Review Article

Neuroprotection in Glaucoma

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Abstract

Glaucoma is a degenerative optic neuropathy characterized by retinal ganglion cell (RGC) loss and visual field defects. It is known that in some glaucoma patients, death of RGCs continues despite intraocular pressure (IOP) reduction. Neuroprotection in the field of glaucoma is defined as any treatment, independent of IOP reduction, which prevents RGC death. Glutamate antagonists, ginkgo biloba extract, neurotrophic factors, antioxidants, calcium channel blockers, brimonidine, glaucoma medications with blood regulatory effect and nitric oxide synthase inhibitors are among compounds with possible neuroprotective activity in preclinical studies. A few agents (such as brimonidine or memantine) with neuroprotective effects in experimental studies have advanced to clinical trials; however the results of clinical trials for these agents have not been conclusive. Nevertheless, lack of compelling clinical evidence has not prevented the off-label use of some of these compounds in glaucoma practice. Stem cell transplantation has been reported to halt experimental neurodegenerative disease processes in the absence of cell replacement. It has been hypothesized that transplantation of some types of stem cells activates multiple neuroprotective pathways via secretion of various factors. The advantage of this approach is a prolonged and targeted effect. Important concerns in this field include the secretion of unwanted harmful mediators, graft survival issues and tumorigenesis. Neuroprotection in glaucoma, pharmacologically or by stem cell transplantation, is an interesting subject waiting for broad and multidisciplinary collaborative studies to better clarify its role in clinical practice.

Keywords: Brimonidine; Ginkgo Biloba Extract; Glaucoma; Memantine; Neuroprotection; Stem Cell Transplantation

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INTRODUCTION

Glaucoma is a kind of degenerative optic neuropathy characterized by retinal ganglion cell (RGC) loss and visual field defects.[1] Although high intraocular pressure (IOP) is considered as the most important risk factor for the development of glaucoma, it is neither necessary nor sufficient. RGC loss continues in spite of IOP reduction in some glaucoma patients.[2] The risk of unilateral blindness in patients with treated open-angle glaucoma is estimated to be around 27%, which is higher than previously expected.[3] Thus, IOP reduction may not be sufficient for some glaucoma patients.

The pathophysiology of glaucoma is not completely understood. Clinically, there is progressive loss of the retinal nerve fiber layer (RNFL)[4] leading to axonal degeneration and characteristic optic nerve head cupping.[5] The most susceptible cell to glaucomatous damage is the RGC, which is located in the inner

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retina,[6,7] the axons of which constitute the RNFL and merge to form the optic nerve.

One of the areas of great interest in glaucoma is how RGC death occurs.[8] The molecular basis of RGC death stems from investigations on animal models of glaucoma. Deprivation of neurotrophic factors,[9] elevated concentrations of excitatory aminoacids such as glutamate,[10] and oxidative stress[11] may contribute to RGC apoptosis [Figure 1].

IOP reduction *per se* can prevent or delay RCG death in glaucomas and therefore is indirectly neuroprotective. However, neuroprotection in glaucoma is defined as any intervention, independent of IOP reduction, that can prevent RGC death. Several natural and synthetic compounds, have been reported to possess neuroprotective properties. Neuroprotection can affect glaucoma by direct protection of RGCs or neutralization of the deleterious effects of toxic factors. The present article reviews current evidence on neuroprotective compounds in the treatment of glaucoma.

