



Review

Light at the end of the tunnel? Advances in the understanding and treatment of glaucoma and inherited retinal degeneration

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Abstract

Glaucoma and inherited retinal degeneration/dystrophy are leading causes of blindness in veterinary patients. Currently, there is no treatment for the loss of vision that characterizes both groups of diseases. However, this reality may soon change as recent advances in understanding of the disease processes allow researchers to develop new therapies aimed at preventing blindness and restoring vision to blind patients. Elucidating the molecular mechanisms of retinal ganglion cell death in glaucoma patients has led to the development of neuroprotective drugs which protect retinal cells and their function from the disastrous effects of elevated pressure. Identification of the genetic mutation responsible for inherited degenerations and dystrophies of the outer retina has enabled researchers using gene therapy to restore vision to blind dogs. Other patients may benefit from retinal transplantation, stem cell therapy, neuroprotective drugs, nutritional supplementation and even retinal prostheses. It is possible that soon it will be possible to restore sight to some blind patients. © 2006 Elsevier Ltd. All rights reserved.

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1. Introduction

Loss of vision and blindness in a companion animal is an emotionally devastating event for most pet owners, and a very serious handicap for the patient. Fortunately, some causes of blindness are treatable. A notable example is cataract, with hundreds of surgeries performed every year worldwide to restore vision in cataractous patients (Appel et al., 2006). Similarly, some cases of optic neuritis and other neuro-ophthalmological causes of blindness may be amenable to medical treatment (Shamir and Ofri, 2007). And in the last decade, veterinary ophthalmologists have begun performing surgeries to re-attach retinas, thereby restoring sight to some patients blinded by retinal detachment (Vainisi and Wolfer, 2004).

To date, however, veterinarians seldom have treatment options to offer patients that have been blinded by two very

common groups of diseases: glaucoma and inherited retinal dystrophies/degenerations. Although the former affects the ganglion cell layer and inner retina and the latter affects mostly the photoreceptors and outer retina, the end-stage of both disease processes is irreversible blindness of the patient. Even more frustrating (for the clinician and the owner) is the fact that in inherited retinal dystrophies and degenerations, very little can be done to halt or even delay the inevitable blindness. The clinician can confirm the diagnosis, educate the client about the progressive course of the disease, and provide recommendations regarding behavioural adjustments, but can offer no treatment. In glaucoma, medical and/or surgical treatment to lower intraocular pressure (IOP) may be provided, but the animal is frequently presented when it is already blind, and even if some vision is present, the long-term prognosis for preservation of sight is very poor. In a retrospective study of 93 glaucomatous cat eyes, 67 eyes were blind at presentation, and treatment succeeded in preserving vision in only nine eyes (Blocker and van der Woerd, 2001) (Fig. 1).

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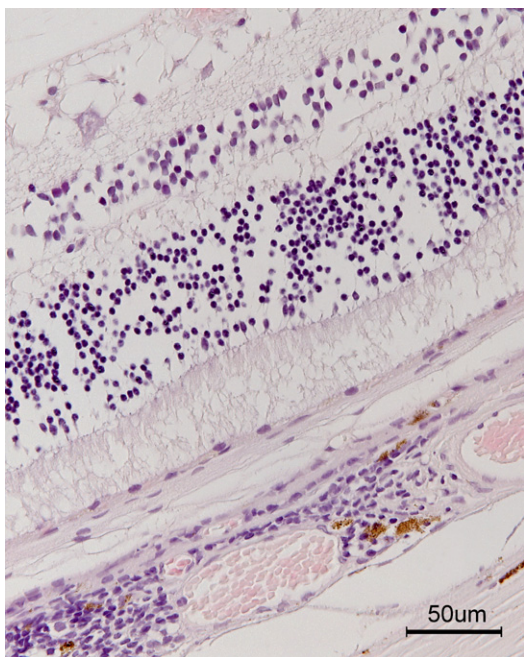


Fig. 1. Histopathology micrograph showing inner retinal atrophy secondary to glaucoma in a dog. Note that except for a few degenerative nuclei, no ganglion cells can be observed in the inner retina. A marked, diffuse edema is also noted. Hematoxylin Eosin. Photo courtesy of Dr. Emmanuel Loeb.

This sad reality may change in the near future. Intense research, using advanced technologies such as gene transfer and stem cell therapy is underway with the aim of preserving sight and/or restoring vision to blind patients. Even though some of the technologies tested today, such as artificial retinas, sound like science fiction and fantasy, some researchers have already succeeded in restoring sight to blind dogs. Much of the work in this area is conducted by veterinary ophthalmologists who are searching for treatment options for their patients, and at the same time using these patients as animal models for related diseases in humans. The aim of this paper is to review the recent progress that has been made in our understanding of glaucoma and inherited retinal degenerations, and the therapeutic advances made possible through this increased understanding. These advances will hopefully one day enable us to treat these causes of blindness.

2. Glaucoma

2.1. New concepts in our understanding of glaucoma: secondary degeneration

Glaucoma is a common and painful cause of blindness. In domestic animals, the disease is most prevalent in dogs. It has recently been shown that breed-related (presumably inherited) glaucoma affects nearly 0.9% of pure bred dogs in North America, with some breeds (e.g. American Cocker Spaniels and Basset Hounds) having a prevalence >5%

(Gelatt and MacKay, 2004a). A similar percentage of the general canine population is affected by secondary glaucoma (Gelatt and MacKay, 2004b). The disease also afflicts cats (Blocker and van der Woerd, 2001), horses (Wilkie and Gilger, 2004), cattle (Mertel et al., 1996), and other species, including wildlife (Ofri, 2002). Glaucoma is also common in humans; over 60 million people are projected to be afflicted with the disease by the end of this decade, and 8.4 million of them will be bilaterally blind (Quigley and Broman, 2006).

