. Because of the lack of recovery, it is easier to detect an effect of neuroprotective therapy in NAION. The incidence rate of NAION has recently been reevaluated using health delivery databases and has been reported to be as high as 82 per hundred thousand (Lee et al. 2011), although a later study demonstrated a much lower crude rate (Cestari et al. 2016).

There are a variety of other optic neuropathies that could be considered for proof-of-concept studies. Examples include Leber hereditary optic neuropathy (LHON), traumatic optic neuropathy, papilledema optic neuropathy, and compressive optic neuropathy. A study was performed to test neuroprotection with brimonidine in LHON (Newman et al. 2005). The rationale was the fact that patients who lose vision in one eye from this disease almost always lose vision in the other eye as well. A significant neuroprotective effect was not observed. Another approach is to look at the effects of a neuroprotective therapy on the visual outcome in LHON, and a prospective study was performed with idebenone (Klopstock et al. 2011). This study failed to meet its prespecified effect on the primary outcome measure, although effects on secondary measures were observed (Klopstock et al. 2013). Traumatic optic neuropathy is also rare, sometimes recovers

spontaneously, and has been studied in several trials using high-dose corticosteroids as a neuroprotective therapy. This has been called into question because high-dose corticosteroids were shown to be associated with increased mortality when given after trauma (Roberts et al. 2004).

Papilledema optic neuropathy is frequently seen with idiopathic intracranial hypertension (IIH), where there is visual loss associated with increased intracranial pressure. IIH most commonly affects young, obese women, although women of other ages and men can also have the disease. IIH is increasing in incidence in many areas of the world where there is a rise in obesity. Standard therapies lower the intracranial pressure and include carbonic anhydrase inhibitors, weight loss, optic nerve sheath fenestration, ventricular peritoneal shunts, and lumbar peritoneal shunts. These therapies clinically decrease the rate of progression, although randomized clinical trial evidence is only available for the carbonic anhydrase inhibitors (Wall et al. 2014). The improvement with standard therapies makes it difficult to conduct neuroprotective studies, given that subjects would normally be treated, thereby decreasing progression rates.

Compressive optic neuropathy can be untreatable when a mass cannot be removed, radiated, or treated with chemotherapy. If a clear baseline rate of progression can be established in subjects with untreatable disease from a compressive optic neuropathy, then a neuroprotective study could be performed, using each subject's progression rate as a baseline. However, the variability of progression and the relatively uncommon prevalence of such patients make this optic neuropathy less preferable.

7.2. Endpoints

A clinical trial is designed with a primary endpoint, usually at a fixed time after randomization, with a measure that is believed to be clinically meaningful. An example is the detection of significant progression of visual-field loss, measured using proprietary software such as Guided Progression Analysis or similar techniques. An endpoint for a clinical trial is not always patient centered, and finding statistical significance does not mean that a treatment would be appreciated by a patient. For regulatory reasons, as well as to improve the ability to analyze data from trials, endpoints should be clinically meaningful and ideally have low variability.

Ideally, a clinical trial design defines endpoints that are highly correlated with patient-relevant outcomes. An example of a dissociation between an endpoint and a patient-relevant outcome is the use of a structural measure of glaucomatous optic neuropathy progression [e.g., thickness of the retinal nerve fiber layer determined by optical coherence tomography (OCT)]. From the point of view of trial design, the measurement variability of the nerve fiber layer is usually low, especially if attention is paid to ensure that the signal-to-noise ratio of the OCT is high and that the technology for segmenting the retinal layers is robust. The retinal nerve fiber layer primarily consists of axons of RGCs, the cells that die in glaucoma, and therefore, its thinning over time reflects that anatomical sequela of glaucomatous optic neuropathy.

But although a structural measure such as retinal nerve fiber layer thickness may have advantages, patients are unaware of its thickness unless told by their physician, because it is not directly correlated with visual function. Regulatory agencies have made clear that until the correlation between a structural endpoint and a functional endpoint is very high, structural endpoints are not themselves enough to qualify as an endpoint for a phase 3 trial leading to approval of a therapy in glaucoma. In some diseases (e.g., retinal diseases such as geographic atrophy), there is an extremely high correlation between the visual-field defect (a functional measure) and the area of structurally evident loss of photoreceptors. In these cases, regulatory agencies have allowed a structural endpoint—namely, the area of geographic atrophy.

7.3. Comparison Groups

A randomized clinical trial has at least two groups, with at least one group receiving the new therapy. In the case of neuroprotection studies in glaucoma, the choice of comparison groups can be complex.

