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ROLE OF BDNF FOR THE TREATMENT OF GLAUCOMA AND RETINITIS PIGMENTOSA

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ABSTRACT

Primary open-angle glaucoma (POAG) is the most common form of glaucoma, representing up to 90% of cases. POAG is described as a multifactorial optic neuropathy that is chronic and progressive with a characteristic acquired loss of optic nerve fibres. The cause for the elevated is generally IOP accepted to be decreased facility of aqueous outflow through the trabecular meshwork**.** Retinitis pigmentosa (RP) is a group of relatively rare, inherited disorders characterized by progressive peripheral vision loss and night vision difficulties (nyctalopia) that can lead to central vision loss due to the degeneration of the rod photoreceptor cells in the retina. RP manifests initial symptoms independent of age; thus, RP diagnosis occurs anywhere from early infancy to late adulthood. Brain-derived neurotrophic factor (BDNF) is a 14 kDa protein belonging to the neurotrophin family of growth factors. BDNF has trophic functions in the hippocampus, cortex, and basal forebrain as well as the retina, motor neurons, the kidneys, saliva and the prostate. The notion that BDNF may have a role in treating the neurodegenerative aspects of POAG is underpinned by convincing preclinical evidence in animal models of glaucoma that BDNF administered intravitreously can, at least in part, rescue retinal ganglion cells (RGCs) under conditions such as those induced by increased intraocular pressure (IOP). Overall and despite a mechanistically sound rationale, the pharmacokinetic challenges indicate that instilled BDNF topical administration is associated with higher than normal risks, likely explaining the emphasis on alternative approaches (such as synthetic small molecule BDNF analogues or gene therapy approaches) in the last decade. Moreover, the high BDNF doses required to drive efficacy (and low target tissue penetration) also raise concerns over promotion of tumour growth resulting from BDNF taken up from non-retinal tissues. In conclusion, even if preliminary evidences are available, further studies are necessary in order to clarify the role of BDNF for the treatment of glaucoma and retinitis pigmentosa.

KEYWORDS: Brain-derived neurotrophic factor, Glaucoma, Retinitis Pigmentosa, increased intraocular pressure, Neurotrophins.

INTRODUCTION

Primary Open-Angle Glaucoma (POAG). Primary open-angle glaucoma (POAG) is the most common form of glaucoma, representing up to 90% of cases. POAG is described as a multifactorial optic neuropathy that is chronic and progressive with a characteristic acquired loss of optic nerve fibres. This loss develops in the presence of open anterior chamber angles, characteristic visual field abnormalities, and increased intraocular pressure (IOP). POAG manifests by cupping and atrophy of the optic disc, in the absence of other known causes of glaucomatous disease.[1,2] The exact cause of glaucomatous optic neuropathy is not known, although many risk factors have been identified, including the following: elevated IOP, family history, race, age older than 40 years and myopia. Elevated IOP is the most studied of these risk factors because it is the main clinically treatable risk factor for glaucoma and may cause neuropathy *via* vascular dysfunction causing ischemia to the optic nerve or cribriform plate compression of the axons. Other factors may include excitotoxic damage from excessive retinal glutamate, deprivation of neuronal growth factors, peroxynitrite toxicity from increased nitric oxide synthase activity, immune-mediated nerve damage and oxidative stress. The exact role that IOP plays in combination with these other factors and their significance to the initiation and progression of subsequent glaucomatous neuronal damage and cell death over time is still under debate.[3,4]

Several large studies[4,5] have shown that as IOP rises above 21 mmHg, the percentage of patients developing visual field loss increases sharply, most notably at pressures higher than 26-30 mmHg. A patient with an IOP of 28 mmHg is about 15 times more likely to develop field loss than a patient with a pressure of 22 mmHg. Therefore, a patient population of those with elevated IOP should not be thought of as homogeneous. Disc cupping and nerve fibre layer losses of up to 40% have been shown to occur before actual visual field loss

has been detected. Therefore, visual field examination cannot be the sole tool used to determine when a patient has begun to sustain undeniable glaucomatous damage and it should not be used in isolation as the benchmark for treatment.[6]

The cause for the elevated is generally IOP accepted to be decreased facility of aqueous outflow through the trabecular meshwork. This increase in resistance to flow may be related to an obstruction of the trabecular meshwork by accumulated material, loss of trabecular endothelial cells, reduction in trabecular pore density and size in the inner wall endothelium of the Schlemm canal, loss of giant vacuoles in the endothelium of the Schlemm canal, loss of normal phagocytic activity or a combination of these factors. Other processes may involve altered corticosteroid metabolism, dysfunctional adrenergic control, abnormal immunologic processes and oxidative damage to the meshwork.

