Symposium

Neuroprotection in glaucoma

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Glaucoma is a neurodegenerative disease characterized by loss of retinal ganglion cells and their axons. Recent evidence suggests that intraocular pressure (IOP) is only one of the many risk factors for this disease. Current treatment options for this disease have been limited to the reduction of IOP; however, it is clear now that the disease progression continues in many patients despite effective lowering of IOP. In the search for newer modalities in treating this disease, much data have emerged from experimental research the world over, suggesting various pathological processes involved in this disease and newer possible strategies to treat it. This review article looks into the current understanding of the pathophysiology of glaucoma, the importance of neuroprotection, the various possible pharmacological approaches for neuroprotection and evidence of current available medications.

Key words: Glaucoma, neuroprotection, pathophysiology, pharmacological approach

Indian J Ophthalmol: 2011;59 Suppl 1:S102-113

DOI: 10.4103/0301-4738.73700

Glaucoma is an optic neuropathy, specifically a neurodegenerative disease characterized by loss of retinal ganglion cells (RGCs) and their axons. In the past, glaucoma was viewed as a disease of raised intraocular pressure (IOP); however, it has become increasingly clear that elevated IOP is only one of the risk factors for this disease.

Recent evidence indicates that lowering IOP does not prevent progression in all patients and that progression can continue despite effective lowering of IOP. This was clearly depicted in the Advanced Glaucoma Intervention Study (AGIS) trial,[1,2] Collaborative Normal Tension Glaucoma Study,[3,4] the Collaborative Initial Glaucoma Treatment Study Trial,[5] and the Early Manifest Glaucoma Trial.[6] As RGCs cannot divide and regenerate, optic nerve damage is irreversible. It is therefore imperative that these cells are kept alive.

The term neuroprotection refers to mechanisms within the nervous system, which protect neurons from apoptosis or degeneration, for example, following a brain injury or as a result of chronic neurodegenerative diseases. It has been a common approach that has been used to treat a variety of chronic neurodegenerative diseases such as Parkinson's and Alzheimer's disease, to name a few. Numerous theories of neuroprotection in glaucoma have been drawn from these neurodegenerative conditions, where the loss of the cells is targeted instead of the disease process by which these losses occur. This approach attempts to accelerate or impede specific biochemical pathways that may prevent neuronal injury or accelerate neuronal recovery. Hence, any therapy that prevents, retards or reverses apoptosis-associated neuronal cell death resulting from primary neuronal lesions is neuroprotective.

Therefore, neuroprotection in glaucoma is aimed at protecting those neurons that are damaged or likely to be damaged in glaucomatous optic neuropathy which consists of neurons along the entire visual pathway, chiefly the RGC axons. This strategy is an addition to that achieved by IOP lowering alone. Even though any treatment approach that preserves RGCs in glaucoma could be described as neuroprotective, the term has been limited by many researchers to describe a drug that directly interacts with neuronal or glial elements within the optic nerve head.

Consequently, the endpoint of neuroprotection in glaucoma offers a means to prevent the irreversible loss of those cells in glaucoma, especially where the particular etiology is either idiopathic or differs from patients to patients. Hypothetically, neuroprotection should be beneficial irrespective of the pathophysiology of the disease.[7-9]

Pathophysiology of Glaucomatous Damage and Importance of Neuroprotection

The pathogenesis of RGC loss in glaucoma remains incompletely understood. A diverse range of mechanisms have been postulated, and whether the site of primary damage is the ganglion cell body or their axons remains disputable. Axonal or white matter diseases include anterior ischemic optic neuropathy (AION) and glaucoma, while central retinal arterial occlusion (CRAO) and high levels of acute IOP are diseases of the cell body or gray matter.[10] In a cell body disease, the cell metabolism is directly affected and for that reason, the window to change the outcome is approximately 45–90 minutes for cell rescue, whereas in axonal injuries, metabolism is indirectly affected, giving a longer window of opportunity, and therapies can hence be delivered after the acute insult.

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Manuscript received: 31.01.10; Revision accepted: 07.10.10

In glaucoma, there is transsynaptic degeneration as evidenced from experimental primate and human glaucoma, which suggests that axonal injuries extend from the optic nerve to visual pathways in the brain. Thus, central neurodegenerative changes in the visual pathway may contribute to the pathophysiology of glaucomatous progression.[11] Accordingly, therapies combining IOP-lowering approaches with neuroprotective agents would confer protection of local and central visual neurons, thus preserving vision. In view of the fact that glaucoma is a long-standing disease with features of axonal damage, it is therefore a good candidate for neuroprotection.

Glutamate induced excitotoxicity

Excitotoxicity has been implicated in almost all chronic neurodegenerative diseases. The amino acid glutamate is an essential neurotransmitter in the central nervous system as well as the retina. Glutamate concentrations higher than the physiological levels are toxic to the neurons depending on the duration and magnitude of the increase. Excitototoxic neuronal injury involves a self-perpetuating cascade of events originating from a persistent activation of ionotropic glutamate receptors.[12] It has been observed that neuronal hypersensitivity to glutamate can occur in certain conditions and, for that reason, elevated glutamate levels are not required to induce neuronal cell injury.

