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Strategies for Neuroprotection in Glaucoma

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1. Introduction

Glaucoma has long been considered an irreversible progressive optic neuropathy with associated visual loss. Elevated intraocular pressure (IOP) was once considered the main modifiable risk for progression of glaucoma and has been the target for treatment. The pathogenesis of glaucoma was originally based on the mechanical and vascular dysregulation theory, however, this has evolved over the past decade. With the classification of low tension glaucoma, it is now recognized that the damage that occurs in the optic disc is not directly due to the elevated IOP and may be independent of this risk factor. Even though clinicians may aim for a target pressure, progression of optic disc cupping and visual field loss can still continue despite normal IOPs.

In contemplating a systematic approach to neuroprotection, the main areas to target include 1) neurotoxic agents such as nitric oxide and glutamate, 2) deprivation of internal neurotrophic factors 3) balancing self-repair with self-destruction in ocular nerve tissue and, 4) ocular blood flow and combating ischemia [1,2,3]. Focus in this chapter is dedicated to reviewing the mechanisms involved in the pathophysiology of neurodegeneration, target processes that offer neuroprotection, and the chemical and genetic interventions bearing potential for increasing retinal ganglion cell (RGC) survival. Glaucoma has cellular and molecular neurodegenerative pathways akin to those of other neurodegenerative disorders such as Alzheimer's and Parkinson's, which increases the accessibility to possible treatment options.

Gene therapy targets increased conventional and uveoscleral outflow, reduced aqueous production and prevention of wound healing in addition to neuroprotection. Interfering with the apoptosis cycle by gene therapy has also been considered by increasing neurotrophic factors [4]. Intravitreal injections of brain-derived neurotrophic factor (BDNF), a neurotrophin that improves neurogenesis and survival are being studied. Interestingly it has recently been noted in animal models that short periods of hyperglycemia may be protective to the retinal ganglion cells during periods of elevated intra ocular pressure [5].

Neuroprotection is the strategy to prevent retinal ganglion cell death. There have been several methods, many still experimental, aimed at reducing glutamate excitotoxicity, nitric oxide, free radical production and tumour necrosis factor (TNF) inhibition [1-4,6]. With the latest research, glaucoma, which was once thought to be an optic neuropathy, then a retinal disease, is now being considered a neurodegenerative disease, like alzheimer and parkinson [7].

This chapter will review the present pathophysiology theories of neurodegeneration in glaucoma and highlight the latest updates in neuroprotection strategies, mechanisms that block apoptosis and improving the survival and functionality of the retinal ganglion cell.

2. Retinal ganglion cell death

The neurodegeneration seen in glaucoma is as an end result of apoptosis (programmed cell death) of the retinal ganglion cell (RGC). When the retinal ganglion cell dies, there is a degenerative change along the axon with the resulting clinical findings including thinning of the retinal nerve fiber layer (objectively measured by Optical Coherence Tomography, Heidelberg Retinal Tomography or GDx) and increased optic disc cupping. Retinal ganglion cell apoptosis results in visual field loss and ultimately loss of vision in glaucoma. There are several etiologies for retinal ganglion cell (RGC) death which occurs with and without elevated intraocular pressures.

Retinal ganglion cell apoptosis is thought to be a result of several factors:

- increased intraocular pressure (IOP)
- glutamate excitotoxicity
- oxidative stress: free radical induced apoptosis (nitric oxide)
- neurotrophic factors deprivation
- glial cell activation
- abnormal immune response
- hypo perfusion

The glutamate and nitric oxide (NO) theories were the early proposed mechanisms for neurodegeneration. There is a proposed oxidative component which results in oxidative stress on the RGC due to increased IOP and hypoxia leading to apoptosis.

3. Increased intraocular pressure

Although neurodegeneration theories were considered because of progression despite normal IOPs, increased IOP does have a role in RGC death. Increased IOP can block axonal transport of the excitotoxic transmitter, glutamate, at the level of the lamina cribrosa, leading to depre-

vation of neurotrophic factors. It is also theorized that a secondary release or decreased uptake of glutamate via the müller cells is another cause for retinal ganglion cell apoptosis. It has been noted that retinal ganglion cell death has been associated with elevated IOP with positive correlation with an increase in matrix metalloproteinase-9 (MMP-9) activity ($P < 0.001$), tissue inhibitor of matrix metalloproteinase (TIMP-1) ($P < 0.05$) and collagen 1 ($P < 0.01$) [8].

With increased IOP, structural changes occur in the optic nerve head. There are several proposed theories for this effect. The mechanical bowing of the lamina cribrosa and loss of the axons may occur because of the hypo perfusion secondary to increased IOP. Optic nerve damage may be more prominent in hypotensive patients which may in part be due to reduced perfusion and resulting oxidative stress from the induced hypoxia associated with reduced blood flow. In addition to this elevated IOP results in remodelling of the lamina cribrosa which may be a result of an increased synthesis of extracellular matrix; matrix metalloproteinases (MMP), collagen I and IV and elastin [9-11].

The upregulation of MMP may be due to either the vascular insufficiency with resulting ischemia or secondary to increased endothelin and TNF α production [12]. There is a significant correlation between MMP-9 activity and both RGC apoptosis ($P < 0.001$) and loss of laminin ($P < 0.01$) [8,9]. This change in the structure of the lamina cribrosa may result in damage to the retinal ganglion cell axons as they traverse it [13]. Astrocyte activation can result from ischemia, increased hydrostatic pressure or damaged axons and this can propagate the process of structurally changing the lamina cribrosa, resulting in further damage to the transversing ganglion cell axons [14,15].

4. Glutamate

Glutamate is an excitatory neurotransmitter that is continuously released by photoreceptors and OFF bipolar cells in the dark which results in the dark current. Light stimuli start the process of phototransduction which leads to reduced glutamate concentration in the synaptic cleft. Glutamate transporters allow for the uptake of glutamate by müller cells which is converted by glutamine synthetase into glutamine which is then released by the glial cells. This glutamine is taken up by the neurons and hydrolysed by glutaminase to glutamate again. Glutamate allows the influx of calcium, resulting in high intracellular calcium levels which promote apoptosis. Glutamate in excess is neurotoxic, due to its induced excitotoxicity. The glutamate-glutamine cycle allows for natural homeostasis between the neurons and the glial cells (Figure 1).