GLUTAMATE ANTAGONISTS

Glutamate-induced exitotoxicity has been implicated as a common pathogenic mechanism in a broad variety of neurological diseases, including Alzheimer's disease and glaucoma.[12-14] The detrimental effect of glutamate on RGCs has been documented by exposing the retina to high glutamate levels both *in vitro*[15] and *in vivo*.[16] This effect of glutamate on RGCs occurs through interaction with glutamate receptors. Excitatory receptors are abundant in RGCs.[17] However, under normal conditions, homeostatic mechanisms prevent overexpression of the receptors.[18] Glutamate-induced excitotoxicity develops when extracelullar glutamate levels are increased.[19] Accumulation of excessive glutamate results in overstimulation of N-methyl-D-aspartate (NMDA) receptors, which in turn causes intracellular calcium influx leading to the activation of a complex cascade which attacks cell components and produces free radicals,[20] followed by programmed cell death or apoptosis.[21-23]

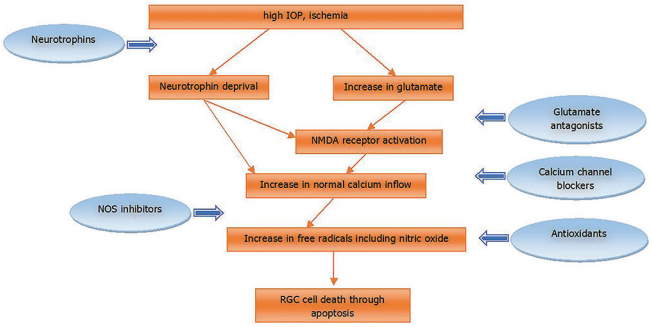

Figure 1. Simplified pathway of RGC death and assumed mechanisms of neuroprotective agents. IOP, intraocular pressure; NMDA, n-methyl-D-aspartate; NOS, nitric oxide synthase; RGC, retinal ganglion cell.

It has been shown in experimental models that after acute IOP elevation, there is an increase in intraocular glutamate levels.[24] In addition, analysis of the composition of vitreous fluid from dogs with glaucoma, experimental monkey glaucoma models and glaucomatous human eyes have revealed high levels of glutamate.[25,26] However, Carter-Dawson et al found normal levels of glutamate in the vitreous of monkeys with experimental glaucoma.[27] Based on these observations, inhibition of glutamate activity by modulation of NMDA-type receptors has been advocated as an important strategy for neuroprotection.[28]

MK801 (dizocilpine maleate), an uncompetitive NMDA antagonist, may be the most potent glutamate inhibitor[29] and neuroprotective agent in experimental glaucoma.[29,30] Nevertheless, because of the high affinity of the compound for the NMDA receptor, its long half-life and interference with normal physiologic functions of glutamate, MK801 is neurotoxic[31] and has never been evaluated in higher-level clinical trials.[32,33]

Memantine is a selective, non-competitive blocker of the NMDA receptor with moderate affinity.[28] In a study to assess the effect of glutamate and its antagonist (memantine) on RGCs, three groups of rats were studied; in the first group, the animals received serial intravitreal injections of glutamate to induce chronic elevations in glutamate levels. The second group of rats was treated with intraperitoneal memantine and glutamate, while the third group received vehicle injection with or without concurrent memantine. After 3 months, RGC survival was evaluated: Intravitreal injections of glutamate had raised its intravitreal levels up to 3 to 5 times its normal endogenous concentration. Glutamate elevation caused death of 42% of RGCs after 3 months. When memantine was administered alongside low-dose glutamate, it exhibited a partial protective effect against glutamate toxicity. However, memantine treatment alone, without concurrent injection of glutamate had no effect on ganglion cell survival.[22]

Although subsequent preclinical and experimental research with memantine appeared promising,[23,34,35] the phase III randomized, double-masked, placebo-controlled clinical trial conducted to test the efficacy of memantine as a neuroprotective agent in glaucoma, found no significant effect in preserving visual function.[36] These results came as a great disappointment for memantine, which had initially raised high hopes. It is possible that memantine may have actually benefited patients but to a level which was difficult to detect clinically, as observed in the study conducted by Hare et al In an experimental monkey model of glaucoma induced by laser destruction of the anterior chamber angle, Hare et al showed that memantine enhanced RGC survival only in animals with

moderately high IOP. Furthermore, although memantine treatment had reduced the rate of RGC loss based on electroretinographic (ERG) measurements early during the study, this beneficial effect could not be observed if the injury was allowed to progress too long.[35] These observations suggest limited efficacy of memantine for reducing RGC death in glaucoma patients.