N-methyl-ᴅ-aspartate (NMDA) receptor activation leads to opening of associated ion channels in the neurons and the entry of extracellular calcium and sodium. Glutamate-mediated neuronal toxicity is dependent on the influx of extracellular calcium, which in turn acts as second messenger to activate a cascade [Fig. 1], consequently leading to neuronal cell death.[13] In order to maintain the physiological concentrations and to protect ganglion cells from excitotoxic cell death, appropriate removal of synaptic glutamate is necessary. Müller cells and astrocytes surrounding the synapses express the glutamate transporter which transports extracellular glutamate into the glial cells. Within the glial cells, glutamine synthetase converts glutamate to glutamine which is non-toxic and is released by glial cells to be taken up by neuronal cells

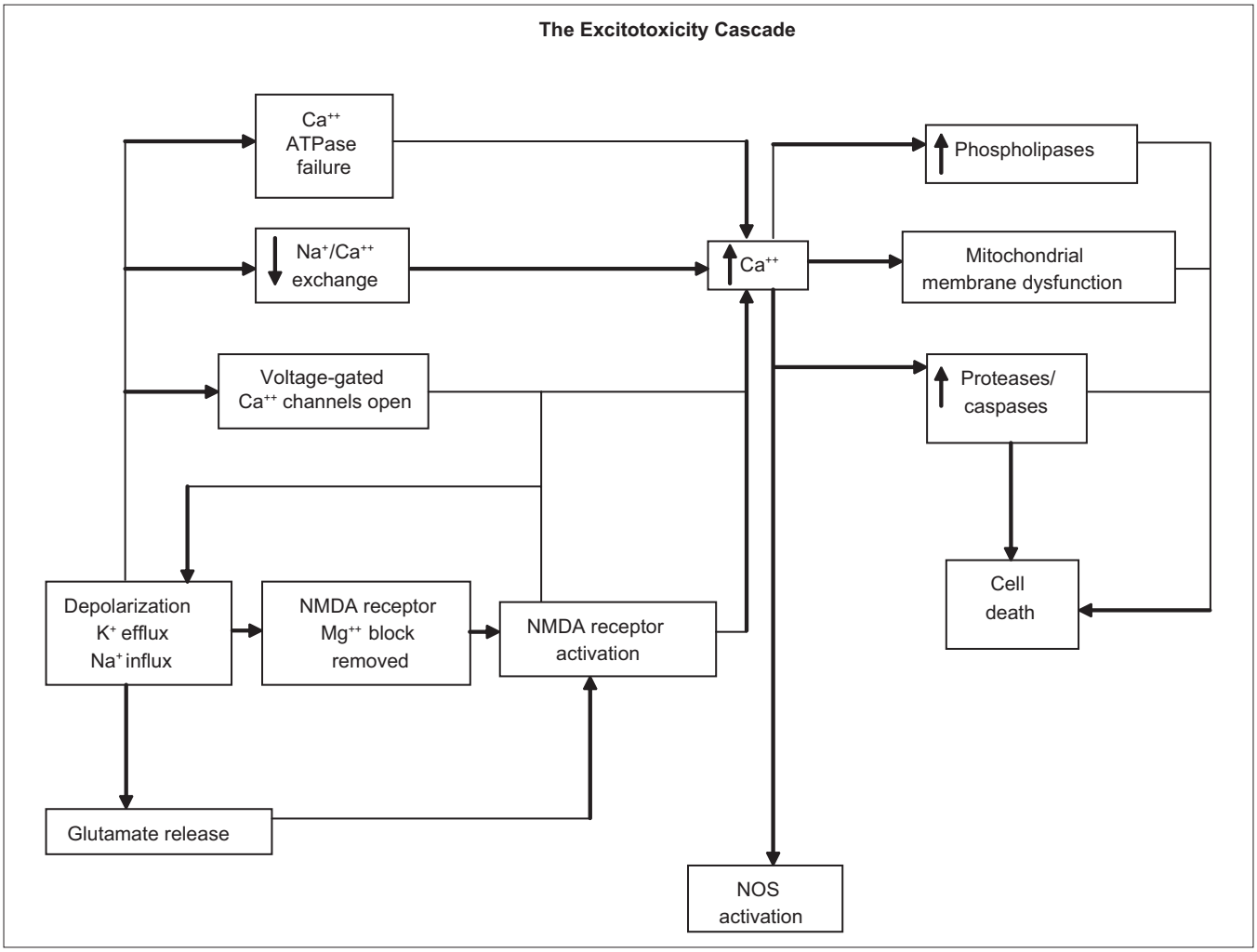

Figure 1: Excitotoxicity cascade leading to neuronal injury or death is caused by excessive exposure to the neurotransmitter glutamate and uncontrolled stimulation of its membrane receptors, mainly the NMDA-sensitive channel (Source: Adriana Di Polo, PhD and Leonard A Levin, MD, PhD. Strategies for prevention of neural injury in glaucoma. In: Levin LA WR, Weinreb RN, Di Polo A, editors. A Pocket Guide of Neuroprotection in Glaucoma. New York: Ethis Communications; 2007. pp.36)

where it is again converted to glutamate in the presence of glutaminase. This cycle replenishes the neurotransmitter stores and glutamate toxicity is averted.