Glutamate is released from degenerating cells or reduced uptake from müller's cells can increase the presence of glutamate. RGC may undergo apoptosis directly because of increased glutamate excitotoxicity. Müller cells can be injured by the excess glutamate which results in a secondary RGC death [16].

Glutamate ionotropic receptors are found on the post synaptic bipolar, horizontal, amacrine and ganglion cells. They are gating cation channels that are classified into 3 groups; N-methyl-D-aspartate (NMDA) receptors, α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid

(AMPA) receptors and kainite receptors. In view of this glutamate receptor antagonists have been found to reduce the neurotoxic effect of increased glutamate levels.

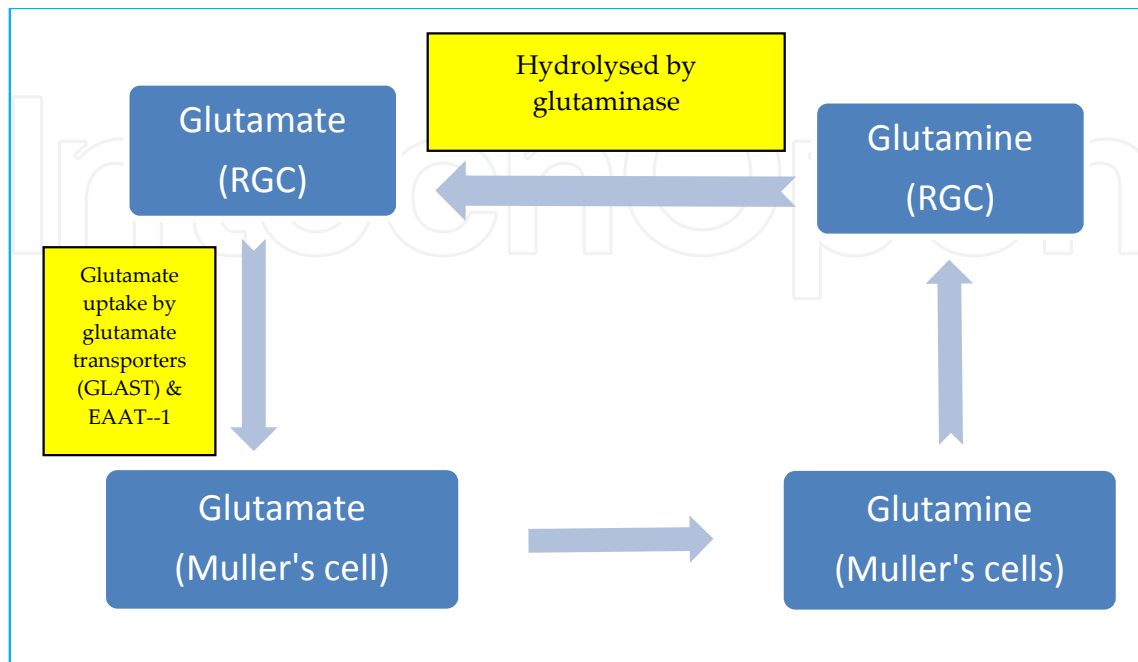


Figure 1. The glutamate-glutamine cycle (RGC= Retinal Ganglion cell, GLAST = Glutamate Aspartate transporter, EAAT-1 =Excitatory Amino Acid Transporter))

Increased glutamate has been noted in the vitreous of glaucoma patients [17]. However the glutamate transporters; glutamate aspartate transporter (GLAST) and excitatory amino acid transporter-1 (EAAT-1) are localized exclusively to müller's cells and glutamate transporter -1 (GLT-1) and excitatory amino acid transporter- 2 (EAAT-2) in the brain, decreased with increasing IOPs [18,19,20] (Figure 1). Therefore, the increase IOP effect on the glutamate transporters can further aggravate glutamine neurotoxicity.

Mice deficient in excitatory amino acid carrier-1 (EAAC1) or GLAST had RGC apoptosis in the absence of elevated IOP. Neuronal EAAC1 does not play a direct role in glutamate transport but transports cysteine much more than GLAST. This is important for the glutathione synthesis. Lack of glutathione made the RGCs more susceptible to oxidative stress [21].

5. Glutamate receptor antagonists

The NMDA ionotropic glutamate receptor has been shown to have an important role in the mechanisms of certain CNS disorders, eg alzheimer's and parkinson's disease as seen in rat and human models [22,23,24]. Glutamate opens calcium and sodium channels after binding to the NMDA receptor, which results in a high intracellular influx of calcium which starts the cascade of apoptosis. Therefore, NMDA receptor blockers have been investigated for counteracting possible glutamate excitotoxicity.

5.1. Memantine

Memantine, is a NMDA receptor blocker which is approved in the USA for dementia associated with Alzheimer's disease. Although oral memantine clinically showed a protective effect on visual function and structural damage on macaque monkeys, it did not persist in long term treatment (>5 months) on ERG findings [25,26]. It has been used at doses of 4mg/kg po daily reaching concentrations of 0.3-1.8µM in the monkey vitreous [25,26]. The second phase III clinical trial showed that although the progression of disease was significantly lower in patients receiving the higher dose of memantine compared to patients receiving the low dose of memantine, there was no significant benefit compared to patients receiving placebo [27].

Latest animal studies have shown that using memantine in monkeys will result in an overall higher mean multifocal visual evoked potential (VEP) amplitudes than the non treated memantine monkeys when experimental glaucoma has been induced [28]. However it was not significant from baseline in the former. The use of the GDx in future studies will also allow more sensitive changes in retinal nerve fiber layer to be detected however, this may not directly be translated into functional damage, which in humans can be assessed with visual fields.