7.3.1. Putative neuroprotective drugs that also lower intraocular pressure. Neuroprotection therapies act independently of IOP lowering. Yet several drugs that have been proposed as clinically neuroprotective also have IOP-lowering effects. An example is brimonidine, which both lowers IOP and was shown in animal models to be neuroprotective (WoldeMussie et al. 2001). Clinical studies of brimonidine as a neuroprotective agent therefore require an equivalent amount of IOP lowering in the other arms of the studies. If this is not done, then the effects on progression from the drug may be ascribed to neuroprotection, when it is from IOP lowering. It is difficult to balance the amount of IOP lowering between arms when different drugs are used and even more difficult to balance the amount of IOP lowering over a 24-h cycle or other effects of the drug, such as those on ocular blood flow. To mitigate this problem, the neuroprotective drug can be delivered systemically or through some route that does lead to significant lowering of IOP.

7.3.2. Placebo arms in neuroprotection studies. If a drug is thought to be neuroprotective and does not lower IOP, then the comparison arms do not require IOP lowering. By contrast, it is questionable whether an arm with placebo alone should be used in patients who have progressive glaucoma. It has been considered ethically appropriate to do short-term studies of glaucoma with a placebo arm [e.g., the Early Manifest Glaucoma Trial (Heijl et al. 2002) and the United Kingdom Glaucoma Treatment Study (Garway-Heath et al. 2015)]. But these are of a short enough duration that the risk of visual loss is low and involves careful patient monitoring within the trial. As is discussed elsewhere, most experts in the field consider that a neuroprotective study in progressive glaucoma requires at least 12 months and possibly 18–24 months, a period of time during which a placebo arm is ethically problematic because of the risk of permanent visual loss.

7.4. Inclusion Criteria

Optimal design of a clinical trial for neuroprotection includes minimizing the number of subjects needed and the length of time over which the trial takes place yet maintaining the ability to detect a clinically meaningful difference. These design issues have recently been reviewed in detail (De Moraes et al. 2017), and some of the key points are presented here. The goal is not only to demonstrate a high effect size, which may be limited by the nature of the neuroprotective therapy, but also to decrease the variability of the response, enabling a greater power to detect differences. In other words, the signal-to-noise ratio should be made as high as possible.

7.4.1. Homogeneity and rate of progression. Given that most neuroprotection studies focus on progression of visual-field loss, the initial progression rates of the subjects should ideally be as similar as possible. The baseline rate of progression should also be high enough that the effect of the neuroprotective therapy on progression rate can be clinically meaningful. A subject progressing at 0.1 dB per year, whose rate is decreased by 50% with a neuroprotective therapy to 0.05 dB per year, has only a small change in absolute progression rate (i.e., 0.05 dB per year), which is not clinically meaningful. It will also be difficult to demonstrate statistically because of the variability of detecting these low rates of progression over short periods of time.

7.4.2. Subjects representative of population likely to be treated. Another criterion for inclusion is that subjects are representative of the population likely to be treated. Ideally, subjects have progression rates that are high enough to be detected in clinical trials of reasonable duration. Yet most patients with glaucoma do not have high progression rates. The ability to extrapolate from subjects with medium progression rates to a population with lower progression rates is necessary for a clinical trial of the former to be successfully used in a population of the latter. This extrapolation may break down when subjects with extremely high progression rates are used in a trial because they may differ pathophysiologically from the typical patient with glaucoma.

7.5. Analytical Techniques

Several new concepts for the design and analysis of data from neuroprotection studies have been proposed in the last few years. One concept was using futility analysis as a way of decreasing the number of subjects needed (Quigley 2012). In a futility analysis, usually performed early to midway in the trial, the data collected up to that point are analyzed. If it is clear that a treatment effect is unlikely to be seen by the end of the study, on the basis of a lack of effect thus far, the study is stopped. The benefit is in decreasing the time and expense of a study of a therapy that is unlikely to work. The disadvantage is that an incorrect (false negative) decision can be made because a lack of effect at an early time point does not perfectly predict a lack of effect when more subjects are enrolled. Another disadvantage is that the futility analysis, assuming an early look at the data, can slightly increase the number of subjects needed for the end of the study (i.e., spending alpha).

Futility analysis is a subset of the more general concept of adaptive trial design, where the nature of this trial is prospectively decided to change, depending upon data that are analyzed earlier in the stages of the study. Such approaches have been commonly used in other areas of medicine (e.g., oncology) and are welcomed by regulatory agencies, as long as the adaptive aspect is clearly defined prospectively. The use of adaptive trial designs would also be helpful in improving the efficiency of studies of neuroprotection. One approach is to plan interim analyses based on a structural measure (or other measure with low variability) to help in changing the number of subjects or other trial parameters. Single-cell RGC in vivo imaging (discussed below in Section 8.1) is an example of one such approach; however, any approach using an analysis not directly linked to a clinically meaningful outcome, but that is predictive of such an outcome, would increase the efficiency of an adaptive-design neuroprotective study.