Estimates of the prevalence of glaucoma suggest that glaucoma is the second cause of irreversible blindness, after macular degeneration. As of 2010, there were 44.7 million people in the world with open angle glaucoma.[7] In a Caucasian population, the incidence of new onset of glaucomatous damage in previously unaffected patients is 2.6-3% for IOPs 21-25 mm Hg, 12-26% incidence for IOPs 26-30 mm Hg, and approximately 42% for those higher than 30 mm Hg. Visual field loss can be expected to develop in about 3% of subjects over 10 years of follow up without treatment.[5] Risk increases with age and IOP.[6] Prevalence of POAG is 3-4 times higher in Africans than in Caucasians and up to 6 times more susceptible to optic disc nerve damage than Caucasians as a result of a higher prevalence of larger cup-to-disc ratios in the normal African population. Reports on gender predilection are less clear, while age is an independent risk factor for POAG; up to 15% of people will be affected by the seventh decade of life, even after compensating for the slow increase in IOP slowly with increasing age.

Retinitis Pigmentosa (RP). Retinitis pigmentosa (RP) is a group of relatively rare, inherited disorders characterized by progressive peripheral vision loss and night vision difficulties (nyctalopia) that can lead to central vision loss due to the degeneration of the rod photoreceptor cells in the retina. RP manifests initial symptoms independent of age; thus, RP diagnosis occurs anywhere from early infancy to late adulthood.[8] Patients in the early stages of RP first notice compromised peripheral and dim light vision due to the decline of the rod photoreceptors. The progressive rod degeneration is followed by abnormalities in the adjacent retinal pigment epithelium (RPE) and the deterioration of cone photoreceptor cells. As peripheral vision becomes increasingly compromised, patients experience progressive tunnel vision and eventual blindness.[9] Affected individuals may additionally experience defective light-dark adaptations, nyctalopia (night blindness) and the accumulation of bone spicules in the fundus.

RP can be non-syndromic or related to other neurological disorders, developmental disturbances or secondary to systemic diseases.[10] Genetically, RP constitutes a wide variety of retinal dystrophies and retinal pigment epithelium (RPE) dystrophies. More than 40 different genes for isolated RP and more than 50 different genes for syndromic RP have been characterised. Not only is the genotype heterogeneous, but patients with the same mutation can phenotypically have different disease manifestations. RP occurring with congenital or progressive with deafness is called Usher syndrome; RP is often seen together with ophthalmoplegia, dysphagia, ataxia and cardiac conduction defects in the mitochondrial DNA disorder Kearns-Sayre syndrome (ragged red fibre myopathy). A-betalipoprotenaemia can present with mental retardation, peripheral neuropathy, acanthotic RBCs, ataxia and steatorrhea. RP may also present as part of the clinical picture in McLeod syndrome, an X-linked recessive phenotype characterized by a complete absence of XK cell surface proteins, hypogonadism and developmental delay with an autosomal recessive inheritance pattern (Bardet-Biedl syndrome), neuro-syphilis, toxoplasmosis, Waardenburg and Alport syndromes, Refsum disease etc. RP can be passed on by all types of inheritance: approximately 20% of RP is autosomal dominant, 20% is autosomal recessive and 10% is X linked, while the remaining 50% is sporadic.[10]

RP is typically thought of as a rod-cone dystrophy in which the genetic defects cause apoptosis, predominantly in the rod photoreceptors; less commonly, the genetic defects affect the RPE and cone photoreceptors.[11] Regardless of aetiology, the final common pathway remains photoreceptor cell death by apoptosis. The first histologic change found in the photoreceptors is shortening of the rod outer segments. The outer segments progressively shorten, followed by loss of the rod photoreceptor. This occurs most significantly in the mid-periphery of the retina. These regions of the retina reflect the cell apoptosis by having decreased nuclei in the outer nuclear layer. In many cases, the degeneration tends to be worse in the inferior retina. The histological hallmark is loss of the rod photoreceptors. As rods are most densely found in the mid-peripheral retina, cell loss in this area tends to lead to peripheral vision loss and night vision loss. Cone photoreceptor loss occurs in a similar manner, with shortening of the outer segments followed by cell loss.

Prevalence is reported to 1:4,000-5,000.[12] No specific gender predilection exists; X-linked RP is expressed only in males; therefore, because of these X-linked varieties, men may be affected slightly more than women. The age of onset can vary. RP usually is diagnosed in young adulthood, although it can present anywhere from infancy to the mid-30s to 50s.

Current Standard of Care. Current therapy for POAG is limited to lowering the IOP. However and despite several large studies[3,5,7], no simple consensus exists in terms of what is an appropriate IPO target for preventing or delaying POAG in the absence of other risk factors. Most investigators agree that an initial goal of 20-25% reduction in IOP is beneficial, but also that the target IOP should be set independently for each patient, depending on the risk factors (age, race, medical status, concomitant medication etc). The target IOP should be re-evaluated periodically and regular review of IOP trends should be performed to determine whether the patient is maintaining the target level.

