Chapter 6

Restoring Vision to the Blind: Neuroprotection

The Lasker/IRRF Initiative for Innovation in Vision Science

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Introduction

Retinal cell death is the leading cause of vision loss in the majority of blinding conditions. Research focused on understanding the mechanism by which retinal cells die provides the cornerstone on which we will build and develop therapies meant to target those mechanisms. Neuroprotective therapies are one approach that is aimed at preventing, slowing, and ultimately, reversing the neurodegenerative processes that drive retinal diseases.

Targeting Cell Death Mechanisms

There are many different diseases that cause neurodegeneration in the eye, some of which are inherited, such as X-linked retinitis pigmentosa (XLRP), and others that are widespread throughout the population with their underlying causes poorly understood, such as age-related macular degeneration (AMD). Regardless, they, as well as many other retinal degenerations, have one thing in common, cell death (Huckfeldt & Vavvas, 2013). It has been shown that in early cases of diabetic retinopathy, there is a significant increase in the activation of proapoptotic signaling, suggesting that these target molecules are some of the first responders in the early stage of retinal degeneration, thus making them excellent targets for therapy (Valverde et al., 2013). Programmed cell death, or apoptosis, has been and will continue to be a promising target in the search for neuroprotective therapies. Several neuroprotective agents from monoclonal antibodies to small inhibitory RNAs to small peptide inhibitors have been developed to target both intrinsic and extrinsic apoptotic pathways. Such targets include caspases, Fas ligand/receptor complex, BAX/Bcl2, and various components of the PI3K pathway including ERK- and Akt-dependent neuroprotection via erythropoietin (EPO) (Chinskey, Besirli, & Zacks, 2014; Ha, Shanmugam et al., 2014; Huang, Li, Qui, Gonzalez, & Challa, 2013; Mo et al., 2013; Shen et al., 2010; Zacks, Boehlke, Richards, & Zheng, 2007). A highly informative review by Chinskey et al. (2014) outlines the current strategies in retinal neuroprotection, spanning several cellular mechanisms including apoptosis, necrosis, autophagy, and inflammation, as well as targeting calpains.

Retinal Ganglion Cells or Microglia?

Given the many cell–cell interactions in the central nervous system, including the retina, the targeting of cells other than degenerating retinal neurons or photoreceptors must be considered. For example, there is an increasingly large amount of recent research in the field of glaucoma that supports the idea that microglia are actually a more promising target for neuroprotection than are retinal ganglion cells (Rojas et al., 2014). There is a considerable body of data suggesting that microglia, in fact, maintain important neuroprotective functions that are specific to the different stages of

Table 6.1. Examples of Neuroprotective Agents That Have Been Effective in Photoreceptor and Retinal Ganglion Cell Degeneration

FGF-2, FGF-5, FGF-18	α_2 -adrenergic agonists
BDNF (and related)	Melatonin receptor antagonists
NT-3	Melatonin
CNTF (and related)	Levobetaxalol
IL-1 β	Calcium channel blockers
GDNF	PBN (free-radical scavenger)
PEDE	Caspase-3 inhibitors
LEDGF	Dimethylthiourea
HSP 25/HSP-70	Ginko biloba extract
RdCVF	Estradiol & its derivatives
Erythropoietin	Antioxidants

microglial cells (M1, M2, etc.) (Ardeljan & Chan, 2013). It will be important to determine what microglial cells are secreting at the different stages and attempt to harness their protective reactivity. It is unknown how microglia assemble/disassemble their scaffolding to maintain such a rapid and dynamic response following their activation. Microglia make a promising target, because every time the retina is damaged, regardless of the type of damage, there is robust activation of these glial cells (Harada & Harada, 2004). Thus, the response of microglia may hold the key to identifying a compound, or pool of compounds, that can be utilized to provide neuroprotective treatment for retinal degenerations, regardless of the type of mutation, stage of progression, or underlying pathology. In some disease states, however, subpopulations of microglia have been shown to promote neurodegeneration, making them excellent potential targets for therapy (Arroba, Alvarez-Lindo, van Rooijen, & de la Rosa, 2011; Arroba, Alvarez-Lindo, van Rooijen, & de la Rosa, 2014). Thus, the activation of glial cells can be harmful at times.