Over the years, our understanding and definition of glaucoma has evolved. While traditionally the disease has been defined as elevation in intraocular pressure (IOP), today there is growing recognition that increased pressure is only a primary risk factor in the pathogenesis of glaucoma-induced damage. Other factors, such as extracellular matrix abnormalities in the lamina cribrosa, or perfusion defects in the optic nerve head (ONH) circulation, may also contribute to the optic nerve damage in glaucoma. An extreme example of the effect of these IOP-independent factors is *normotensive glaucoma*, a disease that bears all the features of glaucoma (including decreased retinal ganglion cell sensitivity and function, ganglion cell death and ONH cup enlargement, incremental reduction in visual fields, and blindness), yet IOP is normal. Normotensive glaucoma is a well-recognized entity in humans (Trick, 1993) and non-human primates (Komaromy et al., 1998), and probably exists in dogs as well (D.E. Brooks, personal communication).

Another pathological process that is probably responsible for the progressive loss of optic nerve axons that is the hallmark of glaucomatous neuropathy is *secondary degeneration*. A similar pathogenesis of axonal damage, which progresses even after the initial insult has been alleviated, is observed in many neurological disorders including stroke, hypoglycemia, trauma and epilepsy. It is suggested that in all of these diseases, as well as in glaucoma, axons damaged by the initial insult release various substances into their immediate surroundings. The localized high concentrations of these substances create a hostile microenvironment. Adjacent axons, which were not damaged during the initial insult, undergo secondary degeneration as a result of being immersed in this toxic milieu. This creates a “domino effect” in which (in the case of glaucoma) optic nerve axons will continue to degenerate even after IOP has been successfully lowered, resulting in further loss of vision (Schwartz, 2005).

In searching for mediators of secondary degeneration, much of the early attention has focused on the role of glutamate, an amino acid that normally functions as an excitatory neurotransmitter in the central nervous system. However, following neuronal injury, intracellular glutamate is released by damaged axons into the immediate surroundings. The resulting locally elevated concentration of glutamate causes overstimulation of the *N*-methyl-D-aspartate (NMDA) subtype of glutamate receptors in neighboring (undamaged) neurons. This stimulation in turn, leads

to increased calcium influx, thereby starting an intracellular enzymatic cascade progressing to apoptosis and cell death (*excitotoxicity*) (Kuehn et al., 2005).

Indeed, loss of glutamate from ganglion cells has been demonstrated in dogs with glaucoma, with consequent elevated levels of the neurotransmitter in the vitreous humor and Müller cells of the patients (Brooks et al., 1997; McEllinay et al., 2004). In animal models, elevated glutamate levels have been demonstrated in monkeys (Wamsley et al., 2005) and rats (Martin et al., 2002) with experimental glaucoma. Furthermore, intravitreal injection of glutamate in rodents resulted in glaucomatous-like damage to the retina and optic nerve, and the damage was inhibited by injection of memantine, a glutamate antagonist (Vorwerk et al., 1996). Therefore, even though glutamate is not an infective agent, its role in the pathogenesis of glaucomatous optic neuropathy has been supported by three of Koch's postulates; it is found in glaucoma patients, its injection into normal subjects induces disease, and the disease can be prevented by inhibiting its action. The only postulate that remains to be proven is that inhibition of glutamate in naturally occurring cases will also prevent glaucomatous damage (see below).

Another compound that probably plays an important role in the pathogenesis of glaucomatous damage is endothelin 1. Elevated levels of this potent vasoconstrictor have been shown in the aqueous humor of glaucomatous dogs (Kallberg et al., 2002), as well as in humans suffering from normotensive (Kaiser et al., 1995) and hypertensive (Tezel et al., 1997) glaucoma. These findings suggest that abnormalities in retinal and optic nerve head (ONH) circulation may also play a role in the disease process. It is hypothesized that elevated endothelin levels cause microcirculation dysfunction in the canine eye, thus inducing ischemic conditions in the retina and ONH (Kallberg et al., 2002). The resulting anterior ONH ischemia, in turn, releases more glutamate (Kim et al., 2000), thus further continuing the damage cascade.

However, it is important to note that glutamate and endothelin are far from being the only compounds involved in the pathogenesis of glaucomatous optic neuropathy. Since the mid-1990s, a number of compounds and genes have been implicated in the process of secondary degeneration. These include D-serine, nitric oxide, tumor necrosis factors α and β , and plasminogen activators, to name just a few (Osborne et al., 2004; Mali et al., 2005). Indeed, the length of the list of factors involved in the disease process is probably why glaucoma remains such a frustrating syndrome to understand and to treat, and why so many human and animal patients still lose their sight as a consequence of the disease.

2.2. Neuroprotection and the prevention of glaucomatous loss of vision

An obvious implication of the process of secondary degeneration is that treatment with compounds which inhi-

bit the excitotoxic factors may slow or stop the cascade of secondary degeneration, and protect the undamaged neighboring axons. This therapeutic approach is known as *neuroprotection*. Obviously, such drugs are not expected to restore vision which has been lost prior to initiation of treatment. For example, in the retrospective study of feline glaucoma cited in Section 1 (Blocker and van der Woerd, 2001), neuroprotection will not help the 67 glaucomatous cats that were blind at presentation (Fig. 1). However, it may very well help preserve vision in the 26 cats that still had some vision at presentation.

To this end, several therapeutic strategies are attempted. One target for neuroprotective intervention is inhibition of the toxic compounds. Inhibition of glutamate toxicity was probably the first approach to be evaluated, with numerous studies of the beneficial effect of inhibiting glutamatergic NMDA receptors (Lipton, 2003). Memantine, an NMDA channel blocker, has been shown to be neuroprotective in both rodent (Schuettauf et al., 2002) and monkey (Hare et al., 2004) models of glaucoma. Indeed, in both Europe and the USA memantine is already undergoing advanced clinical testing in humans suffering from neurological disorders that may have a similar pathogenesis to glaucomatous neuropathy, such as Alzheimer's disease (Lipton, 2005). Inhibition of other glutamatergic receptors, such as metabotropic receptors, has likewise been shown to be neuroprotective in a rat model of glaucoma (Guo et al., 2006).