5.2. Eliprodil

This NMDA antagonist acts at the polyamine binding site of the NMDA receptor (NR2B subunit), blocking voltage dependent calcium channels. It has been shown to be neuroprotective in cultured neurons of brain and retina from excitotoxic and ischemia damage at doses of 1-10mg/kg [29]. Eliprodil has shown reduction in the NMDA currents by 78% in a glutamate induced cytotoxicity model [30]. Although there has been promise of this drug in animal studies, clinical trials have not been undertaken for glaucoma in humans.

5.3. Nitric oxide

Nitric oxide (NO) is a neurotransmitter, vasodilator and neuromodulator and can be neurotoxic. Nitric oxide is found at the post junctional area of glutaminergic junctions (rods, bipolar, amacrine and ganglion cells) and acts as an intracellular mediator for glutamate. Excessive production of nitric oxide by astrocytes has been shown to play a role in cell death in both the optic nerve head and the RGC [2,3,6,31,32]. Reactive oxidative species (ROS) may play a role in neurodegeneration as a result of apoptosis (Figure 2).

5.3.1. Oxidative stress

Nitric oxide is produced by nitric oxide synthase (NOS-2). NOS has 3 isoforms inducible NO (iNO), endothelial NO (eNO) and neuronal NO (nNO). These oxidize L-arginine to L-citrulline, producing NO. Nitric oxide freely diffuses to adjacent neurons and combines with O₂ – to form peroxynitrite anions (ONOO⁻) which is a potential toxin, setting into motion neuronal apoptosis. It can be induced by injury or cytokines, such as interleukin 1 beta, tumour necrosis factor alpha, resulting in high concentrations of nitric oxide [32,33]. Increased levels of NOS are seen in the optic nerve head of glaucoma patients [32]. Tumour necrosis factor (TNF) α is upregulated in the glaucomatous optic nerve head and induces NOS in the astrocytes [34].

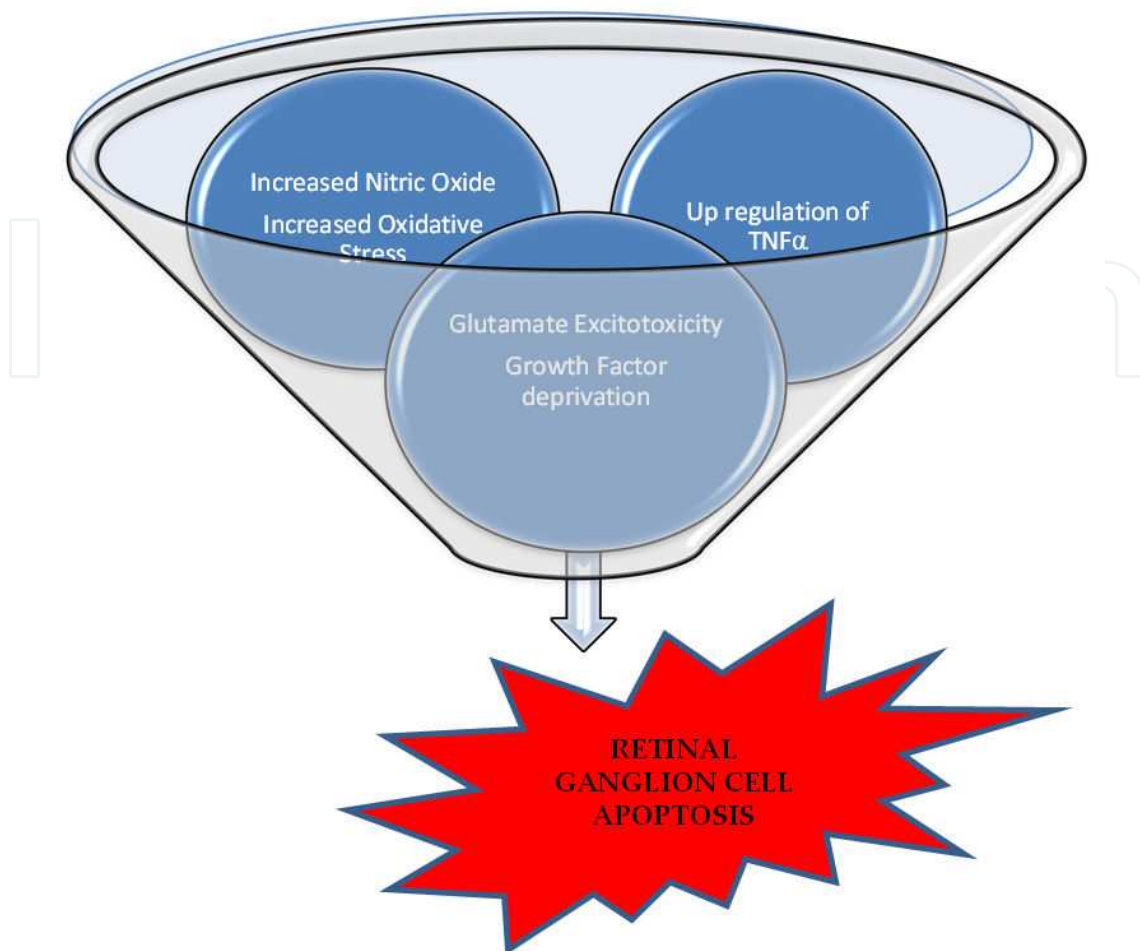


Figure 2. Multiple mechanisms for neurodegeneration which may be aggravated by vascular dysregulation, hypoxia and elevated IOP

nNO and iNO are expressed in reactive astrocytes. Increased NO reacts with a superoxide anion which can be toxic to the axons of the retinal ganglion cell. Motallebipour et al, showed a genetic association between iNO and primary open angle glaucoma (POAG) using genetic analysis and nuclear factor [35]. iNO is located in the astrocytes and microglial in the optic nerve head and expresses more activity with exposure to increased intraocular pressure and cytokines. This results in increased in NO production and the induction of the apoptotic cascade [36]. The NO oxide has its effect in both the astrocytes of the optic nerve head and the pericytes of the vasculature [32].