First-line treatment comprises topical pharmacological monotherapy. If one medication is insufficient to reach the target pressure, a second medication with a different mechanism of action is added. Common first-line agents include beta-adrenergics such as timolol or levobunolol, adrenergic agonists such as brimonidine or apraclonidine, carbonic anhydrase inhibitors (*e.g.* dorzolamide). Combinations can include latanoprost/timolol or a beta-blocker/alpha agonist combination (*e.g.* brimonidine/timolol). Non-selective sympathomimetics (*e.g.* dipivefrin, epinephrine, memantine) are also used.

Surgery is indicated in POAG as a second-line therapy when neuropathy worsens (or is expected to worsen) at any given level of intraocular pressure and the patient is on maximum tolerated pharmacological therapy. Laser trabeculoplasty, drainage implants (i.e. seton/tube/shunt) surgery or ciliary body ablation have all been used and provide shorter- or longer-term benefit but are all associated with moderate risks.[13,14] Newer techniques that hold potential as surgical options in primary open-angle glaucoma include deep sclerectomy/viscocanalostomy with or without collagen implant and 360-degree suture canaloplasty are being evaluated.

No therapy for RP is available and treatment is supportive (psychological and situational).[12,15] A comprehensive epidemiologic study concluded that very high daily doses of vitamin A palmitate (15,000 U/day) slow the progress of RP by about 2% per year. The effects are modest; therefore, this treatment must be weighed against the uncertain risk of long-term adverse effects from large chronic doses of vitamin A.[16,17] Trials with docosahexaenoic acid (DHA)[18], acetazolamide[19], corticosteroids, calcium channel blockers such as diltiazem, lutein/zeaxanthin, valproate have been essentially negative. Isotretinoin, sildenafil and vitamin E are believed to exacerbate RP and should be avoided.

BDNF Background. Brain-derived neurotrophic factor (BDNF) is a 14 kDa protein belonging to the neurotrophin family of growth factors. BDNF has trophic functions in the hippocampus, cortex, and basal forebrain[20-24] as well as the retina, motor neurons, the kidneys, saliva and the prostate.[25] The trophic effects of BDNF are mediated *via* the tropomyosine receptor kinase B (TrkB) and possibly also the low-affinity nerve growth factor receptor (LNGFR, aka p75).[26] BDNF may also modulate the activity of the alpha-7 nicotinic receptor.[27] Functionally, BDNF has important functions in synaptic transmission by modulating NMDA receptor activity[28-30] and by suppressing post-synaptic GABAergic signalling.[31] In addition, BDNF promotes neurogenesis by promoting neural stem cell and neural progenitor cell proliferation through Akt activation and PTEN inactivation[32] BDNF also promotes neuronal differentiation.[33,34] Against this background, it is not surprising that BDNF-signalling has been implicated in a range of neurodegenerative conditions (Alzheimer's[35], Huntington's[36], Rett[37]) and psychiatric conditions (depression[38], schizophrenia[39], obsessive-compulsive disorder[40] and anorexia nervosa.[41]

BDNF and POAG. The notion of a neuroprotectant role for BDNF in POAG derives from the observation that death of retinal ganglion cells (RGCs) induced by axotomy of optic nerves is rescued by intravitreal BDNF administration in rodents[42,43] and cats.[44] RGCs and optic nerve fibres express TrkB in the adult retina[45-48] and it is known that BDNF is transported by both antero-grade and retrograde axonal transport in these cells.[49-53] A major destructive effect of increased or fluctuating IOP is deformation of the *lamina cribrosa*, mechanically compressing RGC axons. This reduces or blocks the retrograde transport of essential neurotrophic factors such as BDNF. A lack of appropriate target-derived trophic support is therefore thought to cause cells to undergo apoptotic degeneration in a manner similar to neuronal death during embryonic development or following spinal cord injuries. Supplementation of these neurotrophic factors has been suggested to protect neurons from such degeneration.

Experimentally, the neuroprotective role of BDNF in retinal degeneration was first reported in 1993 by Mey and Tanos[54], who performed intravitreal injections of BDNF in axotomised RGCs from adult rats. This obser-vation was reproduced by other groups[*e.g.* 42,55,56], but results also triggered scepticism regarding the magnitude and duration of exogenously administered BDNF[57-59]; these latter workers also attempted co-administration with radical scavengers, gene therapy and more long-lasting synthetic TrkB agonists and despite positive data in these animal models, no clinical studies with topical instillation of BDNF have been carried out.