7.5.1. Robust regression. A useful approach for analyzing effects on progression rates is robust regression, which is an improvement on linear or other standard regression techniques (Zhu et al. 2014, 2015). The advantage of these techniques is that they account for the variability associated with different stages of disease. Although not yet used in published clinical trials of drug therapy, their adoption will lead to decreased time and number of subjects needed for neuroprotective studies.

7.5.2. Larger visual-field stimuli. Automated visual fields are usually performed with Goldmann size III stimuli. Recent data have demonstrated that the much larger size V stimuli have a lower variability than size III stimuli. The biggest advantage of the size V stimulus is that at the lower range of sensitivity of the visual field (e.g., 10 dB or less), the size III stimulus is highly variable and the size V markedly less so (Wall et al. 1997). Clinical trials using size V stimuli in subjects with progressive glaucoma and areas of the visual field that are in this range would have lower variability, and therefore, almost certainly, the power of the trial to detect differences is greater

(Gardiner et al. 2015). The severely damaged areas can also be weighted less or excluded (Gardiner & Mansberger 2016).

8. BRIDGING THE TRANSLATIONAL DIVIDE

Previous sections have discussed the difficulties in translating preclinical studies of neuroprotection in glaucoma to successful clinical trials. The problem is not specific to neuroprotection, nor to glaucoma; translational research in general frequently fails because of a variety of problems discussed previously (Ergorul & Levin 2013, Levin 2016, Levin & Danesh-Meyer 2010). In this section, we discuss some of the strategies that can be used to mitigate these translational problems.

8.1. Biomarkers

One of the greatest difficulties in translational research is the significant jump from the laboratory to the clinic. The most noteworthy is the change in species, from a usually small laboratory animal that has low variability with respect to size, metabolism, anatomy, and physiology to the human, who has highly variable biological measures and will be at different stages of disease. In clinical trials, the variability usually means that large numbers of subjects must be enrolled in studies to statistically detect significant outcomes. This makes the translational process high risk.

One way to mitigate this risk is to use biomarkers that can serve as interim measures for assessing efficacy of the neuroprotective treatment. A biomarker can be relevant to a final outcome measure, as long as it can be assessed early and accurately during the trial. An example of such a biomarker is the study of the retinal nerve fiber layer, which may undergo detectable changes earlier than changes associated with visual fields because of the lower variability in the structural measure. Below, we discuss some novel biomarkers.

An exciting new biomarker on the horizon is the use of in vivo imaging of dying RGCs. First reported by Cordeiro's group, using a rat model of glaucoma and other injuries to RGCs, this technique demonstrates individual RGCs undergoing cell death (Cordeiro et al. 2010). A fluorophore-labeled annexin V is injected intravitreally and binds to phosphatidylserine exposed on the outer leaflet of the membrane of an apoptotic cell. Localization of externalized phosphatidylserine on the cell surface allows quantitation of the number of dying cells (**Figure 5**). If a neuroprotective agent decreases the rate of cell death, then presumably, this can be detected with annexin V binding. Furthermore, it can be performed on a daily or weekly basis, detecting neuronal death far earlier than the bulk changes associated with loss of axons in the retinal nerve fiber layer and earlier than changes in the visual field. This work underwent phase 1 studies (Cordeiro et al. 2017), and phase 2 studies are planned (M.F. Cordeiro, personal communication).

Alternatively, the number of live RGCs can be detected using modified adaptive optics scanning light ophthalmoscopy (Rossi et al. 2017). Other approaches to in vivo biomarkers of RGC disease in glaucoma include detecting the signaling of cell death before apoptosis (Tsuda et al. 2016). One approach has been the induction of superoxide, which increases in the cell body after axonal injury to RGCs (Catrinescu et al. 2012, Kanamori et al. 2010a, Lieven et al. 2006). Similar changes can presumably be detected by focusing on individual axon bundles (Kanamori et al. 2010b, 2012). Using this approach, a proof-of-concept clinical trial could be performed on the basis of the in vivo detection of sick or dying RGCs and the changes induced by a putative neuroprotective drug. If there were to be an effect on dying cells or their axons, then one could more confidently make the decision to move to longer-term studies with more subjects, using standard endpoints such as visual fields.