5.3.2. Vascular modulation

The endothelial NO synthase (eNOS) is expressed in the trabecular membrane and schlem's canal cells. eNOS produces nitric oxide which regulates the vascular tone causing smooth muscle relaxation and relaxation of the trabecular meshwork which improves aqueous humour outflow [37]. Elevation of the IOP increases the shear stress which activates eNOS which results in increase in the pressure dependent outflow.

5.3.3. *The future*

Krauss 2011 and Impagnatiello 2011 have had success in lowering IOP in preclinical trials with a nitric oxide donating prostaglandin F2 agonist (BOL-303259-X) more than with latanoprost (prostaglandin F2 agonist) alone [38,39]. Fabrizio 2012, also had some success with combining a carbonic anhydrase inhibitor with a nitric oxide moiety, NCX250 in lowering IOP compared with the a carbonic anhydrase inhibitor alone [40].

6. Alpha adrenergic receptor agonist

α_2 adrenergic agonists are a known group of anti glaucoma drugs that inhibit adenylate cyclase, reducing cAMP, thereby decreasing aqueous production. They also act by increasing uveoscleral outflow. α_{2A} receptors can be found in non pigmented ciliary epithelium, α_{2B} receptors on neuronal dendrites and α_{2C} receptors on photoreceptors cell bodies and inner segments [41]. α_2 agonists have been shown to have secondary neuroprotective effects [42,43,44].

6.1. Brimonidine

α_2 adrenergic receptors can modulate the release of neurotransmitters such as glutamate [45]. NMDA receptors when stimulated results in an increase in intracellular Ca^{2+} and an inward current in the RGC. Brimonidine, an α_2 agonist, can block the NMDA receptors which results in controlling the intracellular calcium, hereby allowing neuroprotection [23,46]. Brimonidine is also thought to up regulate brain derived neurotrophic factor (BDNF), activating anti apoptotic genes and the cell survival signaling pathway. It is also thought to modulate the N methyl-D-aspartate receptors [43,46-48].

Brimonidine is also known to upregulate not only BDNF, but prosurvival factors, such as anti apoptotic factors B-cell lymphoma -2 (Bcl-2) and B-cell lymphoma extra large (bcl-xl), basic fibroblastic growth factor (bFGF) and extracellular signal regulated kinases (ERKs). These actions assist in the prevention of neuronal death and promotes cell survival [49].

7. Selective beta receptor blockade

Beta blockers have a long history of use in reducing the IOP in glaucoma by reducing the production of aqueous humour. Levobetaxolol, timolol and metipranolol have been shown to have secondary neuroprotective effect by reducing sodium and calcium influx, which reduces the release of glutamate with levobetaxolol being more effective than timolol [50,51,52].

7.1. Betaxolol

Betaxolol has been shown to reduce the spontaneous firing rate by suppressing glutamate-gated current and in effect Na currents in the ganglion cells [52]. By doing this it also reversibly

blocks the voltage gated calcium current. High intracellular calcium can be neurotoxic. Due to the Ca²⁺ channel blockage activity by the selective beta 1 beta blocker, betaxolol exerts a neuroprotective effect on the retinal ganglion cells. This effect can be seen at 2-50uM concentration [53]. Timolol is not effective even in higher concentrations (100uM) and clinically betaxolol is more efficacious in preserving visual fields in glaucoma patients compared to timolol [54]. It has been demonstrated in human cryopreserved retinal arterioles that intraluminal betaxolol caused a significant greater dilatation than timolol, this may be due to the selective nature of the beta blocker [55]

Betaxolol 0.5% also upregulates the neurotrophic factor BDNF in retinal glia cells [56]. By its action on vascular smooth muscle relaxation this improves blood flow and reduces ischemia induced RGC apoptosis [52, 57]. Retinal ganglion cells protection has been shown using rat experimental model and the preservation of the a and b waves in the electroretinogram in both ischemic-reperfusion and glutamate toxicity models [56,57,58]. This has also been seen in light response experiments on tiger salamander flat mounted retinas [53].

8. Calcium channel blockade

As high intracellular calcium can be neurotoxic reducing this effect can be neuroprotective to the cells. This effect can be seen in both beta blockers and alpha agonists [2] (Table 1).

9. Prostaglandin analogs

Prostaglandin analogs are known as a first line treatment for reducing the IOP. However, latanoprost and bimatoprost acid have shown a neuroprotective on hypoxic induced or glutamate excitotoxicity on RGCs [58,59]. This was IOP independent and is not thought to be associated with normal mechanism to lower IOP [59]. Acting via prostaglandin F2 receptors, it has been suggested that latanoprost may have a COX 2 feedback inhibition resulting in neuroprotection [58]. It has also been shown that it inhibits inducible NOS [56]. Latanoprost may also be combined with the NO moiety as previously mentioned [38,39]. Further, it has been theorized that it may have an anti apoptotic effect through the inhibition of caspase-3 [58,60].

10. Carbonic anhydrase inhibitors

The hypotensive effect of carbonic anhydrase inhibitors is a result of reduction of aqueous humour production at the ciliary epithelium level. However in cultured retinal cells, RGC death is prevented by dorzolamide because of its anti-apoptotic pathway [60].