As noted previously, glutamate is not the only compound shown to be neurotoxic to retinal ganglion cells. Nitric oxide and endothelin-1 have also been demonstrated to play a role in the pathogenesis of glaucomatous damage, and consequently their inhibition was demonstrated to have a beneficial, neuroprotective effect. Inhibition of nitric-oxide synthase 2 by aminoguanidine has been shown to be neuroprotective in a rat model of glaucoma (Neufeld, 2004). Unoprostone, a potent vasorelaxant, was shown to antagonize the vasoconstrictive effect of Endothelin-1 and to improve retinal blood flow in human glaucoma patients (Melamed, 2002).

The efficacy of neuroprotective compounds in preventing glaucoma-induced loss of vision may be demonstrated morphologically through increased survival of ganglion cells in animal models of the disease. Alternatively, it can be demonstrated using electroretinography (ERG), which is an objective measure of retinal function. Preservation of inner retinal function following neuroprotective treatment in glaucomatous rats has recently been reported (Ben-Shlomo et al., 2005) (Fig. 2).

Another neuroprotective approach is to prevent the molecular cascade that leads to cell death by inhibiting the gene activation or the signal transduction that is triggered by the neurotoxic compounds. This approach is particularly promising for glaucoma patients, as there is mounting evidence that many glaucoma drugs which are already approved for use as IOP-lowering medications may also have a neuroprotective effect. These drugs include commonly used glaucoma medications such as brimonidine,

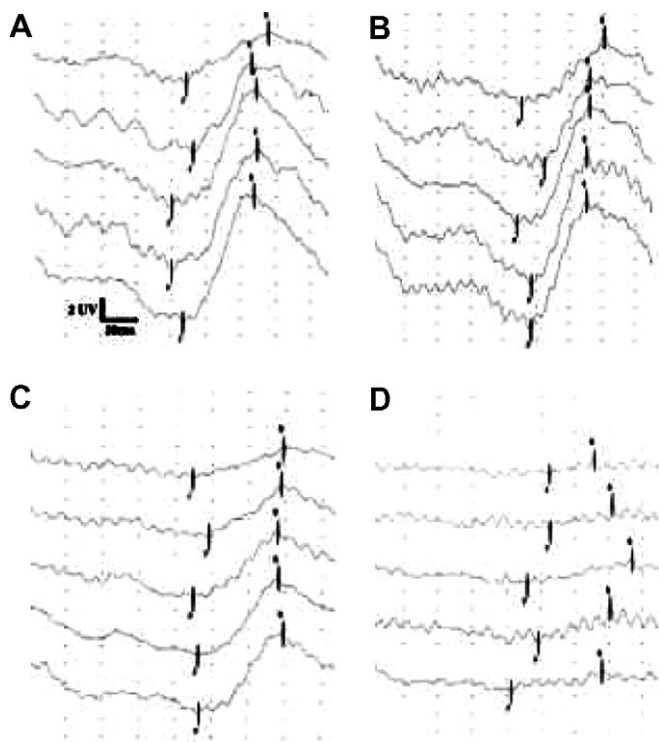


Fig. 2. Electrophysiological evidence for preservation of retinal function in glaucoma using neuroprotective treatment (COP-1, injected intraperitoneally). Pattern ERG recordings, indicative of ganglion cell function, were conducted in two rats in which glaucoma was induced by limbal plexus laser photocoagulation. Signals were recorded in response to patterns of increasing width. In each set of signals presented, the top trace was recorded in response to the smallest stimulus (82 min of arc/phase). The size of the stimulus was doubled in each subsequent recording, reaching a maximal size of 1304 min of arc/phase in the bottom trace of each set. (A) Baseline recordings (prior to glaucoma induction) in a rat treated with COP-1. (B) Baseline recordings (prior to glaucoma induction) in a control rat. (C) Recordings 4 weeks after glaucoma induction in a rat treated with COP-1. (D) Recordings 4 weeks after glaucoma induction in a control rat. Note that there is no difference in baseline recordings between the treated and the control animals (A, B, respectively). Glaucoma caused a significant reduction of retinal function in both animals. However, because of COP-1 administration, the reduction in the treated animal (C) is noticeably smaller than in the control animal (D). For additional details on the study and the neuroprotective properties of COP-1, see Bakalash et al., 2005.

an α -2 receptor agonist (Wheeler et al., 2003), and various β -adrenoreceptor antagonists, including betaxolol, metipranolol and timolol (Wood et al., 2003). It is suggested that these drugs, used at their therapeutic (hypotensive) doses, also act to prevent calcium and sodium influx into neurons, thereby protecting them from apoptosis (Wheeler et al., 2003; Wood et al., 2003). Gene therapy, using the anti-apoptotic gene XIAP has a similar protective effect (Straten et al., 2002).

Recently, a third approach has been proposed for preserving vision in glaucoma patients. This approach, involving autoimmunity, aims to increase the resistance of optic nerve neurons to the injurious conditions by harnessing the body's immune system (Schwartz, 2001). Use of immuno-modulating drugs such as copaxone has been demonstrated to recruit T cells to the glaucomatous eye,

resulting in functional and morphological protection of the optic nerve from the effect of elevated IOP (Bakalash et al., 2005) (Fig. 2).

As noted, many of the neuroprotective compounds being evaluated are already approved for use in glaucoma patients. It is hoped that one day this group of drugs will not only lower IOP, but will also prevent the neuronal death and the progressive loss of vision that are the scourge of glaucoma.

3. Inherited retinal degenerations and dystrophies

3.1. Progress in understanding inherited outer retinal diseases

Harald Magnusson described the first case of progressive retinal atrophy (PRA) in the Gordon Setter dog in Sweden in 1909 (Magnusson, 1909). Since then more than 100 breeds of dog have been shown to be affected by this bilateral, progressively blinding disease of the outer retina and the number of dog breeds afflicted by this devastating disorder appears to be continuously increasing (see Petersen-Jones, 1998, for a review). Cats are also affected by a similar disease (Fig. 3) (Narfström, 1985).