However, it still remains to be ascertained whether glutamate excitotoxicity is an initial response to elevated pressure and ischemia or secondary to glutamate release from dying ganglion cells. While there is substantive evidence that high doses or prolonged exposure to glutamate promotes RGC death via overactivation of ionotropic glutamate receptors,[14] no consensus has been reached in answering the question on whether the level of glutamate is truly elevated in glaucoma.

Neurotrophin withdrawal

As the nervous system develops, the surplus of neurons produced is subsequently eradicated by apoptosis. Neurons require neurotrophic growth factors, which are acquired by retrograde axoplasmic transport. These growth factors, known as neurotrophins, regulate cellular metabolism hence maintaining the normal cellular milieu.[15] Thus, where neurotrophic support is absent due to retrograde axonal transport block, RGCs die. This group of small growth peptides comprises brain-derived neurotrophic factors (BDNF), nerve growth factors (NGF), neurotrophin-3 (NT-3) and neurotrophin-4 (NT-4).[16,17] Lack of BDNF and NGF secreted by RGC targets results in apoptosis of developing RGC though it is postulated that it has almost no effect on the survival of mature RGCs since retrograde transport of neurotrophin factors persists along adult RGC axons. Hence, in glaucoma, blockade of axonal transport results in neurotrophin deprivation leading to neuronal cell death.[17-29]

Apoptosis

Apoptosis refers to a common mode of cell death. It is a subtle process where the cell initiates a death program and commits suicide resulting in cell shrinkage, genomic fragmentation and nuclear pyknosis.[30] In necrosis on the other hand, destruction of the cell membrane leads to spillage of cellular contents into the extracellular matrix (ECM), hence damaging and killing other cells. This sequential occurrence of cell death processes appears to be biphasic, and research in optic nerve injury models has shown both fast and slow phases of RGC degeneration.[31] It may therefore be reasonable to theorize that the early phase of degeneration, i.e. from 1 to 3 days, could represent necrosis, followed by apoptosis.

There are many triggering factors for apoptosis, be it extracellular or intracellular events. These include trophic factor deprivation or oxidative damage, both of which have been postulated to induce RGC apoptosis in glaucoma. A principle class of intracellular apoptotic regulators is the Bcl-2 family of mitochondrial membrane-bound proteins. While some proteins in this family inhibit apoptosis (e.g. Bcl-2, Bcl-X_L), others promote it (e.g. Bax, Bad, Bid).

Caspases are proteases that execute the dismantling and demolition of apoptotic cells. Caspases are categorized into two broad groups: initiators (e.g. caspases 8 and 9) which activate other caspases and effectors (caspase 3) which cleave specific substrates involved in cellular disassembly. Some experimental glaucoma models have shown that the initiator caspases are activated, while inhibition of the effector caspases can be neuroprotective.[32-40] Calcium overload is also responsible for activation of calpane and caspase cascades, leading to apoptosis.[41]

Nitric oxide

Nitric oxide (NO), a free radical which is formed from L-arginine by NO synthetase (NOS), is thought to play a role in many neurodegenerative diseases including glaucoma, Alzheimer disease, multiple sclerosis and cerebral-cardiovascular diseases.[42] There are three isoforms of NOS: NOS-1 neuronal, a constitutive enzyme that been detected in the diminished nerve fiber bundles at the prelaminar region and lamina cribrosa of glaucomatous eyes;[43] NOS-2, an inducible enzyme (iNOS) produced in response to high IOP, which also has a genetic association in patients with primary open angle glaucoma (POAG); and NOS-3 endothelial, another constitutive enzyme found in the prelaminar region of the optic nerve, which functions as a vasodilator.[44]

It has been proposed that damage to optic nerve axons at the level of lamina cribrosa is caused by excessive NO production by reactive astrocytes and microglia in the optic nerve heads. Studies have shown that NOS-1 and NOS-3 are upregulated in POAG patients, and NOS-2, absent in healthy individuals, is expressed in the optic nerve head of POAG patients. Excitation of NMDA receptors has also been shown to trigger NOS-1, which in turn upregulates NOS-2, leading to a large increase in cellular NO level, while induction of NOS-3 can provide neuroprotective effects by causing vasodilatation and increased blood flow to the optic nerve head.[43-46]

A number of pathogenic insults including ischemia, reperfusion, inflammation and excitotoxicity have been linked with raised levels of NO in the retina. NO has the ability to pass from one neuron to another bypassing synapses, leading to rapid intra and intercellular diffusion. Being a free radical with moderate activity, it causes the formation of free radicals that contribute to RGC demise.[47-50]

Free radical generation and oxidative stress

Free radicals are a byproduct of oxidative metabolism. The high metabolic activity of retinal tissues render RGCs especially vulnerable to oxidative stress.[51] Free radicals interfere with macromolecular cellular constituents of the cells and further lead to derangement of protein breakdown, lipid peroxidation and nucleic acid degeneration, resulting in cell death.[52] To counteract this, ocular tissues have highly efficient antioxidant mechanisms that include the superoxide dismutase–catalase system, ascorbic acid and reduced glutathione.