Bis(7)-tacrine is a newer NMDA receptor antagonist which possesses remarkable neuroprotective activity through concurrent inhibition of acetylcholinesterase[37,38] and nitric oxide synthase,[39] in addition to NMDA receptor blockade. Bis(7)-tacrine demonstrated more potent neuroprotective effect as compared to memantine in a study on cultured RGCs.[23] This agent still awaits further experimental and clinical studies for evaluation as an effective neuroprotective agent in glaucoma. Amantadine,[40] psychotropic tetrahydrobannabinol, and non-psychotropic cannabinol[41,42] are other potential neuroprotective agents that act via attenuation of NMDA activity.

GINKGO BILOBA EXTRACT

Ginkgo is an ancient species of tree similar to plants which were living 270 million years ago. This tree is widely grown in China and was introduced early in traditional Eastern medicine to treat a variety of problems such as asthma, vertigo, fatigue and tinnitus or circulatory disorders. In modern medical science, the extract from the leaves of ginkgo biloba, named as ginkgo biloba extract 761 (EGb761), has been shown to be beneficial for cognitive impairment and dementia.[43]

Because of biological and mechanistic similarities between Alzheimer's dementia and glaucoma,[44] investigators have studied ginkgo for glaucoma. Several studies have illustrated the role of mitochondrial dysfunction in the pathogenesis of glaucoma.[45] Only anti-oxidants capable of penetrating into the mitochondria can be of benefit as neuroprotective agents. Ginkgo contains certain substances, including poly-phenolic flavonoids which may theoretically prevent oxidative stress in the mitochondria and thereby protect RGCs.[46-48]

In a crossover randomized clinical trial, ginkgo biloba extract (GBE) improved pre-existing visual field defects of NTG patients. Twenty-seven NTG patients were included. Forty milligrams of GBE, three times a day, prescribed orally for 4 weeks, followed by 8 weeks washout period and then 4 weeks of placebo treatment were given. The other group of NTG patients were given the placebo first and GBE later on. Visual fields were examined at the end of each phase of the study and compared with the baseline perimetry. A significant improvement in visual field indices was recorded with GBE treatment in NTG patients.[49] More recently and in another short course placebo-controlled, crossover clinical trial, GBE could not improve contrast sensitivity or visual field damage in Chinese patients with NTG.[50]

The duration of follow-up in the above-mentioned studies was only 4 months; considering the chronic course of glaucoma, these studies were limited by short follow up period and small sample size. Despite the inconclusive results of clinical studies regarding the neuroprotective effect of GBE, because of its relatively safe profile,[51] some glaucomatologists have been prescribing GBE for their patients as adjuvant therapy for several years.[52] However, increasing risk of bleeding during surgery has been a cause of concern in patients using ginkgo.[53]

Efficacy and safety reports have recommended a daily dose of 120 mg of GBE.[51] Because of the beneficial effect of IOP reduction in most glaucoma cases and the economic burden associated with the use of GBE, its administration is recommended only in subjects with normal-tension glaucoma or in patients with high-pressure glaucoma whose condition progresses despite apparently adequate IOP reduction.[52]

NEUROTROPHIC FACTORS

Disruption of axonal transport has been demonstrated in experimental glaucoma models in monkeys and in human glaucoma.[5,54,55] These results suggest that interruption of the retrograde supply of a trophic factor to RGCs may play role in the RGC death observed in glaucomatous optic neuropathy.[56,57] *In vivo* and *in vitro* studies have revealed that neurons and glial cells within the mammalian retina possess receptors for different trophic factors, and that direct application of these factors may enhance the survival of injured ganglion cells.[58,59]