In recent years, however, there has been a tremendous advancement in the understanding of the hereditary disease processes affecting the outermost portion of the retina: the photoreceptors and the retinal pigment epithelium (RPE). Many of the diseases previously simply called PRA have now been further characterized biochemically, electrophysiologically and morphologically. There has further been a tremendous progress in molecular genetic studies with the elucidation of causative mutant genes for several hereditary retinal disorders of many animal species, including dogs and humans. The recent availability of the canine genome sequence (Lindblad-Toh et al., 2005) (<http://www.genome.gov/12511476>) has greatly simplified the task of identifying genes responsible for diseases and traits in dogs. In cases where mutations responsible for human inherited retinal degenerations are known, the canine orthologs can be identified as candidate genes for sequence analysis in dogs that have similar disease phenotypes. In other cases, the canine disease gene may be identified by positional cloning and lead to the identification of the mutation causing the corresponding human disorder. It is likely that within a relatively short period of time the mutations responsible for most Mendelian inherited diseases in purebred dogs will be known.

A number of the PRAs have been designated gene symbols reflecting either the specific cells involved in the hereditary retinal dystrophy or the protein involved in the degenerative condition. For instance, the Miniature Poodle, Labrador Retriever and the English and American Cocker Spaniel are among the 14 breeds presently known to have allelic forms of slowly progressive degeneration of the rods and cones (Acland et al., 1998; Narfström and Petersen-Jones, 2007). The disease in these breeds has been given the

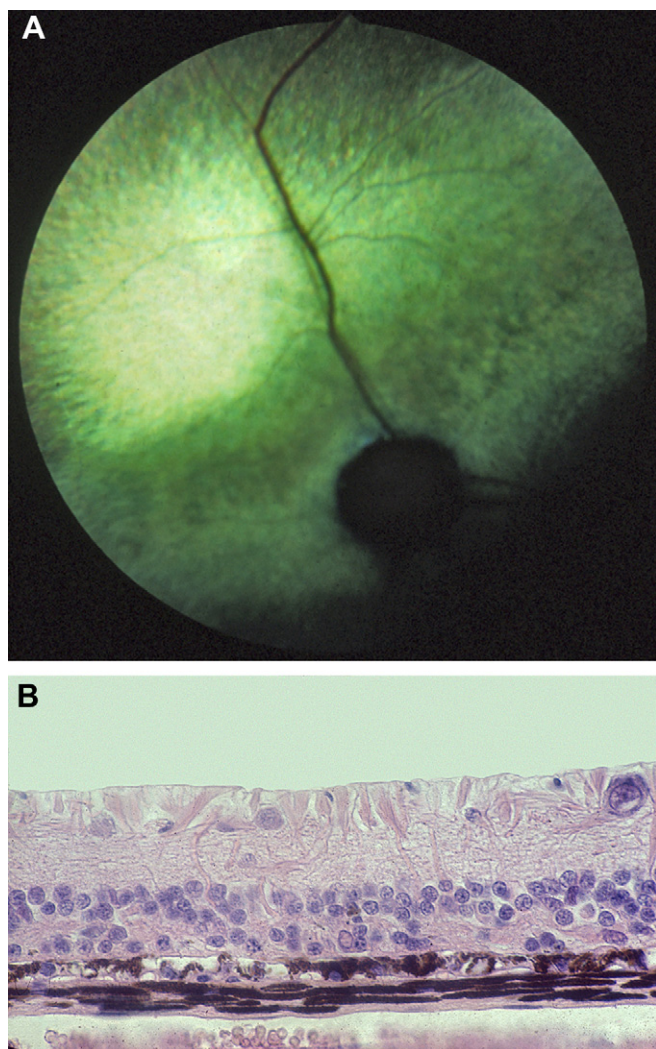


Fig. 3. (A) Fundus picture of a moderately advanced case of PRA in a 3-year-old Abyssinian cat, with clinical similarities to *prcd* in dogs and RP in humans. Note the severe vascular attenuation and the hyperreflectivity of the tapetal fundus, observed most clearly in the upper midperipheral area. (B) Light micrograph of the inferior non-tapetal retina, midperipheral area from the same case. Note the severe atrophy of the outer retina with only some sparse nuclei remaining of the photoreceptor cells, while the inner retina is normal appearing. Hematoxylin and Eosin.

gene symbol *prcd* for progressive rod cone degeneration. Very recently, the causative mutation for this defect was elucidated (www.optigen.com), although at the time of writing not published. To date, gene tests are available for approximately 30 different diseases affecting the outer retina of dogs (Narfström and Petersen-Jones, 2007).

A comparable disease in humans is Retinitis Pigmentosa (RP) (Phelan and Bok, 2000). Early symptoms include night blindness and loss of peripheral vision. Eventually visual acuity is lost with central visual defects, and patients become legally blind at varying age depending on the genetic type of RP. The inheritance of the disease may be either autosomal dominant, autosomal recessive, sex-linked, mitochondrial or be isolated, i.e. not apparently hereditary. Up to 1 in 3000 people on a worldwide basis are affected by the disorder (Mohand-Said et al., 2000).

Although the precise mechanism for different forms of RP is still under investigation, many different genes are mutated in RP patients. For example, only in the rhodopsin gene more than 100 mutations have been identified, leading to production of aberrant protein (Gal et al., 1996).

Canine forms of PRA were for many years all thought to be inherited as a simple autosomal recessive trait. Sex-linked (X-linked) inheritance for retinal degenerative disease in two breeds were then discovered, namely in the Siberian Husky and in the Samoyed (Zhang et al., 2002), with the gene symbols *x/PRA*, and due to two different mutations in the RPGR gene. Further, an interesting dominantly inherited disease was recently elucidated in the Bull Mastiff and in the Old English Mastiff dog (Kijas et al., 2002). A mutation in the rhodopsin gene was described with unusual consequences. Research showed that affected dogs were sensitive to light exposure and the retinal degenerative process could be focally enhanced by simple fundus photography! This finding may have important implications for both humans affected with RP and for dogs with PRA affected by the rhodopsin mutation. Recommendations regarding examination procedures may be warranted, in order not to inadvertently cause an unnecessary fast increase in disease progression (Cideciyan et al., 2005).