Domenici *et al.*[60] investigated the utility of exogenous BDNF in the DBA/2J mouse, which develops chronic IOP elevation and subsequent loss of vision. Visual function was monitored using electroretinography (ERG) and visual cortical evoked potentials (VEPs). RGC alterations were assessed using Brn3 immunohistochemistry and confocal microscope analysis. In these studies, rhBDNF was dissolved in

physiological saline solution and administered both by intravitreal injection and by topical instillation. A progressive decline in ERG and VEP parameters was seen in control animals between 4 and 7 months of age, with clear correlations to the IOP and the reduction of Brn3 immunopositive RGCs. Following repeated administration (once every four days over two weeks for intravitreal; every other day for two weeks for topical instillation), visual responses were significantly improved in terms of ERG, VEP and the number of Brn3 immunopositive RGCs, independently of reduction in IOP.

The same group reported the effects of topical instillation of BDNF on de-generative responses to continuous light exposure in albino rats. Results suggest that BDNF before light exposure prevented impairment of both ERG- and histological parameters.[61] In these studies, BDNF was formulated both in physiological saline, carboxymethyl cellulose (CMC) and the tamarind seed polysaccharide (TSP), the currently proposed and patented vehicle-BDNF combination. Pharmacokinetic results suggest that the relative retinal bioavailability of the TSP-BDNF is approximately twice that of a saline solution, possibly on account of an increased mean residence time caused by the higher viscosity of the TSP.

CONCLUSION

Rationale for BDNF in POAG. The notion that BDNF may have a role in treating the neurodegenerative aspects of POAG is underpinned by convincing preclinical evidence in animal models of glaucoma that BDNF administered intravitreously can, at least in part, rescue RGCs under conditions such as those induced by increased IOP. However, given that POAG is a chronic condition, developing over several years, the prospect of chronic, intravitreous administration of BDNF is not realistic.

As an alternative, some groups propose to deliver BDNF by topical instillation via a transconjunctival route, which however poses significant pharmacokinetic challenges; to reach the posterior retinal target, BDNF - a poorly permeable protein - has to cross several barriers (the conjunctiva, the sclera, the choroid and the retinal pigment epithelium) to reach the target tissue. To deliver therapeutically meaningful concentrations, a steep concentration gradient is required, translating into high and sustained concentrations at the point of dosing (the cornea/conjunctiva). While this route of administration is well established for highly permeable and lipophilic low molecular weight molecules like timolol or latanoprost, BDNF has physico-chemical properties entirely unsuitable for instilled eye delivery. This also becomes evident from the data from ITH, showing that a 500-fold higher dose is required to achieve comparable retinal concentrations by the instilled route as compared to the intravitreous route.

In an attempt to increase the retinal bioavailability by increasing the mean residence time at the conjunctival mucosa, BDNF was formulated in tamarind seed polysaccharide (TSP), a viscous and highly branched polysaccharide consisting of a cellulose-like backbone that carries xylose and galactoxylose substitution at the glucan chain. TSP has achieved GRAS status[62] and has been proposed as an excipient for various topical ocular therapeutics, but appears not to be the constituent of any registered drug products. The TSP-BDNF combination appears to confer a slightly higher relative bioavailability than normal saline, but is still in the region of 0.5% vs. intra-vitreal administration. Another limiting factor is the geometric differences in eye size between rodents and humans (a human eye is 10-fold bigger than a rat eye and 50-fold bigger than a mouse eye), suggesting that even higher doses are required to maintain the concentration gradient required to drive penetration of therapeutic BDNF levels to the retina. Overall, and despite a mechanistically sound rationale, the pharmacokinetic challenges indicate that instilled BDNF topical administration is associated with higher than normal risks, likely explaining the emphasis on alternative approaches (such as synthetic small molecule BDNF analogues or gene therapy approaches) in the last decade.

The high BDNF doses required to drive efficacy (and low target tissue penetration) also raise concerns over promotion of tumour growth resulting from BDNF taken up from non-retinal tissues. Tumorigenicity is a concern with all growth factors, and BDNF has been associated with tumour promotion, poor prognosis and more invasive disease across a wide range of malignancies, including squamous cell-, colorectal-, hepatocellular-, breast- and cervix cancer as well as neuroendocrine tumours.[63-70] Tumour promotion with other growth factors used topically has been de-scribed and can occur also at sites distal from the site of administration.

For both POAG and RP and like any other slowly progressive neurodegenerative disease, it is challenging to identify clinical outcome measures for use in shorter-term proof-of-concept studies; most likely, the only acceptable endpoint for regulatory approval in POAG will likely be demonstration of slowing of progression of visual field loss, which will presumably require studies of long (more than 2 years) duration.[71] Mechanistic markers could include ERG or cup-to-disc ratio by fundoscopy; the predictivity of these measures to clinical outcome is however uncertain. Another complicating factor is the obligate co-therapy with an IOP-lowering medication, which may introduce confounding factors.

In conclusion, even if preliminary evidences are available, further studies are necessary in order to clarify the role of BDNF for the treatment of glaucoma and retinitis pigmentosa.

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