Among a variety of candidate growth and trophic factors for RGCs, brain-derived neurotrophic factor (BDNF), as a member of the nerve growth factor proteins, appears to be of particular importance to RGC function and survival.[60-64] BDNF has been shown to undergo both anterograde and retrograde axonal transport,[65] and has been effective in preventing lesion-induced axonal die-back in the rat optic nerve; however, it could not prevent the rapidly progressive degeneration of RGCs after axotomy. Weibel et al reported that BDNF has a selective influence on mechanisms responsible for survival of optic nerve axons.[66] Presence of the BDNF receptor, TrkB, in optic nerve axons and a change in its distribution with acute and chronic glaucoma in rats and monkeys was shown later by Pease et al.[57]

Therefore, disruption of BDNF supply to RGCs could be considered as a contributing factor in glaucomatous damage.[56] Several experimental investigations have demonstrated the protective effect of intravitreal injection of BDNF on RGCs in rat and primate models of optic nerve damage.[67-69] Di Polo et al observed a protective influence on RGCs by adenovirus-infected

retinal Muller cells through production and release of BDNF.[70] Quigley et al suggested the optimal dose of BDNF to be 0.01 mg per milliliter of vitreous volume for intravitreal injections and found that higher intravitreal doses decrease the protective effect of BDNF on RGCs possibly due to down-regulation of Trk B, the BDNF receptor.[56]

In all preclinical studies mentioned above, the neuroprotective effect of BDNF on RGCs was assessed in the setting of optic nerve lesions such as transection and crushing.[59,71] However, experimental studies for demonstrating the protective effect of exogenous BDNF in models simulating glaucoma are scarce.

Another trophic factor undergoing preclinical investigation is the human ciliary neurotrophic factor (CNTF), which also showed a neurotrophic effect on RGCs. A single injection of CNTF protein into the vitreous significantly protected RGCs in a rat model of optic nerve axotomy[61,72] and against nitric oxide (NO) induced cell death.[73] CNTF promoted the survival of purified rat RGCs in culture[74] and it showed a promising effect on RGC protection after optic nerve axotomy when transferred by adenovirus vectors.[75]

Pease et al assessed virally-mediated over-expression of CNTF and BDNF in an experimental model of laser-induced glaucoma in rats. Loss of RGC axons was 15% lower in CNTF-treated retinas than in controls; however, neither the combined CNTF-BDNF group nor the BDNF over-expression group showed any significant improvement in RGC survival.[76]

Artemin,[77] basic fibroblast growth factor,[78] interleukin-6[79] and erythropoietin[80] are other trophic factors or cytokines for which a neuroprotective effect has been proposed.

The challenge facing the application and efficacy of these trophic factors is how to accomplish effective and sustainable delivery to the retina. The blood-retina barrier impedes such large proteins from reaching the retina with systemic administration. Intravitreal injection is an alternative route to deliver purified recombinant trophic factors to the retina, but this may not be feasible for life-long administration in chronic conditions such as glaucoma. The integration of neurotrophic factors in drug delivery devices for intraocular implantation is one possible approach for long-term provision of such agents.

Although viral vector-delivery of trophic factors in animal models of retinal degeneration have demonstrated protective effects, certain issues such as precise control of dosage make the clinical application of this approach questionable.[81]

CALCIUM CHANNEL BLOCKERS

The neurotoxic effect of NMDA is mediated by calcium influx into neural cells, followed by apoptosis and cell death.[82] Thus, calcium-channel blockers (CCBs) seem to be a rational alternative for neuroprotection in glaucoma. CCBs theoretically rescue RGCs by prevention of cell death mediated by calcium influx and by improving local blood flow in ischemic tissues by inducing vasodilation.[83]

Different calcium channel blockers such as iganidipine, nimodipine and lomerizine have been shown to significantly increase purified rat RGC viability under hypoxia.[84] In another laboratory study, unlike nilvadipine, diltiazem could not prevent glutamate-induced RGC apoptosis.[85] The effect of topical 2% flunarizine on the rabbit retina under ischemic conditions induced by high IOP was evaluated by ERG; topical flunarizine reduced IOP and attenuated injury to the retina, including RGCs.[86]