A specific outer retinal disorder was discovered in congenitally night-blind Briard dogs almost 20 years ago (Narfström et al., 1989). Affected dogs had a normal fundus appearance up to the age of 3–5 years, had severe visual problems, always at night but in many cases also in daylight. Pupillary light reflexes were abnormal, and nystagmus was sometimes observed in affected dogs (Narfström et al., 1994). Morphological and electrophysiological research showed that the RPE cells were primarily involved in the disease process (Nilsson et al., 1991; Wrigstad et al., 1992). Molecular genetics proceeded and, through candidate gene analysis, a 4 base-pair deletion in the RPE65 gene, causing a loss of RPE65 protein, was found (Veske et al., 1999). This is a 61 KD protein, preferentially expressed in the retinal pigment epithelium (RPE), and involved in a complex of enzymes and proteins participating in the recycling of A-vitamin (Redmond et al., 1998), that is normally occurring in the RPE and photoreceptor outer segments (Kuksa et al., 2003).

A similar disease is known to affect humans, Leber's congenital amaurosis (LCA). Approximately 10% of the LCA cases also have a null mutation in the RPE65 gene (Thompson et al., 2000). Therefore, the canine retinal dystrophy due to the RPE65 null mutation has become an important animal model for LCA.

3.2. Gene therapy for inherited retinal dystrophies

Gene therapy aims at delivering corrective genetic material to cells that are affected by a unique genetic defect (Bennett and Maguire, 2000). The mutation-expressing cells are most often affected but also specific cellular defects can trigger a cascade of events that lead to loss of other,

initially normal cells as well. Through gene therapy, specific genes can be turned on or off, and even be replaced. The eye is particularly amenable to this form of therapy since there has been great progress in delineating the molecular bases of many retinal diseases. Also treatment and subsequent follow-up are simplified since the ocular tissues can be observed directly, followed over time for signs of disease and function evaluated objectively using the ERG. Gene therapy cannot effectively and completely restore a degenerating retina, however, it can halt the progression of disease and could in theory preserve photoreceptors that have not yet been destroyed.

Genetic material is targeted to the retina through enveloping the genetic material (nucleic acids) into a lipid-containing complex or, most often, incorporated into a viral vector (Dejneka and Bennett, 2001). The latter is most effective and prolonged retinal transgene expression is obtained with the use of recombinant viral vectors, such as adeno-associated virus (AAV). Recombinant AAV has shown great promise in ocular gene therapy since it is comparably safe and replication-defective, has a wide host range and has not been associated with human systemic disease. Further, AAV does not appear to provoke a significant immune response to the eye, targets photoreceptors and the RPE, and is effective long-term (Bennett et al., 1999). For intraocular treatment a construct is created, which incorporates the genetic material needed together with the viral vector and a promoter (a helper virus), such as cytomegalovirus (CMV) (Liang et al., 2000).

Proof of principle that the technology works was achieved by two independent groups using different strains of RPE65 null mutation dogs. The first in vivo study with treated affected dogs was performed by Acland et al. (2001). Three dogs were injected subretinally with a rAAV-RPE65 gene construct into one eye and the other eye was either not touched or injected intravitreally (Acland et al., 2001). Approximately 3 months after treatment objective testing using ERGs showed some encouraging post-operative results: there was significant functional improvement in ERG responses in the subretinally gene transfer treated eyes. The second study was performed using similar treatment in 12 affected dogs (Narfström et al., 2003) (Fig. 4). Again, surprisingly good results were obtained and functional improvement was demonstrated clinically, using bilateral ERG recordings and behavioral studies (Fig. 5). Only 3 months following treatment improvement was also demonstrated morphologically by a reduction in the inclusion bodies in the RPE following treatment. Apparently a normalization of the retinoid cycle occurred following treatment, inducing visual processing, at least locally in the treated area. Functional vision was obtained in the affected dogs. Long-term studies showed somewhat reduced visual capacity with time although the dogs were visual for more than 3 years following treatment (Narfström et al., 2005a).

Even though initial studies have shown remarkable results as to visual function (Acland et al., 2005), it is still

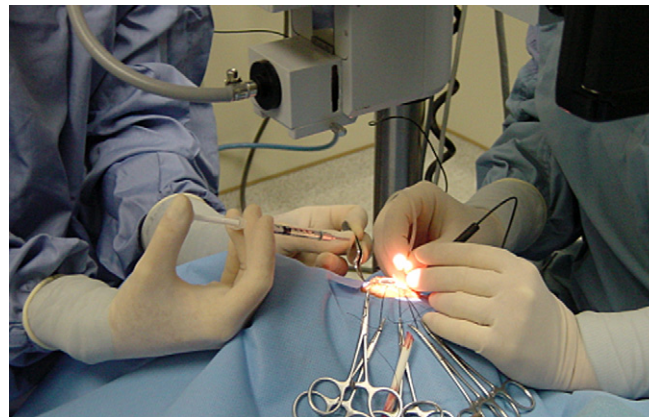


Fig. 4. Gene transfer in progress using aseptic intraocular surgical procedures in an RPE65 null mutation dog, blind due to the retinal dystrophy. A subretinal injection of 100 μ L of the gene construct is given using a hand-drawn glass micropipette.

not clear if cell death by apoptosis or programmed cell death (Portera-Cailliau et al., 1994) continues after treatment (Narfström et al., 2005b). Further safety assessment studies are needed in preparation for human clinical trials. It is very exciting, however, that the research performed so far has proven that corrective gene therapy is feasible for hereditary retinal degenerative disease.

3.3. Retinal transplantation

Diseases affecting the outer retina are incurable once photoreceptors are lost. These diseases usually also cause detrimental effects on the RPE, the outermost cell layer of the retina. The inner retina, on the other hand, may remain functional for an extended time period even though significant retinal remodeling is known to occur in conjunction with death of the photoreceptors (Jones and Marc, 2005). If the degenerated visual cells can be replaced, vision loss may be prevented. Experimentation in this regard includes replacement of visual cells with neuroblastic progenitor (fetal) cells and RPE cells as sheets of normal tissue (Aramant and Seiler, 2002, 2004) (Fig. 6). In fact, it has been shown that retinal transplants can morphologically reconstruct a damaged retina and even restore retinal sensitivity (Thomas et al., 2006).