NEUROPROTECTION STRATEGIES	Substances studied	General Method of Action
Glutamate Receptor Antagonists • NMDA receptors	Memantine Eliprodil	Reduces glutamate excitotoxicity and neurotoxicity
Nitric Oxide Synthase Inhibitors	Combinations of nitric oxide donating prostaglandin F2 agonist and with a carbonic anhydrase inhibitor (experimental)	Reduce oxidative stress
Beta blockade	Selective Beta Blockade (Betaxolol)	Reduces IOP Reduce glutamate production Upregulates BDNF Calcium channel blockade
Alpha adrenergic agonists	Alpha 2 agonist (Brimonidine)	Reduces IOP Reduce glutamate production Upregulates BDNF Calcium channel blockade Increases anti apoptotic genes
Prostaglandin analogue	Latanoprost acid Bimatoprost acid Tafluprost acid	Reduces IOP Inhibition of COX-2 activity Possible caspase 3 inhibition
Carbonic Anhydrase Inhibitor	Dorzolamide	Reduces IOP Reduces apoptosis
Neurotrophic factors (BDNF and CNTF)	Brain Derived Neurotrophic Factor Ciliary Derived Neurotrophic Factor	suppress the intrinsic apoptosis whilst activating the survival signals
Antioxidants: Reactive Oxygen species scavengers	Melatonin Vitamin E Co Q10 cofactor Manganese Tetrakis (in vitro)	activates anti oxidative enzymes Neutralizes free radicals. Oxidative stress can damage the trabecular meshwork, optic nerve head and retina.
Immunomodulators Anti Inflammatory agents TNF- α Inhibitors	Cop-1 (glatiramer acetate) Ethanrecept Agmatine, an aminoguanidine Aspaminergic agent GLC756	Immunization can modulate immune function
Gene Therapy (Mitochondrial Augmentation)	Cycloheximide (CHX)	inducing neuroprotective genes including bcl-2
Apoptosis Inhibitors Inhibition of cytochrome c release Caspase inhibitors	Deprenyl BIRC4	Increase mitochondrial expression of bcl-2 and bcl-x, suppresses bax. Improved neuronal survival
Hypo perfusion	Gingko Bilboa IOP lowering medications	Improved blood flow

Table 1. Pharmacological neuroprotection strategies

11. Antioxidants

Numerous studies have shown that mitochondrial metabolism results in the release of reactive oxidative species that cause damage to lipids, protein, resulting in cell death and neurodegeneration [61]. Hypoxia and ischemia are found to play an important role in the cascade of events leading to oxidative stress, and stimulating delta-opioid receptors (DOR) [62]. DOR has been proven to reduce the build-up of harmful free radicals, glutamate, and pro-inflammatory cytokines [62]. It has been shown that naloxone, an opioid blocker given intraperitoneally 6mg/kg in rabbits can reduce the retinal thickness thinning caused by ischemia [63]. Morphine has been used to pharmacological pre condition rabbit retina and has been shown to reduce acute IOP induced damage [64]

11.1. Coenzyme Q10 (Co Q10)

Coenzyme Q10, either on its own or in combination with vitamin E (alpha -tocopherol) have been shown to reduce intravitreal NMDA mediated damage in mice when administered orally in 10mg/kg dosage [65]. In addition to its effect against oxidative stress its positive effect on the mitochondria may assist in the energy levels within the neuron, protecting it from apoptosis [66]. The RGC requires energy produced from mitochondria to ensure the conduction of currents and normal function of the RGC. Agents that promote the ganglion cell mitochondrial energy production may be neuroprotective in glaucoma. Oral alpha-lipoic acid and nicotinamide have been suggested for further assessment for their neuroprotective effect on light induced neuronal apoptosis [67].

Ginkgo biloba may be useful for treating dementia associated with alzheimer and for vascular insufficiency. Although the mechanism of action remains unknown for its use in glaucoma, it is thought that it causes intracellular signaling and neutralizes reactive oxygen species [13, 68, 69]. It has been shown to reduce the RGC axonal loss in mice compared to controls in a dose dependent manner after intragastral administration [68]. In one prospective randomized placebo controlled cross over trial, Ginkgo biloba extract was used 40mg tds orally for 4 weeks, which resulted in a statistically significant decrease in the corrected pattern standard deviation in visual fields of those patients [70].

Visual field defects have been noted to improve in patients with normal tension glaucoma after 4 weeks on ginkgo biloba and no ocular nor systemic adverse events occurred. Ginkgo biloba may exert multifactorial mechanisms which include increase ocular blood flow, anti oxidant activity, nitric oxide inhibition and improved cognitive function due to improved cerebral blood flow [70]. *Manganese Tetrakis* (4-benzoyl acid) porphyrin, a cell superoxide scavenger can prevent NO mediated motor neuron death in vitro [3]. *Cycloheximide* (CHX), a protein synthesis inhibitor has been used in doses of 50-500nM, to prevent neuronal death and protects against oxidative insults by inducing neuroprotective genes including bcl-2 [3].

12. Neurotrophic factors

12.1. Brain derived neurotrophic factor

Brain derived neurotrophic factor is a neurotrophin derived from the brain (produced in the lateral geniculate body of primates) which moves in a retrograde fashion to bind TrkB receptors on RGC cell body and axon. Its retrograde transport is obstructed in acute and chronic glaucoma models, hence apoptosis occurs, as its neurotrophic support is important in RGC survival [13].

Eyes with chronic glaucoma exhibit loss of physiological neurotrophin levels particularly BDNF. Intravitreal injections of neurotrophins, eg BDNF has shown a reduction in apoptotic RGC death in adult rat models [71]. A recent study considered the cost effective use of serum BDNF as a biomarker for early POAG as its levels were significantly decreased in glaucoma patients compared with controls [72].

12.1.1. Role in neuroprotection

BDNF acts through Trk B receptors; phosphorylating kinase enzyme, activating phosphoinositol 3-kinase thereby inhibiting the activation of capsase 3, an important link in the apoptosis pathway (Figure 3). Experimentally BDNF has shown little effect on RGC survival in a single dose, but repeated intravitreal injections as well as virally mediated over expression has been shown to slow RGC loss [4]. It has been used at doses of 25-100ug/kg in clinical trials [73]

12.2. Ciliary derived neurotrophic factor

Ciliary derived neurotrophic factor (CNTF) is a secretor- protein expressed in cells of all retinal layers and the optic nerve head. The protein shows increased expression in retinal and optic nerve injuries, and is reduced in the presence of increased IOP [4]. The protein demonstrates neuroprotection in virally-mediated overexpression after intravitreal injection. In one study by Pease et al, CNTF showed a 15% less axonal death in experimental induced glaucoma, which was statistically significant over combined CNTF- BDNF and BDNF alone [74]. Intraocular delivery of neurotrophins, BDNF and CNTF, intravitreal or by viral transfer may be a potential future development for neuroprotection [4].