A Focus on Molecules

Several different molecules have been tested and used as neuroprotective agents. The rationale for the use of these has been varied; some have been shown to be neuroprotective in other regions of the nervous system, some are known as growth factors that influence cell survival at different times during development, some are involved in (or inhibitors of) cell death pathways, and some were identified empirically or by serendipity in the past 20 years. Listed in Table 6.1 are some neuroprotective agents that have shown efficacy in treating photoreceptor and retinal ganglion cell degenerations.

Highlighting CNTF

One of the most remarkable breakthroughs in recent years has been the discovery and clinical development of ciliary neurotrophic factor (CNTF). It is one of the best-studied neurotrophic factors for neuroprotection in the retina, and it was first shown by LaVail et al. (1992) to promote rod photoreceptor survival in light-induced degeneration. Several groups have shown this cytokine to be effective in a variety of different animal species and models of inherited retinal degeneration. Specifically, CNTF can rescue photoreceptors in 13 different models of retinal degeneration in four different species of animals (Sieving et al., 2006). As outlined in an excellent review by Wen, Tao, Li, & Sieving (2012), CNTF has shown tremendous therapeutic effects on photoreceptors and retinal ganglion cells (RGCs) in the mammalian retina. Despite the positive effect of CNTF in many retinal degenerations, the mechanism of rescue remains controversial, with the cellular localization of CNTF receptors in the retina and the possible involvement of Müller cells as intermediaries debated (reviewed by Wen et al., 2012). CNTF was predominantly thought to act only on rod photoreceptors until a phase 1 clinical trial for CNTF-encapsulated cell technology (ECT) implants (Sieving et al., 2006) showed that one patient with late-stage retinitis pigmentosa (RP) showed an improvement in his vision based on best-corrected visual acuity (Sieving et al., 2006). The argument was made that since the rods are essentially gone in these patients, the improvement was most likely mediated by enhanced cone function. There are currently patients within this population who have been living with their ECT-CNTF implants for 7 years, but the phase 2 clinical trial ended after 24

Table 6.2. Some Mechanisms and Therapeutic Targets for the Identification of New Neuroprotective Agents

Antioxidants	Inhibitors of RIP kinases
Stimulation of glycolysis, pentose pathway, & oxidation	Inhibitors of the inflammasome
Mitochondrial activity and biogenesis	UPR pathways
PI3K signaling	Chaperones
JAK/STAT signaling pathways	Electron carriers
AMP-activated protein kinase (AMPK)	Kinase inhibitors
Sirtuin (Sirt) family stress regulators	Intrinsic and extrinsic cell death mechanisms

months (Birch et al., 2013), and patients have not been evaluated consistently on a long-term basis after the end of the clinical trial. It will be invaluable to organize a long-term study to determine whether there has been any ongoing improvement/protection in these patients' vision and, more importantly, if none is observed, we need to determine if the ECT implants are still producing and releasing CNTF to the retina.

CNTF has been shown directly to induce cone outer segment regeneration in the S334ter-3 rhodopsin transgenic rat model of retinal degeneration (Li et al., 2010). Consistent with this important finding, CNTF has been shown to reduce the rate of cone cell loss in a subset of participants in a phase 2 clinical trial of long-term CNTF release in human patients with RP (Talcott et al., 2011); this evaluation was possible by using an adaptive optics scanning laser ophthalmoscope (AOSLO) to image individual cone photoreceptors (and other cells) over the course of 24 to 36 months. This technology is currently being used to assess patterns of photoreceptor loss in several types of retinal degeneration (Mkrtchyan et al., 2012; Ratnam, Carroll, Porco, Duncan, & Roorda, 2013; Zayit-Soudry, Duncan, Syed, Menghini, & Roorda, 2013), and a randomized clinical trial of CNTF is underway using AOSLO cone spacing measures as the primary outcome measure (www.clinicaltrials.gov, clinical trial identifier NCT 01530659). The ability to visualize individual photoreceptor cells with AOSLO is a revolutionary imaging technique that has the potential to provide objective, sensitive measures of photoreceptor survival in eyes treated with neuroprotective and other therapeutic approaches.