Synaptic connections between transplants and host retina have been indicated with trans-synaptic tracing. It has also been shown by experiments in blind rats that retinal transplants in sheets can restore and preserve the visual responses in a small area of the superior colliculus corresponding to the placement of the transplant in the retina (Thomas et al., 2004; Seiler and Aramant, 2005). It appears that the beneficial effect of retinal transplantation involves two main mechanisms: (a) trophic effects, which includes partial rescue of host cones. With preservation of photoreceptors, retinal remodeling by itself is slowed down and, (b) synaptic connectivity between transplant and the host retina, needed for a beneficial effect on visual function.

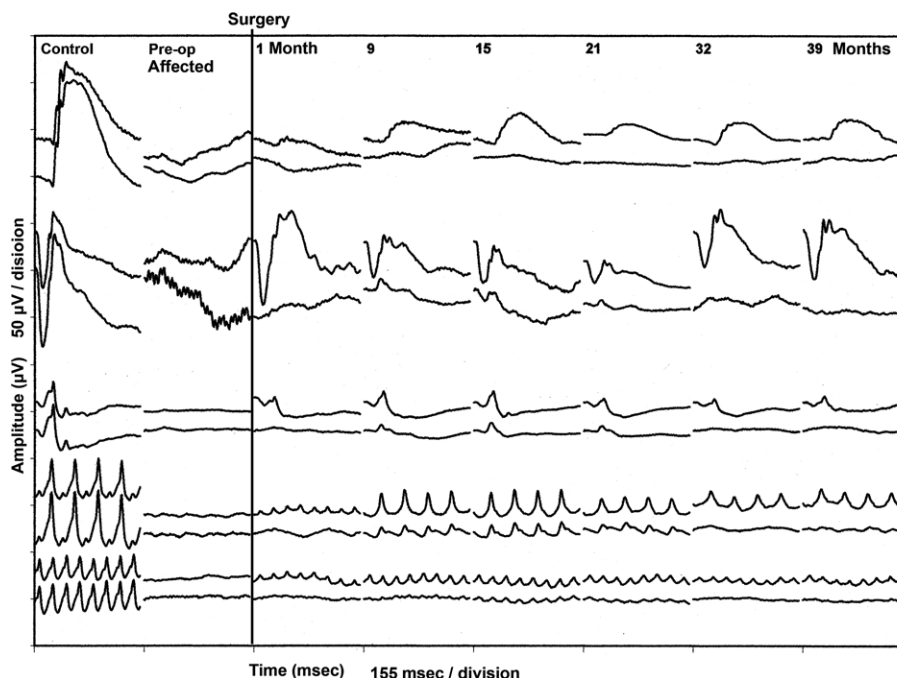


Fig. 5. The actual pre- and post-operative ERG recordings from an RPE65 null mutation dog treated subretinally at the age of 12 months with a gene construct injected subretinally into the right eye. To the far left is a normal control dog injected subretinally with similar amounts of saline also into the right eye. Upper recording from each set of two recordings is from the right, treated eye. Note the increased amplitude responses seen already 1 month after gene transfer surgery from the right, treated eye. Note also that responses are sustained throughout the study period. From the top to bottom in each column, dark-adapted recordings are shown from three different light intensities: 0.01 cd s/m², 1 cd s/m² and 4 cd s/m², respectively, and thereafter photopic recordings from single flashes at 1 cd s/m² light intensity stimulation, then the same light intensity stimulation at 30 and 50 Hz flicker after 10 min of 30 cd/m² background light adaptation.

Clinical trials with transplantation of fetal retina sheets to human patients with Retinitis Pigmentosa (RP) or age-related macular degeneration have been initiated. These studies have included safety evaluations in five patients with advanced disease (“light perception patients”) (Radtke et al., 2002). Currently patients with less reduced visual capacity are being operated and evaluated using sheet transplants. It has been shown that donor tissue is well tolerated in the subretinal space, an immune-privileged site, and can survive without immuno-suppression and is safe to use in human patients. Since retinal sheet transplants have proven to have beneficial effects in several types of animal models of retinal degenerations, including the rat, *rd* mouse and cat, transplantation has thus been established as a realistic treatment modality in end-stage retinal degenerative disease (Aramant and Seiler, 2004). Time will tell if retinal transplantation will result in useful functional vision. An RP patient with 20/800 vision at the time of transplantation improved her visual acuity to 20/160 one year after surgery, while her 20/400 vision in the non-operated eye remained the same. She maintained her improved vision for at least 2.5 years post-operatively (Radtke et al., 2004).

3.4. Stem cell therapy for inherited retinal dystrophies

In general terms, stem cells can be defined as tissue-specific ancestral cell types associated with a specific tissue and these tissue-specific cells have the capacity of self-renewal

(Ahmad, 2001). The latter means that these cells, also called progenitor cells, have the ability of generating a large number of identical multipotent progeny by clonal amplification. Multipotent neuronal progenitor cells can be derived from the mammalian forebrain (Reynolds and Weiss, 1992) and the ocular neuroepithelium, the latter specifically in the ciliary epithelium (Perron and Harris, 2000). Transplanted neural progenitor cells have been shown to migrate extensively throughout the degenerating mammalian retina, differentiate into neurons, and extend processes into the inner retina and even into the optic nerve (Young et al., 2000).

Even though there have been clear demonstrations of graft survival and morphologic development in situ of retinal cell transplants using fetal eye tissue (Fig. 6), and even simple visual functional responses obtained to light stimulation after transplantation (Klassen and Lund, 1990), there have been difficulties in establishing substantial graft-host connectivity in the retina after retinal cell transplantation (Zhang et al., 2003). These problems have frequently been attributed to the formation of a reactive glial barrier to regeneration (Gouras et al., 1992). Important in this regard is that functional benefits have been reported when instead using neural progenitor cells (Teng et al., 2002). Transplanted neural progenitor cells have not only been shown to integrate into the dystrophic retina of mature rodents (Young et al., 2000), but also to differentiate into photoreceptors after transplantation to retinal dystrophic mice (Klassen et al., 2004).