13. Immune modulation

13.1. Anti-inflammatory agents

There is an inflammatory component to the neuronal retinal degeneration in glaucoma [75-78]. Studies have proved an age related susceptibility of glaucoma victims to progressive nerve damage and RGC loss even with single digit IOP [4]. Researchers have also established elements of the complement pathway such as C1q, as markers for astrocyte destruction that

may result in RGC apoptosis [78]. Thus, the future of glaucoma therapy lies in employing additional modalities based on proven mechanisms of RGC loss.

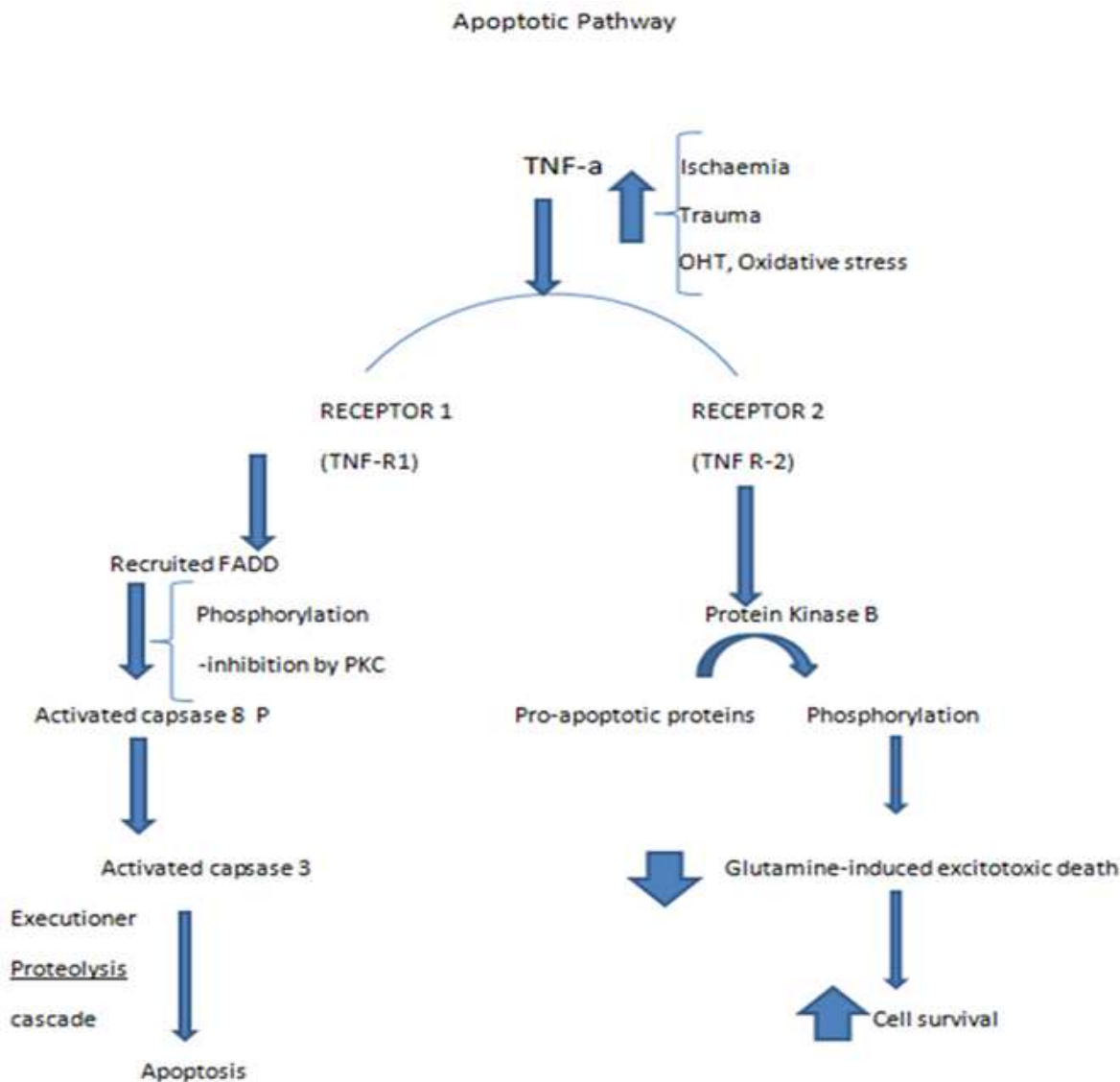


Figure 3. Apoptosis Pathway for Retinal Ganglion Cells

13.2. Tumour Necrosis Factor α (TNF α)

Progression of optic nerve axonal degeneration and retinal ganglion cell (RGC) apoptosis, have been shown to be responsible for progressive visual field loss in glaucoma, with or without ocular hypertension. One mechanism has been linked to tumor necrosis factor (TNF- α) in tissue around the optic nerve head demonstrated during immunostaining of mouse specimen [77]. This protein is a pro-inflammatory cytokine produced in response to trauma and inflammation and can start the apoptotic cascade [77] (Figure 3). TNF- α , secreted by damaged glial cells and through the binding of TNF receptor-1 (TNF-R1) starts the apoptotic process

stimulating caspase ultimately leading to RGC death [79] (Figure 1). However the binding of the TNF-R1 receptor also triggers via heat shock proteins and activation of transcription factor NF-KB, a cell survival pathway. TNF- α levels if at best optimized (kept low) create a homeostasis that facilitates a balance between neuroprotection and neurodegeneration [75,79].