Calcium-dependent pathways

Calcium is an important cation that is usually seized by the mitochondria, endoplasmic reticulum and a number of calcium binding proteins. High levels of calcium are toxic to cells. Three different calcium channels exist: voltage-sensitive calcium channels, store-operated channels and receptor-operated channel such as NMDA receptor. Neuronal death and axonal degeneration are associated with an increase in intracellular and intra-axonal calcium. Calcium-induced neuronal apoptosis is dependent on calcineurin which is a calcium-dependent phosphatase and facilitates the dephosphorylation of the pro-apoptotic mitochondrial membrane-bound proteins i.e. Bad. The dephosphorylation in turn results in its translocation from

the cytoplasm to the mitochondria, where it binds to Bcl-2 or Bcl-$X_{L,}$ forming protein complexes, which raise mitochondrial membrane permeability, release cytochrome C and cause neuronal suicide.[53-55]

Structural abnormalities

The survival of RGC can be jeopardized by changes in the ECM. Matrix metalloproteinases (MMP) degrade ECM proteins. Increased activity of MMP and decreased ECM proteins such as laminin has been observed in the RGC layer in experimental glaucoma models. MMP upregulation may result from high IOP itself, secondary mechanical damage from raised IOP or upregulation of glutamate receptors.[56,57]

Heat shock proteins

Heat shock proteins (HSPs) or stress proteins are expressed in most cells under normal physiological conditions. They play an important role in normal cellular function such as cellular protein maturation. They are also synthesized in large quantities in response to environmental stresses such as heat, anoxia, and exposure to cytokines and are believed to play an important role in re-establishment of normal cellular function and protect against further damage. They have been found to be highly expressed in the eyes of glaucoma patients and animals with chronic ocular hypertension and are hence thought to be a form of an endogenous defense mechanism against trauma. Though they may serve initially to protect cells from further destruction and facilitate repair, there are evidences suggesting that they may later recruit immune responses that contribute to the progression of disease.[58]

Immunology

Studies have been carried out to examine ways of manipulating the immune system to preserve RGCs. One approach has been to determine the effect of focal activation of the immune system in the optic nerve. These studies demonstrated that activated T-lymphocytes that have been primed to the constituents of the optic nerve, such as the myelin basic protein (MBP), would be drawn to areas of injury and release neuroprotective factors. To elucidate this, optic nerve partial crush models were used, where many axons were only partially damaged. This approach was later tested in laser-induced ocular hypertension models in rats with parallel effects. Additionally, it has been learnt that this immune-mediated protection can also be generated by glatiramer acetate (copolymer-1), a synthetic polypeptide.[59-62]

Vascular insufficiency

Impaired blood flow and disruption of autoregulation of the optic nerve blood flow are thought to contribute to the pathogenesis of glaucoma. Vascular insufficiency is also associated with elevated endothelin-1 levels in the aqueous humor and plasma. Endothelin-1 is a potent vasoconstrictor that further compromises blood supply to retinal tissues.[63-65] The resultant ischemic damage triggers astrocytes and microglia to produce tumor necrosis factor (TNF)-α, which leads to apoptosis via caspase-8, indirectly. The interaction of TNF- signaling with other cellular events in glaucomatous neurodegeneration plays a role in the spread of neuronal damage by secondary degeneration. Inhibition of TNF-α death receptors signaling may be a possible treatment modality providing neuroprotection.[66]

Rationale for neuroprotection

The path to clinical use of neuroprotectants has been long and uneven. Although the possibility of non–IOP-lowering therapy for glaucoma was first recognized in 1972 by Becker *et al*. with the use of diphenylhydantoin for treatment of visual field loss in primary open-angle glaucoma, only of late significant advances have been made in the understanding of the mechanisms for death of retinal neurons.[67]

The randomized clinical glaucoma trials have demonstrated progression of the disease despite significant pressure lowering. A retrospective subanalysis of the AGIS data showed that variation of IOP readings across office visits was more important than the absolute level.[68] Asrani *et al*. suggested that the diurnal IOP range and range over multiple days were significant risk factors for progression, even after taking into account office IOP, age, race, gender, and visual field damage at baseline.[69] Thus, glaucoma will progress even in patients with effective IOP lowering, rendering it a good candidate for neuroprotection.