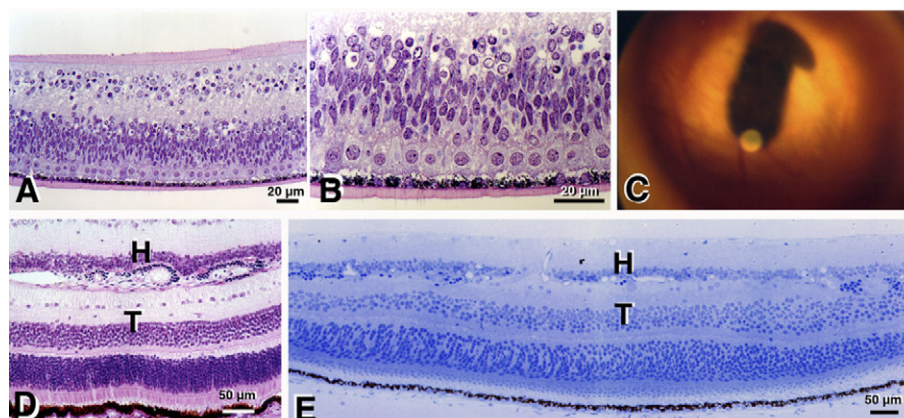


Fig. 6. Donor tissue and morphology of neuroretina and retinal pigment epithelial co-grafts of human fetal retina (A and B) transplanted to athymic nude rat fundus (C). The pigmented transplant is seen in the albino rat fundus. Histology 8.5 months (D) and 8.9 months (E), respectively, after transplantation (Aramant and Seiler, 2002. Used with permission).

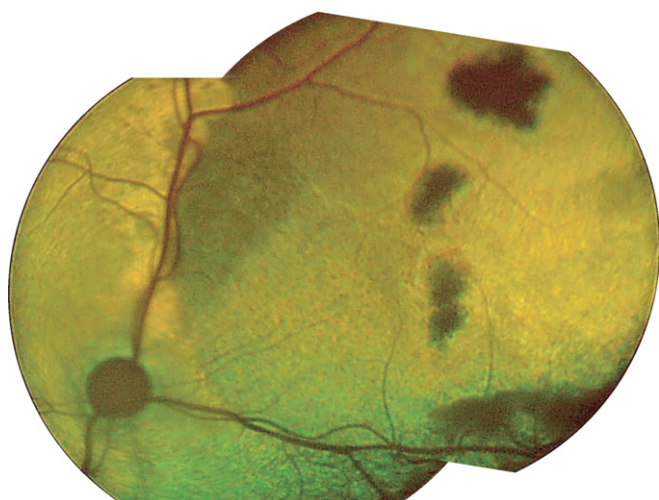


Fig. 7. Composite of fundus photos of stem cell treated cat affected with moderately advanced stage of hereditary retinal degeneration. A suspension of cat neuronal precursor cells was injected subretinally into the superior tapetal nasal fundus. In this area darkly pigmented lesions are observed, some in which stem cell growth was found, observed 4 weeks after surgery. For additional details see Klassen et al. (2005).

It appears that migration and integration of cells are enhanced in traumatized or degenerating retinas as opposed to what is the case in normal retinas (Fig. 7). Further, behavior studies have indicated that grafted progenitor cells ameliorate the loss of luminance detection in dystrophic mice at low light levels. This preservation of visual function was observed for a 25-week period and simultaneously a limited survival of photoreceptors was observed. These findings are extremely encouraging in the search for methods of rescuing photoreceptors and, especially in search for methods of replacing degenerated visual cells.

3.5. Artificial retinal prostheses

Another treatment modality that is being developed to restore vision in patients that were blinded by photoreceptor diseases is intraocular implantation of retinal prosthe-

ses. More specifically, the prostheses are usually implanted epiretinally or subretinally, with the former usually fixed to the scleral wall with tacks. The electrodes on these prostheses emit electrical currents that stimulate bipolar or ganglion cells that remained functional despite the destruction of the photoreceptors. These bipolar and ganglion cells then transmit the signal through the afferent visual pathways, as they would do in a normal eye, and produce a visual sensation.

Evidence of prosthesis functionality is the fact that stimulation of the retinal electrodes elicits recordable electrical activity in the visual cortex of implanted patients (Chowdhury et al., 2005; Walter et al., 2005). These electrically induced visual sensations are called *phosphenes*, and are commonly described as small spots of light in the visual field (Brindley and Lewin, 1968). Retinal prostheses have been successfully implanted in both cats (Chowdhury et al., 2005; Walter et al., 2005) and dogs (Güven et al., 2005) (Fig. 8).

Obviously, in order for the patient to experience (or visualize) more than just spots of light, the prosthesis should be stimulated by light and images. This stimulation may be achieved in a number of ways. A simple approach, adopted in subretinal prostheses, is to mount photodiodes (that capture incoming light and transduce its energy to currents) on the prosthesis. Epiretinal prostheses are more sophisticated, and usually rely on visual input from a small camera that is mounted on the head or on goggles. Real time images of the world, together with the power required for operation, are transmitted to the prosthesis to elicit vision.

Though retinal prosthesis research made considerable progress during the last decade, numerous problems remain. These include the biocompatibility of the prosthesis material; the effect of long-term electrical stimulation on the stimulated retinal tissue; and possible erosion or breakdown of the implant, which would require repeated surgery for replacement (Loewenstein et al., 2004). Also, since retinal implants rely on a functional inner retina, they cannot



Fig. 8. Fundus photo from a cat implanted with a subretinal microphotodiode array or artificial silicon retina device (TM) produced by Optobionics Corporation. Follow-up time is 9 months. The darkly pigmented area adjacent and superior to the implant is probably a tapetal alteration due to minor pressure changes during the surgical procedure. For additional details see Pardue et al. (2001). Figure courtesy of Dr. Mabelle Pardue.

be used in patients blinded by glaucoma or optic nerve disease. Such patients may benefit from cortical implants (Normann et al., 1999).