Other members of this family, brovincamine and nilvadipine, have high blood–brain barrier permeability and are expected to induce favorable effects in the optic nerve or retina with minimal influence on systemic blood pressure.[87] They were shown to improve visual field defects and ocular circulation in NTG patients and diminished the rate of deterioration in visual field sensitivity of NTG patients in randomized clinical trials.[88-90]

There seem to be drawbacks to the use of CCBs in glaucoma. Inadequate perfusion pressure at the ONH, may play a role in the pathogenesis of glaucoma.[91-94] There is concern that although nilvadipine or other CCBs may increase blood flow, these agents may impair the autoregulation of blood circulation at the ONH during acute IOP elevation.[95] One should keep in mind that oral CCBs prescribed for systemic hypertension may be harmful to the optic nerve in glaucoma patients; lower systemic blood pressure seems to reduce ONH blood flow, which is a risk factor in the pathogenesis of glaucoma.[96]

ANTIOXIDANTS

A number of investigations have supported the role of oxidative stress in the pathogenesis of glaucoma.[97] These mainly demonstrated lower levels of antioxidants[98,99] and elevated oxidative stress markers in the aqueous humor of eyes with glaucoma,[99] antibodies against glutathione-S-transferase,[100] decreased plasma levels of glutathione[101] and increased lipid peroxidation products in the plasma of glaucoma patients.[102] Furthermore, tissue analysis studies comparing cultured human trabecular meshwork (TM) from eyes with POAG to that of non-glaucomatous eyes have revealed higher concentrations of reactive oxygen species, decreased cell membrane potentials and reduced ATP production in the TM of eyes with POAG.[103] Insufficiency of reactive oxygen species (ROS)-neutralizing mechanisms has been proposed as the cause of accumulation of oxidative free radicals in the TM.[104-106] Oxidative free radicals

have been implicated in human TM degeneration and subsequent IOP increase and glaucoma.[107] In another study, the correlation between DNA oxidative damage in the TM, increased IOP and visual field defects was reported.[108]

Theoretically, inhibition of ROS and up-regulation of cell defense systems may enhance RGC survival.[109-112] Cell defense mechanisms against oxidative stress include the superoxide dismutase, glutathione (GSH) and thioredoxin (TRX) systems.[110] The TRX system mitigates oxidative damage by scavenging intracellular ROS. The reaction leads to TRX oxidation, which is returned to its reduced form by TRX reductase in the presence of NADPH.

In a rat glaucoma model induced by laser damage to the TM, it was shown that overexpression of thioredoxins 1 and 2 could decrease RGC death following IOP elevation.[110]

In an experimental study, an association was found between a vitamin E-deficient diet and increased RGC death in a rat glaucoma model. The vitamin-E deficient group demonstrated greater lipid peroxidation as compared to rats with the usual diet. This study suggested that accelerated RGC death in the vitamin E-deficient group could be related to increased lipid peroxidation.[113]

Coenzyme Q10 (CoQ10), cofactor of the electron transport chain, is assumed to protect neuronal cells against oxidative stress by stabilizing the mitochondrial membrane potential, supporting ATP synthesis and inhibiting the generation of ROS.[114-116] A study by Nakajima, demonstrated that CoQ10 protected retinal neurons against hydrogen peroxide–induced oxidative stress *in vitro* and NMDA-induced glutamate excitotoxicity *in vivo*.[117] Moreover, CoQ10 prevented retinal damage caused by transient ischemic injury due to acutely elevated IOP.[118,119] The level of CoQ10 in the human retina has been shown to decrease by about 40% with age. The senile decrease in CoQ10 suggested the possibility that it may contribute to age-related RGC loss.[120] In a mouse model of glaucoma, diet supplemented with coenzyme Q10 inhibited glutamate excitotoxicity, and oxidative stress-mediated RGC and axonal degeneration by 29%.[121] To evaluate the effect of antioxidants in a clinical trial, Coqun eye drops (coenzyme Q10 combined with vitamin E) were administrated to 22 glaucoma patients twice daily in addition to beta-blockers. Retinal and cortical evoked responses of treated patients were compared to that of glaucoma patients treated with beta-blockers alone after 6 and 12 months. This topical preparation demonstrated a beneficial effect on inner retinal function as measured by pattern ERG with consequent improvement of visual cortical responses assessed by visually evoked potentials (VEPs).[122]