TNF- α is one of a 19-member family of ligands that exert their inflammatory activities through 29 receptors, triggering a cascade of inflammatory responses. TNF- α has been found to be up-regulated in neurodegenerative diseases such as parkinson's and alzheimer's disease. In studies on the brain tissue of Alzheimer's patients, TNF- α , a mediator of chronic inflammation, has been detected in increased levels [76].

TNF- α in some studies was found in high concentration after laser-induced OHT, along with increased macrophage/microglia near the optic nerve head [77]. The levels of TNF α surpassed those in other inflammatory processes not involving ocular hypertension, validating the protein as a likely mediator of the RGC death, and hence a target for gene therapy. The increase in microglia population in the vitreous surface around the optic nerve head also alludes to an inflammation theory of glaucomatous optic nerve and RGC damage [77]. Research in alzheimer's has established TNF- α as a mediator of chronic inflammation with detection of increased levels in the brain of victims of this neurodegenerative disorder [76]. Serum amyloid A, is another acute-phase inflammatory marker discovered in the retina and trabecular meshwork of glaucoma eyes [78]. An understanding of the molecular processes in parallel disorders will influence the outlook on the future of glaucoma management.

Agarwal et al 2012, reported on extensive research evidence in support of the role of TNF- α in glaucomatous optic nerve degeneration and RGC apoptosis [75]. Research involved eyes with POAG, NTG and exfoliative glaucoma, with cataract eyes as controls [75]. Results showed marked elevation in TNF- α in aqueous samples of all glaucoma groups compared with controls. In addition, optic nerve degeneration and RGC loss were demonstrated in eyes subject to intravitreal injection of TNF- α .

13.2.1. How does TNF- α work?

Under normal conditions there is greater expression of TNF-R1 over TNF- α . Stress factors such as trauma, ischaemia, and elevated hydrostatic pressure result in an increase in expression of TNF-R1 and TNF- α . These have been shown to have several roles including pro-apoptotic and neuroprotective properties depending on the environment in which they are expressed [Figure 3]. Experimental evidence using mouse eyes have shown that in the absence of normal glial cells, the apoptotic effect dominates. Microglial cells are thought to provide survival signals necessary for the neuroprotective effect of TNF- α . Insults such as ischemia, oxidative stress and optic nerve injury increases the expression of cell death signals and reduces the expression of the cell survival signals, thereby potentiating the harmful effects of TNF- α [61].

In contrast, normally functioning glial cells support the neuroprotective effects of TNF- α and TNF-R1. The ischaemic and hydrostatic stress in glaucoma activate microglial activity causing an inflammatory response. Activated glial cells produce TNF- α along with harmful compounds like NO and endothelin 1 (ET-1). In excessive microglial activation, up regulation of

TNF α - causes RGC apoptosis in the absence of normal glial support. If there is significant microglial insult early in the event, TNF α - continues to exert apoptosis even after the stimulus is removed, as has been shown in in-vivo studies with mice, where progression of RGC death was seen on immunostaining even after normal IOPs were reached [75].

13.3. Agmatine, an aminoguanidine

Current research targets TNF- α for neuroprotection by reducing RGC loss (Figure 3). Such agents need to have high selectivity and specificity for excessive TNF- α and TNF-1 expression while preserving local immunity. Agents such as Agmatine, an aminoguanidine, have been shown to protect RGCs against the apoptotic effects of TNF- α , but the effects on other receptors and pathways are yet to be established [44,45,75]. Agmatine has been used at a concentration of 60 mg daily in rat ocular hypertension model [31]. 10(-3) M agmatine solution 4 times a day has shown a high affinity for alpha 2 receptors on the ciliary body, where it exerts its IOP lowering effect which has been seen in the rat model [80]. Amnioguanidine also targets inducible nitric oxide synthase (iNOS) inhibitors [31,73].

13.4. Ethanrecept, the future in neuroprotection

Work done by Roh et. al (2012) demonstrated the ability of ethanrecept, a recombinant chimeric protein, to act as a TNF α inhibitor to reduce RGC loss in the wake of elevated TNF α [77]. This decoy protein selectively binds TNF α , sparing the RGC damage from this and other inflammatory agents such as microglia [77]. Ethanrecept is used in the treatment of juvenile idiopathic arthritis, rheumatoid arthritis, ankylosing spondylitis, and psoriatic arthritis, and has shown no IOP-lowering capabilities. The drug however shows promise as a neuroprotective agent for intravitreal use in the future.

13.5. Copolymer -1 (Cop-1), – A possible vaccination for neuroprotection?

The inflammatory process in neurodegenerative diseases such as alzheimer's and glaucoma has been found to be associated with pro-inflammatory activities mediated in part by T cell activity. Cop-1, a synthetic peptide polymer known to suppress autoimmune encephalomyelitis, modulates this T cell reaction by producing a Th2 anti-inflammatory phenotype with attenuation of normal inflammatory response in neurodegenerative diseases as well as increased neuroprotection [13, 81,82]. Cop-1, glatiramer acetate has been FDA approved in the treatment of multiple sclerosis, a demyelinating disease.

It had been noted experimentally that an eye that had recent glutamate injections had resulting large numbers of lymphocytes present, hence it was theorized that glutamate toxicity induces a T cell lymphocyte reaction [81]. Therefore, by immunizing against this with the correct antigen, theoretically could reduce the damage induced by the glutamate. Cop-1 immunization has shown some protection against glutamate toxicity and elevated IOP in mice retinal ganglion cells [81,82,83]. So T cell mediated immunoprotection may be a future option for glaucoma, however, much research is still to be done.

13.5.1. Opioids

Opioid receptor activation has been shown to reduce the ischemic damage to the retina as demonstrated by ERG [84]. Opioid receptor stimulation and the facilitation of the actions of endogenous opioids show promise in neuroprotection of RGCs in glaucoma [84,85]. In the mice model, glaucoma was induced by raising the IOP above the systolic blood pressure (155-160mmHg) for 45 minutes to induce ischemic retinal injury [84]. The opioid antagonist naloxone (3mg/kg) was given to mice intraperitoneally 24 hours before the ischemic event. Another study group of mice had morphine (0.01-10mg/kg given intraperitoneally 24 hours before the ischemic injury. 7 days after the injury the retina of both groups were assessed by the ERG. The mice that has morphine had greater preservation of their ERG a and b wave amplitudes 7 days after the ischemic event. Further the protective effect of morphine on preservation of ERG amplitudes was dose related with the ED50 of 0.18mg/kg [84]. However, these strategies have not yet been tested in humans or undergone randomized controlled trials.