Pharmacological Approaches

NMDA receptor antagonists

As described in the pathophysiology above, excess glutamate leads to NMDA receptor overactivation as well as excitotoxicity. Hence, using NMDA antagonist would be an efficient way to prevent RGC loss where excitotoxicity is implicated.[14] The earliest experiments used MK-801 which completely blocks normal glutamatergic neurotransmission, which is required for normal central nervous system function, and is therefore inappropriate for clinical use.[70] Experimental models of retinal ischemia induced by transient elevation of IOP have shown that NMDA inhibition with MK-801 confers neuroprotection by decreasing the expression of Bad and transient deactivation of the pro-survival kinase Akt pathway.[71]

The development of NMDA blockers that discriminate excessive NMDA receptor activation without affecting normal function led to clinically viable antagonists. Memantine is a non-competitive, low-affinity, open channel blocker. It exhibits selective blockade of the excessively open channels with a fast-off rate, thus inhibiting excessive NMDA receptor activity while maintaining normal neuronal cell function as it does not accumulate significantly within the channel.[72,73] Memantine, being well tolerated, has been approved for its use in Alzheimer's disease and vascular dementia as it is a highly effective neuroprotective agent as demonstrated in acute animal models of RGC death.[74]

The largest randomized, progressive, Phase 3 clinical trial on neuroprotection studying the safety and efficacy of memantine for open angle glaucoma has been completed, but it disappointingly failed to meet its primary endpoint [Fig. 2].[75,76]

Neurotrophic factors

Various studies in experimental models have shown that neurotrophic factors, especially BDNF and ciliary neurotrophic factor (CNTF), can enhance survival of RGCs after optic nerve injuries but there are to date no adequately powered clinical trials to substantiate this in humans.[77-79] Recent studies have shown that a combination of BDNF and LINGO-1 (a CNS-specific leucine-rich repeat protein) antagonist enhances

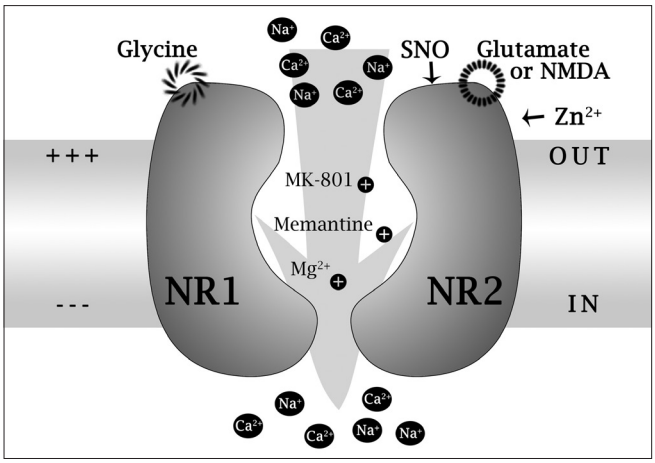

Figure 2: NMDA receptor model illustrating binding and modulatory sites. Glu/NMDA: glutamate/NMDA binding site; Gly: glycine binding site; Zn2+: zinc binding site; NR1 and NR2: NMDA receptor subunits 1 and 2A; SNO: cysteine sulfhydryl reacting with nitric oxide species X (Mg2+, MK-801, and memantine binding sites within the ion channel pore region) (Source: Lipton SA. Paradigm shift in NMDA receptor antagonist drug development: molecular mechanism of uncompetitive inhibition by memantine in the treatment of Alzheimer's disease and other neurologic disorders. J Alzheimer's Dis 2004; 6:S61-S74)

long-term RGC viability. Most of the investigations have been focused on BDNF, and although results are promising, it still remains inconclusive.[76,80]

Anti-apoptotic agents

Several pathogenic mechanisms have been proposed to induce apoptotic RGC death in glaucoma. These include reduced neurotrophic factors and cytokine deprivation to neurons, altered intracellular calcium levels, reactive oxygen species and excitotoxicity due to raised extracellular levels of certain neurotransmitters and neuromodulators.[26,81-84] Enhancing mitochondrial function may also inhibit apoptosis. Recent studies have shown that supplements of creatine, α-lipoic acid, nicotinamide and epigallocatechin-gallate (EGCG), which act by counteracting oxidative stress, promote mitochondrial function and confer neuroprotection.[85]

Two approaches for inhibiting apoptosis have been proposed. The first method is to promote survival pathways. For instance, the use of brimonidine activates anti-apoptotic extracellular signal regulated kinase (ERK) and Akt, which in turn enhance the production of Bcl-2 and Bcl-X_L.[86] A second approach is to block the apoptotic machinery by using caspase inhibitors.[87-91] Caspases are the effector enzymes that disassemble cellular contents during apoptosis. Calpeptin, a calpain-specific inhibitor, has been shown in glaucoma experimental models to confer neuroprotection.[92] As other death processes such as autophagy and necrosis may also play a role in RGC death in glaucoma, inhibition of apoptosis alone may not completely prevent glaucoma progression.[76]