Natural substances such as polyphenolic flavoids in green tea, coffee, wine and dark chocolate; anthocyanosides in bilberry; vitamins including thiamin (vitamin B1) and even melatonin have been suggested to possess antioxidant activity.[123] Further studies are required to investigate the effect of antioxidants in glaucoma. Another open question is whether antioxidants are beneficial for all glaucoma patients or only those with reduced antioxidant reserve.

ALPHA 2 ADRENERGIC AGONISTS INCLUDING BRIMONIDINE

The presence of alpha-adrenergic receptors in human, bovine and porcine retinas, particularly in RGCs and the inner nuclear layer of the rat retina has been demonstrated by immunohistochemical studies.[124,125] In a histological study, brimonidine (a selective alpha-2 receptor adrenergic agonist) increased retinal metabolism and promoted neuronal growth in cultured retinal cells.[126]

It has been suggested that brimonidine may prevent RGC death by direct interaction with alpha-2 adrenergic receptors, leading to reduced accumulation of extracellular glutamate and blockade of NMDA receptors; this protective effect is thought to be independent of IOP reducing mechanisms attributed to this agent.[127-129] Elimination of the protective effect of brimonidine by co-administration of an alpha 2-antagonist confirms that the mentioned effect is secondary to alpha-2 receptor activation.[127,130]

In a pre-clinical study, continuous subcutaneous treatment with brimonidine significantly improved RGC survival exposed to elevated IOP for 8 weeks. Brimonidine treatment also preserved morphology, density and the total number of axons in the optic nerve subjected to high IOP.[131]

It is increasingly recognized that ocular blood flow alteration is involved in the pathogenesis of glaucomatous optic neuropathy.[132,133] In contrast to alpha-1 receptor activation which leads to vasoconstriction of ocular and systemic blood vessels, there is no evidence that alpha-2 agonists alter optic nerve, retinal, choroidal or retrobulbar blood flow.[134,135]

A randomized clinical trial from Singapore compared the effect of brimonidine and timolol on the incidence of glaucomatous visual field defects and the rate of visual field deterioration after acute IOP rise. Evaluation of the visual field tests during the 16-week follow-up period did not show any protective effect from brimonidine.[136] In another study, however, measurement of RNFL thickness by scanning laser polarimetry (GDx) demonstrated less RNFL loss with brimonidine in comparison to timolol 0.5% in ocular hypertensive patients over 12 months of treatment.[137]

The industry-supported "Low-Pressure Glaucoma Treatment Study (LoGTS)" evaluated the neuroprotective effect of brimonidine versus timolol in 190 NTG patients over four-year follow-up.[138] This study suggested that brimonidine may halt visual field deterioration more than timolol, but the authors did not consider the higher rate of incomplete follow up in the brimonidine group (55% and 29% missing data in the brimonidine and timolol groups, respectively).[139]