With its ECT delivery platform, Neurotech USA has initiated clinical trials for CNTF in glaucoma (www.clinicaltrials.gov, clinical trial identifier NCT01408472), which could reveal another benefit for this neuro-protectant that goes beyond photoreceptor degenerations.

Identification and Implementation of Novel Neuroprotectants

The most efficient way to identify novel agents may be with high throughput screens of small molecule libraries with read-outs in human primary cell lines and searches for novel agents that can be obtained from biological sources like tissue and plant extracts. In addition, closer evaluation of specific signaling pathways/cellular mechanisms may help us identify new pathways, molecules, and mechanisms of degeneration that can subsequently be targeted. Some excellent and promising examples of known mechanisms that have pointed to therapeutic targets are listed in Table 6.2.

Compound metabolite screening is currently being done by several groups, including work involving a microscale screening protocol with which 1280 different compounds can be screened using primary retinal neurons (Fuller et al., 2014). Another lab has screened over 800 extracts from 30 different plants containing putative molecules that protect cone photoreceptors (Léveillard, unpublished). This is very promising work and could identify several new molecules to be purified and characterized for use in neuroprotective therapies.

In this regard, the models with which we test the efficacy of a neuroprotective agent must be improved and, more importantly, standardized. The mechanism(s) of action of each new agent needs to be tested in models in which the cell death pathways activated at each stage of disease are known. In the future, we may be able to use human induced pluripotent stem (iPS) cells/self-organizing optic cups as a powerful tool for analyzing a variety of different agents in combination with gene therapy, or even in the setting of a specific set of mutations that have already been induced prior to development of these optic cups. The caveat is that to date they are unable to form functional outer segments, but there are several groups working on this. Once that bridge has been crossed, these will serve as excellent *in vitro* models for testing neuroprotective agents and laying extensive ground work in support of those agents, which can then be tested in more advanced model systems (Eiraku et al., 2011).

In addition to providing a valuable testing ground, human stem cell approaches can foster the discovery of novel

neuroprotective agents and mechanisms. While several fundamental pathways cross from animal model to human, it is likely that species differences in neurotrophic support also exist. Pluripotent stem cells offer the great advantage of producing abundant human retinal neurons and retinal pigment epithelial (RPE) cells, which can be cultured as a mixture or purified to identify pertinent cell–cell trophic mechanisms, and enable molecular analysis. Highly pure populations of human RPE can be generated from fetal eyes (Hu & Bok, 2014) and a recently described adult RPE stem cell (Salero et al., 2012), which can also help distinguish human-specific, RPE-derived factors that support rod and cone photoreceptors.

While human stem cell models are developing and can provide great value in vitro, in order to form a bridge to the clinic, a small nonhuman primate would be an ideal midpoint model prior to human clinical trials. There is much focus on preserving cone function and macular vision in humans, yet we have no standardized animal models that allow us to test the ability of neuroprotective agents to preserve/restore this type of visual function.

One of the most frustrating limitations in the generation and identification of novel neuroprotective agents is the constant struggle over intellectual property. A real world example of this is the unfortunate situation surrounding Rod-Derived Cone Viability Factor (Léveillard et al., 2004), which was shown to demonstrate strong preclinical evidence for rescuing cone function (Léveillard & Sahel, 2010; Yang et al., 2009), yet has lacked any apparent further development for 8 years by the company that holds the intellectual property. The high cost of this particular product licensing fee, should the therapy prove effective, has dissuaded other companies and foundations from investing in its clinical development. Cost recovery and a reasonable return on investment are key to the engagement of pharma and investors.