However, the most complex issues remain the related problems of implant power requirements and the heat that is generated. Studies have shown that the implant temperature may rise by as much as 0.8 °C, with potentially disastrous effects on the neighboring retina (Gosalia et al., 2004). The potential thermal injury limits the number of electrodes that can be mounted on the retinal prosthesis. This, in turn, limits the visual resolution and acuity of the “image” generated by the prosthesis. It has been estimated that an array of 60 electrodes is needed for spot reading and object recognition (Loewenstein et al., 2004). And while today there are already designed prosthetic systems with a stimulating pixel density of up to 2500 pix/mm², corresponding geometrically to a maximum visual acuity of 20/80 (Palanker et al., 2005), most devices currently implanted in vivo have an array of 16–25 electrodes. Although the resulting image that can be produced by such prostheses is very crude, they have been implanted in humans blinded by advanced RP (Humayun et al., 2003; Chow et al., 2004). Following surgery, the patient was able to detect room light, locate a flashlight and perceive motion (but not shape) (Humayun et al., 2003). This may not sound like much, but to the human subject who has been blind for 10 years, this perception offers a glimmer of hope.

3.6. Neuroprotection strategies for inherited retinal dystrophies

Neurotrophic factors, the molecules that promote neuronal survival and cell division, can also be used for treat-

ment of inherited retinal dystrophies (Ip and Ynacopoulos, 1996). Specifically, nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), ciliary neurotrophic factor (CNTF), neurotrophin 3 (NT3), neurotrophin 4–5 (NT4-5) and the growth factors; fibroblast growth factor (FGFs), interleukin (IL)-1b, transforming growth factor (TGF-β2) have been delivered intraocularly in animal models for retinal degenerative disease (Dejneka and Bennett, 2001). For instance, a CNTF analogue was injected intravitreally in the dominantly inherited feline rod cone dysplasia model (Rdy) (Leon and Curtis, 1990). Injected kittens were 1.5–5.5 weeks old (Chong et al., 1999). Results from morphometric and immunocytochemical studies showed significantly increased photoreceptor cell survival up to age 17.5 weeks.

In recent years a new technology has been developed, that of encapsulated cell-based delivery of neurotrophic factors. Using this technique, initial studies were performed in dogs for delivery of CNTF (Tao et al., 2002) into the vitreous. Results showed sparing of photoreceptors in the *rd1* mutation dog with early onset retinal degeneration. Presently safety studies using similar techniques are ongoing in human RP-patients (Sieving et al., 2007).

Stem cells can be maintained in culture and manipulated. These genetically engineered progenitor cells can be used to target gene products to sites of retinal degeneration (Ahmad, 2001). The gene products can also include survival-promoting factors, such as FGF2, NGF, CNTF and BDNF (Lambiase and Aloe, 1996; LaVail et al., 1998; Cayoette et al., 1998; Clarke et al., 2000). All have been shown to significantly slow the process of cell death in animal models of retinal degeneration. Similarly, neuroprotectants can be delivered by gene transfer, in suitable constructs injected intraocularly (Di Polo et al., 1998).

Finally, an approach that may prove effective is the combination of ex vivo gene delivery with cell transplantation. Retinal pigment epithelium cells for example, are easily harvested, grown in culture, infected and then transplanted to the subretinal space (Lai et al., 1999), near the dying photoreceptors. By infecting such cells with a therapeutic virus, carrying neurotrophic factors, neuronal cell survival may be promoted when the cells are returned to their native surroundings. Transplanted cells can thus be engineered to deliver secreted trophic factors.

3.7. Nutritional supplementation for hereditary retinal disease

Nutritional intervention is currently an important subject of research in both veterinary and human medicine. There is increasing awareness that supplementation with dietary antioxidants, such as vitamin E and beta-carotene, together with omega-3 fatty acids enhance the development of cognitive and visual function (Milgram et al., 2002). There are results that indicate that maintenance of foods fortified with mixtures of antioxidants can partially counteract the deleterious effects of aging and appears to play

a role for longevity. Studies have shown significantly increased life span of cats on a supplemented diet (Cupp et al., 2006), as well as positive indicators of reduced disease incidence and improved intestinal health.

A series of human clinical trials have been testing nutritional and other supplements as potential therapies for RP. Since docosahexanoic acid (DHA) has been shown to play a demonstrable role in the development of vision in infants (D. Birch: presentation at the Retina International Scientific and Medical Advisory Board, Fort Lauderdale, 2005) the possible role of DHA levels in patients with severe forms of hereditary retinal degeneration was studied clinically. During a 4-year period patients on DHA showed less change in funduscopic progression of disease, although cone function tested in supplemented and in placebo groups showed no statistical significant benefit between groups (Hoffman et al., 2004). Further, supplementation with vitamins A and E has been investigated in RP patients. The results showed a beneficial effect of 15,000 IU/day of vitamin A, but suggested an adverse effect of 400 IU/day of vitamin E on the clinical course of RP (Berson et al., 1993). RP patients receiving a combination of vitamin A (dosage as above) and 12 mg/day of DHA, an omega-3 fatty acid, did not show a reduction in the progression of disease (Berson et al., 2004).

4. Conclusions and future directions

Improved understanding of the pathogenesis of vision loss has led to the development of new and exciting treatment modalities. Unraveling the molecular mechanisms responsible for ganglion cell death in glaucoma patients led to neuroprotective treatment which preserves retinal function in patients. Similarly, with the elucidation of causative mutations and their detrimental effects on retinal cell function, much needed new discoveries and insights into retinal degenerative disease mechanisms have been gained. It is now possible to aim therapies at correcting disease mutations directly or indirectly in the eye: proof of principle was obtained through the successful corrective gene therapy performed in dogs blinded by hereditary retinal dystrophy and degeneration.

Future research will be focused in more than one direction; gene therapy will continue to be a major area for further work into the correction of disease processes in which the specific mutation is known. For other diseases in which the precise mechanism has been elucidated but not the specific gene defect, neuro-protective therapy will probably evolve further. For those diseases nutritional supplementation will be initiated as well. For end stage retinal degenerations cell replacement strategies, such as retinal transplantation, stem cell therapies and the application of artificial retinal prosthesis will be further developed. There definitely is some light at the end of the tunnel!

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