NITRIC OXIDE SYNTHASE INHIBITORS

Evidence in the literature points to a possible role for NO in RGC degeneration.[140-142] Increased levels of NO to twice normal values was shown in rat retinas with induced glaucoma.[143] Aslan et al suggested that excessive NO could result in apoptosis and necrosis of RGCs.[144] There are three forms of nitric oxide synthase (NOS): NOS-1 (neuronal NOS) and NOS-3 (constitutive NOS) act as vasodilators or neurotransmitters in normal retinal tissue, however NOS-2 (inducible NOS) contributes to RGC neurotoxicity.[145] An increased expression of NOS has been shown in optic nerve head (ONH) of glaucoma patients.[146,147] In an experimental study, *in vitro* elevation of hydrostatic pressure upregulated NOS-2 expression in cultured rat RGCs and astrocytes of the human lamina Cribrosa.[148,149] The ability of aminoguanidine as a NOS-2 inhibitor in protecting RGCs in the rat cautery model of retinopathy led to the suggestion that NOS-2 inhibition may be protective in glaucoma.[141,150] The possibility that NOS-2 inhibition could be neuroprotective in glaucoma was strengthened by reports showing that another NOS-2 inhibitor (N-nitro-L-arginine) delayed RGC degeneration.[151] The non-psychotropic component of marijuana, cannabidiol (CBD), and the synthetic cannabinoids, tetrahydrocannabinol and HU-211 have been demonstrated to possess protective actions in part due to an effect on reducing formation of lipid peroxides, nitrite/nitrate and nitrotyrosine.[42,152,153]

These data suggest that activation of NOS, especially NOS-2, may play a significant role in glaucomatous optic neuropathy and that nitric oxide synthase inhibitors could halt neurodegeneration.

On the other hand, the role of NOS-2 in optic neuropathy has been argued by subsequent studies. Pang et al did not find any evidence for NOS-2 in glaucomatous neurodegeneration in their study. They induced elevated IOP in rats by injection of hypertonic saline into episcleral veins. No significant increase in NOS-2 expression was found in the optic nerve head. Furthermore, aminoguanidine treatment had no effect on glaucomatous damage in rats.[154] In another study conducted by Libby et al with the same IOP elevation method, a similar result was achieved.[155] Kasmala et al found that oral administration of another inhibitor of NOS2, SC-5, did not prevent optic neuropathy induced by saline injection ocular hypertension.[156]

Technical differences in the simulation of glaucoma and different mouse races in experimental studies may explain the discrepancy between different investigations.[157] In short, preclinical evidence regarding the effect of NOS in neurodegeneration is inconclusive and NOS inhibitors have not yet been tested in any clinical study.

ANTI-GLAUCOMA MEDICATIONS WITH BLOOD REGULATION EFFECT

Vascular dysregulation has been implicated in the pathogenesis of glaucoma,[158] therefore a neuroprotective effect has been suggested for agents which can improve regulation of ocular blood perfusion.[159] Some anti-glaucoma medications have additional ocular blood perfusion effects. For instance, carbonic anhydrase inhibitors increase ocular perfusion.[160] Improvement of ocular blood has also been reported with latanoprost.[161,162]

Betaxolol is a putative selective B1-adrenoceptor blocker. Experimental studies have suggested a neuroprotective effect from betaxolol in animal models of retinal ischemia.[163,164] However, the reports did not provide evidence on how betaxolol modulates neurodegeneration and which types of retinal cell are affected by betaxolol.[165,166] Some studies have suggested that betaxolol reduces the NMDA-stimulated influx of calcium into isolated cells of rat retinas by direct interaction with voltage-dependent calcium channels or sodium channels.[167]

Anti-glaucoma medications have a large preservative effect on RGCs by IOP reduction, therefore clinical studies to evaluate their action as a "neuroprotective" agent independent of their protective action due to IOP reduction are difficult to conduct and interpret.

STEM CELL TRANSPLANTATION FOR RGC NEUROPROTECTION

Stem cell transplantation has gained significant interest because of its potential to treat neuro-degenerative diseases such as glaucoma. There are two mechanisms by which stem cell therapy might be applicable to glaucoma. The most important therapeutic power of stem cells lies in their ability to generate new cells of many types and to induce RGC regeneration.[168] Nevertheless, RGC replacement would require that the cells become integrated into the complex circuitry and be capable of synapsing at precise brain locations. Thus, protection of RGCs from degeneration might be a more accessible goal in glaucoma therapy in the short term.[169]