Extending the use of drugs already approved for different indications to the retina is another route worth pursuing, when they impact pathways of interest. There are several challenges in this. While still under intellectual property (IP) protection, the companies marketing the drugs need to support the effort. The size of the orphan market relative to the larger market they already have; a lack of expertise in orphan and/or retinal diseases; a fear of revealing toxicities that might impact the existing indication; a lack of sufficiently convincing retinal efficacy data in animals (especially since there are no nonhuman primate models of retinal disease); and simply the length of time and costs of running orphan drug trials in slowly progressing diseases are significant barriers to companies. Foundations and governments can help address these concerns by providing funding but also in providing natural history studies and well-designed registries that help accelerate trial enrollment and definition of endpoints. These remain important because currently the Food and Drug Administration usually does not accept unilateral eye treatment in which the contralateral eye can serve as an internal control for the eye that receives the treatment. Since the variation between patients is often much greater than the variation in disease severity and progression between eyes of a given patient, any efforts that can help address this challenge are welcome.

There is a substantial need for the identification of new neuroprotective agents; however, these intellectual property walls must be torn down or at least made substantially easier to navigate if we are to see significant translation from the identification/screening process to clinical trials with real-life applications.

Recommendations

1. New models need to be developed and standardized:
 - a. Ultimately, we need to develop the means to test neuroprotective agents in human cell products in two-dimensional (2D) and 3D configurations and subsequently human patients in well-organized clinical studies that will allow us to drive our research toward application in human patients.
 - b. As an alternative to larger nonhuman primates, which are costly and difficult to manage, we suggest using small nonhuman primates, which can be used as a starting/intermediate model for therapies and treatment; new gene editing approaches (e.g., CRISPR technology, could be highly beneficial in building disease-relevant animal models).
 - c. Addressing the challenges surrounding improving the self-organizing optic cups from iPS cells as a starting model will be important. This is potentially an extremely useful tool, and if photoreceptor outer segments can be generated, these iPS cells can be engineered to carry mutations associated with a specific disease that will allow investigators to target therapies and isolate changes in neuroprotective molecules in response to a single disease state.
2. A focus on mechanisms:
 - a. It will be critically important to focus time and resources to generate a better understanding of cell death

mechanisms and pathways that respond to various insults, as well as identifying and targeting the upstream events that lead to the activation of those mechanisms.

3. Improved organization, standardization, and accessibility of data via establishment of an international data base and encouraging a more open/collaborative effort between research groups:
 - a. Develop an international repository of data for: delivery methods, bioavailability, biodistribution, negative results, animal models, vectors used and their efficiency, technical data for methods that have been shown to work, as well as array/transcriptome data.
 - b. An example of this type of concept is the Knowledge Base for Sensory Systems, which is based at the Institut de la Vision in France.
 - c. Consider formation of a national core facility that would specialize in performing tests with novel compounds to create standardization in protocols (i.e., means of injection/injury) and avoid failure to reproduce results from one group to another.
4. Identification of new neuroprotective agents based on known mechanisms/therapeutic targets:
 - a. Based on known mechanisms/therapeutic targets: these include traditional neuroprotective molecules/growth factors; antioxidation enzymes; agents that stimulate glycolysis, pentose pathway and oxidation; mitochondria activity, PI3K, JAK/STAT, AMPK, SIRT; inhibitors of RIPK; as well as inhibitors of the inflammasome.
 - b. Based on studies of human stem cell-derived products aimed at seeking novel neurotrophic agents.

This chapter is part of the Restoring Vision to the Blind report by the Lasker/IRRF Initiative for Innovation in Vision Science. The full report, Restoring Vision to the Blind, including a complete list of contributors, is available in the [Supplementary Material](#).

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Received: 24 October 2014

Accepted: 13 November 2014

Published: 30 December 2014

Citation: The Lasker/IRRF Initiative for Innovation in Vision Science. Restoring vision to the blind: Neuroprotection. *Trans Vis Sci Tech.* 2014;3(7):8, <http://tvstjournal.org/doi/full/10.1167/tvst.3.7.8>, doi:10.1167/tvst.3.7.8

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