Nitric oxide synthase antagonists

Inhibition of NOS using 2-aminoguanidine, i-NOS and L-N^6-(1-iminoethyl) lysine 5-tetrazole amide has been shown to be neuroprotective in experimental glaucoma models.[93] Nipradilol, a β- and α_1 antagonist has also been shown to be neuroprotective.[94,95] However, Pang *et al*., in a recent study, found no proof for the release of NOS-2 by astrocytes in patients or models. In addition, they documented that there were no neuroprotective properties in aminoguanidine.[76,96]

Antioxidants

RGC death by NMDA-induced toxicity may be reduced by antioxidants and free radical scavengers such as vitamins C and E (α-tocopherol),[97,98] superoxide dismutase and catalase.[52,76] *Gingko biloba* (EGb761), apart from increasing blood flow, has been also found to have a free radical scavenger property.[99] Its extract is also known to preserve mitochondrial metabolism and enhance ATP production in various tissues.

Calcium channel blockers

Calcium channel blockers like nifedipine and verapamil may exert neuroprotection by increasing blood flow to the RGCs.[100] In addition, they also improve glutamate metabolism and hence cause efficient homeostasis in the optic nerve head.[101] However, there are concerns that by also causing systemic hypotension these agents can worsen retinal ischemia due to a reduction in perfusion pressure.

A recent study in a rat chronic glaucoma model has shown that continuous treatment using candesartan (angiotensin II type I receptor blocker) provided substantial neuroprotection against RGC loss.[76,102]

Gene therapy

The current core of gene therapy is targeted against apoptotic factors. Candidate agents are deprenyl, a monoamine oxidase inhibitor (anti-parkinsonism drug) which increases the gene expression of factors that halt apoptosis, and flunarizine and aurintricarboxylic acid, which have shown promising results in retarding apoptosis following light-induced photoreceptor cell death.[76,103]

Immunomodulators and vaccination

The objective of vaccination is not only to ameliorate disease propagation but also to decrease the secondary degeneration of neurons following the acute insult. Passive transfer of T cells specific to MBP is one such approach. In order to withstand any insult, the retina and optic nerve require an intact peripheral immune system. These T cells are required to identify site-specific self-antigens**.** They activate resident microglia and harness blood borne monocytes which have been shown by some investigators to support regrowth of axons and arrest degeneration. Glatiramer acetate (copolymer-1/cop-1), a synthetic oligopeptide, is being studied as a possible vaccine used for neuroprotection.[104-106]

The locally activated anti-self T cells target the injury and supply cytokines and growth factors which govern sentinel cells, microglia and enlist macrophages bequeathing the eye with a protective phenotype. These cells arrest the production of TNF-α, as well as remove glutamate and debris and generate growth factors.[59,107-112]

Geranylgeranylacetone

As discussed earlier, the role of the HSPs in the pathophysiology

of glaucoma has led to evaluation of geranylgeranylacetone (GGA) which is used clinically in peptic ulcer disease. It has been observed to evoke the synthesis of HSP70, thus rendering it potentially neuroprotective.[76,113]

Stem cell therapies

Stem cell transplantation is another promising modality being researched for many neurodegenerative diseases. Stem cells are thought to exert neuroprotective effects by generating neurotrophic factors, modulating MMP and other aspects of the CNS environment that may promote endogenous healing.[114] Research on stem cell mobilization and the possible neuroprotective contribution of granulocyte-colony stimulating factor (G-CSF) showed that G-CSF was greatly expressed by the RGCs, thereby providing neuroprotection in neurodegenerative diseases.[115] Also, oligodendrocyte precursor cells (OPCs), a type of neural stem cell, may provide protection to RGCs from damage.[76,116]

Bioenergetics

Bioenergetics is the study concerning metabolic processes that lead to energy utilization in the form of ATP molecules. Emerging evidence points that energy failure and mitochondrial dysfunction at the optic nerve head (ONH) may be involved in glaucoma due to reduced energy and increased free radical production.[117]

Enhancing mitochondrial function or increasing energy supply of neurons may provide an additional method for inducing neuroprotection. Such approaches have been successful in animal models of other neurodegenerative disease including Parkinson's disease and traumatic brain injury. They act by increasing energy buffering capacity of damaged cells which decreases permeability of mitochondrial membrane pore and free radical scavenging. These approaches remain unexplored in glaucoma models.[76,118]

Evidences on Currently Available Topical Medications

A number of large prospective randomized controlled trials have demonstrated the impact of IOP lowering on inhibiting glaucoma progression or preventing conversion of ocular hypertension to glaucoma. IOP reduction is achieved by inhibiting aqueous humor flow (influx) or enhancing aqueous outflow. Among the broad categories of drugs available are the α_2-adrenoceptor agonists, β-adrenoceptor antagonists, prostaglandin derivatives and carbonic anhydrase inhibitors.