14. Stem cells

Much research is still yet to be done on stem cells and neuroprotection. Stem cells can supply neurotrophins and modulate matrix metalloproteinases after an injury which can be neuroprotective and limit neuronal damage [86]. However in a pre-clinical model of glaucoma, intravitreal stem cell injections have been shown to enhance the survival of the RGC [23].

15. Gene therapy

With emerging evidence for the molecular basis in glaucoma- pathophysiology, the disease may be interrupted by targeting key sites once the genetic expression is known. Studies of micro-RNA such as miRNA-125b has led to the understanding of the key sites for targeted down-regulation of messenger RNA which is thought to add to the oxidative stress induction of inflammation and astrogliosis in alzheimer's disease [78]. Alzheimer's disease, parkinson and glaucoma are thought to have a similar neurodegenerative basis (molecular and cellular pathways for neuronal cell loss) [78]. Hence gene therapy for glaucoma and other neurodegenerative disorders may be where medical management is headed. Target sites include uveoscleral outflow site, surgical (trabeculectomy) site, ciliary apparatus, retina and optic nerve head (neuroprotection) [4].

Gene therapy would be helpful in preventing neurodegeneration using anti apoptotic genes, bcl-2 and bcl-x [2]. Another mechanism is blocking the apoptotic pathway with deprenyl (monamine oxidase inhibitor). It is proposed that it stabilizes the mitochondrial membrane potential, preventing the release of cytochrome c which can activate capsases (Figure 1) [2, 87].

Targeting antioxidant genes is a promising strategy for future management of glaucomatous neurodegeneration. Researchers used cloned extracellular superoxide dismutase (ECSOD) or

catalase (CAT), carried on recombinant adeno associated virus intravitreally in mice. The mice were euthanized and optic nerve volume, myelin fibre area, axonal cell loss and RGC loss evaluated. Initial response showed a 15 fold increase in ECSOD and 3.3-fold in CAT [88]. After six months the authors reported 29% reduction in RGC loss, 36% in ON demyelination, and reduction in axonal loss by 44% all compared to control eyes, indicating that antioxidant gene therapy will prove an invaluable adjunct to current glaucoma therapy.

15.1. Administering gene therapy

Administration of gene therapy must ideally be safe, repeatable, have low immunogenicity, and carry low infectious and mutagenic potential, modification of Koch's postulates [4]. Because viral vectors have the ability to maintain stable DNA within the target nucleus, they are preferred over non-viral vectors.

15.1.1. Viral vectors

Adenoviral vectors (Ad), non-enveloped replication-deficient recombinant viruses were the first to be used in gene therapy research [4,89]. They show high level of tropism for post mitotic and highly specialized cells, and have been known to reproduce TM cells with high accuracy. They have application in Muller cell and RPE cell replication as well. Studies have been done with Adenovirus (Ad) mediated intravitreal delivery of BDNF. However, repeated injections have been found to cause severe inflammation in experimental models [2, 90]

Adeno-associated Viruses (AAV). This is an integrating vector known to show efficient delivery to target tissues. AAVs do not carry viral genes, therefore they have no unwanted pathogenicity, immunogenicity, nor significant inflammation upon sub retinal application [2]. In the last 4 years the use of AAV vector in the delivery of gene therapy has met some success in human trials, but the effect is limited to RGC survival. Though the vectors may target trabecular meshwork cells, they are not very active there [89].

Herpes Simplex Virus (HSV). This virus has shown promise in glaucoma research and therapy as it is able to transduce trabecular meshwork, ciliary body epithelial, and retinal ganglion cells. The injected derivative however has been found to carry risks of inflammation, toxicity, and limited duration of gene expression [4].

Lentiviral vectors. These single strand RNA viruses can incorporate trabecular meshwork and RGC DNA by reverse transcription, with both neuroprotective and IOP lowering potential. Combining several enzymes such as cyclooxygenase and prostaglandin pathway enzymes increases their IOP-lowering properties. [90]

15.1.2. Non-viral vectors

Naked DNA Injection. Work done with naked DNA as plasmid vectors expressing chloramphenicol acetyl transferase has shown promise in the possible control of wound healing after trabeculectomy. The plasmid injected in the bleb or under collagen shield has resulted in a 30 fold increase in the activity of the enzyme. [4].

The use of short 21 siRNA by intracameral and intravitreal injection to silence the unwanted expression of glaucoma genes particularly in the trabecular meshwork is being studied. The effects are so far temporary, and such siRNAs need the assistance of developed nanoparticles, such as magnetic nanoparticles, to enter target cells [89]. Chemical approach include the use of *cationic lipids (liposomes)* used in vitro, and shows promise for in vivo application via intracameral route with the target tissue being trabecular meshwork [4].

16. Conclusion

The cause of glaucoma and ultimately retinal ganglion cell death is multifactorial. At present there is no cure for glaucoma and the mainstay of treatment medically and surgically is to control the IOP. However, this conventional approach of lowering IOP is merely a secondary or indirect approach to the real problem. Current studies show that glaucoma is a neurodegenerative disease with neuroprotection and possibly neuroregeneration and neuro enhancement as the future treatment modality. Modified Koch's postulates have been applied in the experimental neuroprotective research. Ultimately the retinal ganglion cell death whether primary or secondary (bystander result) must be stopped and the neurons preserved. The clinical application of most of these experimental neuroprotective strategies still has yet to pass through randomized controlled clinical trials before they can be accepted. The future holds much promise as to possible effective neuroprotective strategies, however, much research is still yet to be done.

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