It has been hypothesized that implantation of some types of stem cells activates multiple neuroprotective pathways simultaneously via secretion of various factors.[170] Transplanted stem cells may be utilized as an intraocular delivery device for diffusible bioactive factors. Supply of various neurotrophic factors is the most widely acceptable mechanism by which transplanted cells can modulate excitotoxicity. This method may provide the advantage of long-lasting and localized effect. Delivery of a single prolonged effective treatment could also prevent the common problem of patient noncompliance with pharmacologic therapy. Even though cells which fulfill all the required criteria for stem cell transplantation have not yet been identified, multiple cells have been suggested in different studies, which can secrete different neurotrophic factors, such as embryonic or adult tissue-derived stem cells.[171]

There are important concerns in this field since implanted cells may secrete other agents with unknown activity, in addition to the desired neurotrophic factor. Some of these factors may even be harmful. Therefore, it is necessary to determine all factors produced by the transplanted stem cells before they come into clinical practice.[172]

Another limitation in this field is graft survival. Prolonged survival is necessary to achieve continuous benefit from stem cell transplantation; however, longer survival times are associated with an increased risk of tumorigenesis.[173] Consequently, careful selection of stem cells, thorough long-term observation, and safety evaluation will be necessary to ensure that the potential benefit of neuroprotection outweighs the risk of inducing tumors. On the other hand, stem cell transplantation has been shown to induce reactive gliosis in the host retina which caused retinal folding, up-regulation of intermediate filaments, and recruitment of macrophages. Inhibition of stem cell-induced reactive gliosis would be fundamental for successful transplantation-based strategies.[174]

Even once these questions are resolved, numerous issues should be addressed during translation of successful laboratory models to the clinic. Difference in animal models used for glaucoma, the rapid time course of optic nerve damage in the laboratory setting and different mechanisms for simulating RCG injuries are some of the issues which should be taken into account in order to achieve the desired results in the clinical setting.[171]

SUMMARY

Over the past 30 years, numerous pharmacologic agents have been advocated as neuroprotective agents in glaucoma, however few of them such as brimonidine or memantine have advanced to clinical trials. In a systematic review by the Cochrane group in 2013 for neuroprotection in glaucoma, from dozens of clinical trials, only one study (LoGTS) fulfilled the criteria for review which itself faced criticism. Through an updated search for the current review, as of July 2015, no more completed clinical trials corroborating neuroprotection in glaucoma have been published after the Cochrane review in October 2012. This has occurred despite encouraging evidence from laboratory and preclinical studies.

Several conceptual and methodological issues hinder the translation of experimental results to clinical glaucoma practice. First of all, glaucoma is a chronic heterogenous group of disorders, and no animal model can fully mimic the course of human disease. Furthermore, considerable disease variability exists in human clinical trials; these include the presence of comorbidities, polypharmacy in elderly glaucoma patients, and minimal control over a myriad of physiologic factors. Another basic difference between animal and human clinical trials in the neuroprotection field is the time of the intervention. In most experimental studies, the neuroprotective agent is given at the time or even prior to injury, unlike human studies, in which the patient is eligible for enrollment after the disease is well establishment. Another basic difference between experimental studies and human clinical trials is outcome measure. Most animal studies employ histopathologic endpoints to assess the treatment efficacies. Clinical trials, however, judge efficacy by using functional outcomes, which most often take months to show any change. Extrapolation of the appropriate dose of a neuroprotective agent for use in humans from animal or laboratory studies is another issue. Many of these agents are toxic or ineffective, at concentrations higher or lower than optimum. Human ocular bioavailability with a given dose also is often difficult to predict.

Broad and multidisciplinary collaborative effort is required to design a set of guidelines for experimental and clinical studies on neuroprotection in ophthalmic disease. A consensus on how to design and execute translational research in neuroprotection in ophthalmic disease would optimize the use of resources and facilitate the development of effective neuroprotective agents.[36] To that day, the main therapeutic option for glaucoma treatment will remain to decrease intraocular pressure and the selection of anti-glaucoma medications should be based on their ability to reduce IOP.

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Conflicts of Interest

There are no conflicts of interest.

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