α_2-Adrenoceptor agonist

α_2-Adrenoceptors are located in the ganglion cell layer of the retina.[119,120]

Activation of these receptors inhibits neuronal cell death via a complex but independent pathway. There is mounting evidence implicating that α_2-adrenoceptors inhibit the pro-apoptotic pathway,[86] trophic factor release,[121] as well as glutamate release,[122] providing neuroprotection. They activate phosphatidyl-inositol-3 (PI3) kinase and protein kinase/Akt pathway, which are the major pathways attributed to cell survival. It blocks apoptosis via phosphorylation-dependent inhibition of pro-apoptotic signaling molecules such as Bad and caspase-9, and activation of anti-apoptotic molecules such as NF-kappaB. Stimulation of the α_2-adrenoceptors also activates ERK and increases the synthesis of survival factors such as bFGF and BCL-2.[123-125]

Recently, it has been shown that α_2 modulation of NMDA receptor function plays an important role in neuroprotection based on neuromodulation instead of direct inhibition of the NMDA receptor for the treatment of glaucoma and other CNS disorders associated with NMDA receptor overstimulation.[126] Brimonidine, being a highly selective α_2-adrenoceptor agonist, which lowers IOP essentially by decreasing aqueous humor inflow, has been established to be neuroprotective to RGCs in this manner. There is an ongoing large randomized controlled clinical trial of neuroprotection called the Low-pressure Glaucoma Treatment Study (LoGTS) comparing brimonidine and timolol. However, the results are not yet available.[76,127]

β-Adrenoceptor antagonists

Another category of widely available drugs is the β-adrenoceptor antagonists, which is further subdivided into the β_1-selective (e.g. betaxolol) and non-selective (e.g. timolol) β-blockers. All β-blockers lower IOP via inhibition of β_2-adrenoceptors present on the ciliary epithelium, thus reducing aqueous humor flow. The neuroprotective elements of β-blockers are believed to be mediated by inhibition of calcium and sodium ion influx into neurons, which occurs in hypoxia, ischemia and excitotoxicity. NMDA and glutamate affinity is also reduced, thereby further reducing calcium influx into the RGCs.[128-135] Timolol binds to voltage-gated calcium and sodium channels, which in turn reduces NMDA stimulated calcium influx, however, to a much lower affinity in comparison to betaxolol. Although the systemic route is just as important as the topical route, betaxolol seems to accumulate in membranes as it is highly lipophilic. Hence, concentration is appreciably lower in vitreous or retina. Correspondingly, high doses of timolol are required to be absorbed systemically. Hence, the topical route may have a better efficacy in reducing IOP and RGC loss though absorption of timolol into systemic circulation which plays an equally vital role.[136] To date, there have been no large clinical trials of β-blockers for neuroprotection in glaucoma.[136-139] Though no large randomized clinical trials have been performed, a number of small clinical trials which compare the effects of betaxolol and timolol on visual field progression in patients with POAG have shown that betaxolol seems to confer better visual field preservation though its ability to reduce IOP is less compared to timolol.[76,140-148]

Prostaglandins

It is well accepted that in the pathogenesis of ischemic and inflammatory injuries, prostaglandins including $PGF_{2\alpha}$ are implicated. They are potent vasoconstrictors and can possibly play a role in the pathogenesis of ischemia and inflammation; however, there is currently no strong evidence to suggest that they are toxic to the retina or optic nerve. Drugs such as latanoprost, travoprost, bimatoprost and unoprostone enhance aqueous outflow, thus reducing IOP. Latanoprost exerts its neuroprotective effects by impeding glutamate and hypoxia-induced apoptosis and is postulated to act via negative feedback on cyclooxygenase-2 activity. Though intravitreal administration of latanoprost has demonstrated an increase in RGC survival following trans-section of the optic nerve,

no electrophysiological data have been documented with regard to the mechanism of action. Again, there have been no large clinical trials focusing on the neuroprotective effects of prostaglandins.[76,149-151]

Carbonic anhydrase inhibitors

Another major group of drugs is the carbonic anhydrase inhibitors, e.g. dorzolamide and brinzolamide, which are selective carbonic anhydrase isoenzyme II inhibitors located in the ciliary epithelium. Carbonic anhydrase isoenzyme II inhibition ensues a reduction in aqueous humor formation as well as increases blood supply to the choroid and optic nerve head, regardless of IOP, though the mechanism is unknown.[152,153] Although it seemed to be neuroprotective in a rat hypertension model, the level of neuroprotection correlated with the level of IOP reduction which might mean that the neuroprotection conferred by these agents may be due to the IOP reduction rather than its direct neuroprotective properties. To date, there is inadequate evidence to show that this group of drugs is successful in providing neuroprotection.[76,154,155]

Other compounds and alternative therapies

Erythropoietin, being a hematopoietic cytokine, has shown to hold amazing neuroprotective properties in pre-clinical models.[156] Endocannabinoids play a big role in central nervous system neurodegenerative diseases. They have vasodilatation properties giving them neuroprotective effects. There are also many natural compounds like omega-3 fatty acids, carnitine, coenzyme Q10, citicoline, curcumin, danshen (*Salvia miltiorrhiza*) and resveratrol that potentially confer neuroprotection via various mechanisms. However, there are no large clinical trials to date which support the use of these compounds in the treatment of glaucoma.[157] Reflexology, the science of stimulating the body's healing forces, is known to increase blood circulation[158] and may be helpful to patients with glaucoma.