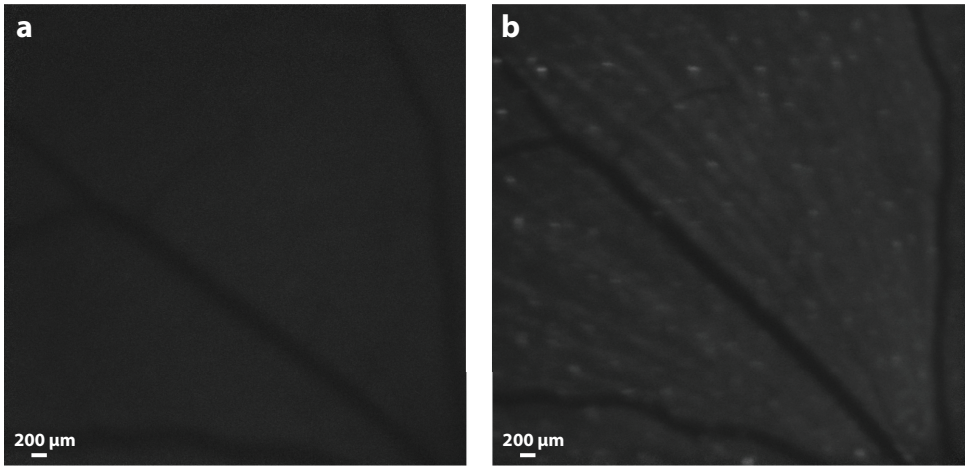

Figure 5

In vivo image of a retina with annexin V labeling. (*a*) There was no detectable fluorescence following intravitreal injection of annexin V into an intact rat eye. (*b*) Optic nerve transection followed by injection of annexin V 24 h later resulted in labeling of retinal ganglion cells and axon bundles with annexin V, indicating exposure of phosphatidylserine in the outer leaflet of the membrane of an apoptotic cell.

8.2. Objective Functional Testing

One of the problems with assessing visual fields as endpoints in clinical trials is their high variability, especially in some patients who have difficulty doing the test or where visual loss is severe. As mentioned earlier, the use of a large stimulus (e.g., size V) can help minimize the variability associated with decreased frequency of seeing when sensitivity levels are low. However, there still is variability associated with the fact that subjects can be tired, the tests are boring, and the presence of mental status changes in the elderly population, who make up the bulk of patients with progressive glaucoma.

In response to the problem associated with variability of visual-field testing, there has been research on objective functional testing in visual fields. One approach is pupillometry, where pupillary miosis is used as a measure when stimuli are presented in different parts of the visual fields (Kardon et al. 1991, Maddess et al. 2013). One of the problems with pupillometry is that the signal-to-noise ratio may theoretically be low. Another is that different diseases may differentially affect RGCs carrying information from the pupillary response (destined for the pretectal nuclei) versus those carrying information used for conscious vision (destined for the lateral geniculate nucleus) (Nadal-Nicolas et al. 2015, Wakakura & Yokoe 1995). The response to neuroprotection may also be different between those classes of RGCs (Valiente-Soriano et al. 2015). A pupil-based visual-field mapping should not be confused with the mass pupil response, which may be less sensitive to small changes (Chang et al. 2013).

Another objective functional test is the multifocal visual-evoked response, which is helpful in detecting changes in RGC activity on the basis of detection of electrical activity recorded at the scalp overlying the occipital cortex. Resolution is not high, the signal-to-noise ratio is low, and, therefore, the effect size of a neuroprotective drug would have to be great to detect meaningful improvement. Developing better testing methods in this and related areas would obviously have great impact on the field.

8.3. The Two-Culture Problem

One of the great difficulties in translational research has been referred to as the two-culture problem (Levin & Danesh-Meyer 2010). Although initially referring to the differences between the humanities and sciences in a seminal work by C.P. Snow (1959), the problem refers to the cultures associated with translational research. In the culture of the basic scientists, experiments are done using sets of approaches that may have different standards and protocols compared to those used in the culture of the clinical trialists; those of the clinical trialists are highly regulated because of the need for approval by regulatory agencies. The result is a failure to translate (Bebarta et al. 2003, Perel et al. 2007, Pound et al. 2004). Both laboratory and clinical research have standards, referred to as good laboratory practice (GCP) and good clinical practice (GCP); most exploratory and discovery work in basic science laboratories does not fall under the former. One of the ways of bringing these two cultures together is to involve basic scientists more in clinical trial design and to have clinical trialists interpret and make decisions based on basic research data. This has been extensively discussed elsewhere (Ergorul & Levin 2013). Another approach is to apply the strict approach used in clinical trial methods to the processes, analyses, and reporting of animal research (Fisher et al. 2009, Kilkenny et al. 2010).

9. SUMMARY

Demonstrating that neuroprotective therapies are efficacious for glaucoma would have a tremendous impact on patients with this blinding disease. The history of neuroprotection in glaucoma has been difficult, but the variety of new animal models, techniques, and understanding of translational research should have a beneficial effect in this area over the next decade. Many of the approaches outlined previously are applicable not only to neuroprotection but to other novel therapies, and not only to glaucoma but to other diseases affecting the optic nerve and retina.

DISCLOSURE STATEMENT

L.A.L. is a consultant to Aerie, Inotek, Quark, Regenera, and Teva on neuroprotection and is an inventor of patented neuroprotective compounds. The patents have been assigned to the Wisconsin Alumni Research Foundation.

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