Clinical Trials for Glaucoma Neuroprotection

In the past, studies on neuroprotectants have had low success rates in the transition from the laboratory to human trials. Despite successful preclinical cell culture and animal model experiments, most of the Phase 2 and virtually all of the Phase 3 clinical trials of more than 100 neuroprotective drug candidates have failed to demonstrate efficacy, acceptable safety, or patient benefit.

A) Memantine trial

The two-part memantine trial was designed to test the safety and efficacy of oral memantine as a treatment for glaucoma. The first Phase 3 trial did not meet the primary endpoint, but showed a statistically significant difference in a secondary functional endpoint. The second Phase 3 trial did not replicate the results of the secondary endpoint of the initial trial, and although the study showed that the progression of disease was substantially lower in patients receiving a higher dose of memantine compared to patients receiving the lower dose, there was no statistical significance when compared with patients receiving placebo. Therefore, the study did not meet its primary endpoint and did not sufficiently replicate the results of the initial Phase 3 trial. Additional analyses are still ongoing in compliance with safety and regulatory requirements.[159]

B) Brimonidine

A small study of topical brimonidine in nonarteritic ischemic optic neuropathy reported that visual fields tend to be slightly better in the treated group than in the untreated group, but the difference was not statistically significant.[160] Treatment with brimonidine is also currently being studied in comparison to timolol in the LoGTS.[127] The common rationale for human trial failures is that the animal models do not properly simulate the human disease or that the variability of the disease in patients is much higher than the variability of the disease in laboratory animals.

The most challenging issue is the endpoint when designing the clinical trial for neuroprotection. There are several endpoints that could be applied and visual function is an important outcome to consider. However, there is no optimum way to measure the visual function due to inconsistent variables. Whether the endpoint to consider should be an event or the rate of progression remains a vital question that needs to be answered in a neuroprotection trial.

Proposed Guidelines for Use of Neuroprotective Agents

Patients who may benefit from a proven neuroprotectant are suggested to be based upon the stage of the disease.[161] In advanced glaucoma where fixation and/or patient mobility is threatened, and moderate damage with high risk of progression, conventional ocular hypotensive therapy and the neuroprotectant agents should be employed. In glaucoma with moderate damage but low risk of progression, conventional ocular hypotensive therapy should be used and neuroprotective agents should be considered if there is an increase in damage or risk for progression.

What the Future Has to Offer

Extensive research has been conducted on the pathophysiology of glaucoma with the hope of discovering new treatment modalities to this incapacitating disease. Recently, in 2009, A. Di Polo and Y. Yücel introduced the concept of neuroregeneration. It is defined as any strategy that promotes regrowth of axons or dendrites and restores connections with postsynaptic neurons, thereby restoring cell function. Neurons must first be healthy to regenerate, i.e. neuroprotection must have already occurred despite a stressful milieu. Understanding neuroregeneartion remains an active area of neurobiology research which may help gain insight into neuroprotection and eventually be applied to glaucoma.

Although many studies are still inconclusive, the potential of neuroprotective agents of glatiramer acetate, immunomodulators as well as gene and stem cell therapy as adjuvant therapy to IOP lowering agents in glaucoma should not be disregarded all together. Further research for continued understanding of these agents should persist in the hope that this will create a paradigm shift for the future of glaucoma.

Conclusions

It has become increasingly obvious that glaucoma represents a

complex multifactorial disease that produces an accelerated rate of ganglion cell atrophy related to a consortium of pathogenic mechanisms that not only most certainly involve IOP but also include defective autoregulation and ischemia, neurotrophic factor deficiency, glutamate-mediated excitotoxicity, immune-related phenomenon, weak collagenous support at the lamina cribrosa, intracellular calcium influx, and free radical damage. IOP lowering will continue to be the mainstay treatment for glaucoma. The question of alternative non–IOP-lowering therapies directed at preventing further progression has become of interest to both the desperate patient and the treating physician. Based on new, emerging research, neuroprotection has promise for preventing RGC death, independent of IOP. It is evident that pharmacological neuroprotection for glaucoma without a doubt represents an exciting development in the pursuit for a treatment modality for this debilitating disease.

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Publication of the supplement was not supported by any external funding. The Editors, Authors and others involved with the journal did not get any financial or non-financial benefit from any sponsors, unless specified otherwise in the Source of Support at the end of individual articles. I confirm that none of the articles appearing in the Glaucoma Supplement are sponsored articles.

Source of Support: Nil, **Conflict of Interest:** None declared.