Chapter II

Current Trends in Glaucoma: What about Neuroprotection?

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Abstract

Glaucoma is an optic neuropathy, considered as the second leading cause of blindness worldwide. Glaucoma is characterized by selective death of retinal ganglion cells (RGC) and a progressive loss of vision. Elevated intraocular pressure (IOP) is one of the most important risk factors for developing glaucoma, so we mainly focus on lowering IOP to arrest the progression of glaucoma. However, many patients continue to demonstrate a clinically downhill course despite the control of initially raised IOP. In fact, some patients develop what is called Normal Tension Glaucoma, not associated to an increased IOP. This emphasizes that several pressure-independent mechanisms are responsible for the development and progression of glaucomatous neuropathy and that high intraocular pressure (IOP) and vascular insufficiency in the optic nerve head are only risk factors for the development of glaucoma, and are not the only target for the treatment of glaucoma. The reason is that the process of RGC death is thought to be biphasic, and the primary injury is followed by a slower secondary degeneration related to a noxious environment surrounding the apoptotic cells. This environment is characterized by changes in the extra-cellular ionic concentrations, increased amounts of free radicals, neurotrophins depletion and increased glutamate induced excitotoxicity due to high extra-cellular glutamate levels, which binds

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to N-methyl-D-aspartate (NMDA) receptors leading to an abnormally high intracellular Ca\(^{2+}\) concentration.

Neuroprotection is a process that attempts to preserve the remaining cells that are still vulnerable to damage, and the main aim of neuroprotective therapy is to employ pharmacologic or other means to attenuate the hostility of the environment surrounding the degenerating cells, or to supply the cells with the tools to deal with this aggression, providing resilience to the insult.

Several agents have been reported neuroprotective in glaucoma, both in clinical assays, such as Ca\(^{2+}\) channel blockers, and in experimental studies, such as betaxolol, brimonidine, NMDA antagonists, Nitric Oxide Synthase inhibitors, neurotrophins and ginkgo biloba extract.

Most neuroprotective agents for glaucoma have probed beneficial effects over RGC, not showing effects over IOP. However, when analyzing classically used medications for glaucoma, it becomes difficult to understand if its effect over the progression of glaucoma is due to neuroprotective pathways or by means of lowering IOP.

The ideal anti-glaucoma drug would be one that when applied topically, reduces IOP, but also probes to reach the retina in appropriate amounts, and activates specific receptors in the retina to attenuate retinal ganglion cell death.

1. Introduction

1.1. Glaucoma

Glaucoma is an optic neuropathy that affects nearly 60 million people, being expected to reach 79.6 million by year 2020 [Quigley et al., 2006]. Nowadays it is considered the second leading cause of blindness worldwide. Glaucoma is associated with selective death of retinal ganglion cells (RGC) [García-Valenzuela et al., 2005; Quigley, 1995; Mittag et al., 2000; Cordeiro et al., 2004; Libby et al., 2005; Urcola et al., 2006; Hernández et al., 2008] and structural changes in the optic nerve head (fig. 1). This loss of RGC and previous axonal dysfunction are thought to be the cause of visual field changes in those affected by glaucoma [Buckingham et al., 2008].

The cause of glaucoma is not yet well known. Elevated intraocular pressure (IOP) has been established as the main risk factor for developing glaucoma. However, glaucoma is more than just an elevated IOP; it is considered as a multifactorial disease. IOP is regulated by aqueous humour dynamics in the front of the eye, and the neuropathological changes in glaucoma occur in the posterior portion of the eye. It is known that ocular hypertension is due to impairment in the removal of aqueous humour via the trabecular meshwork of multiple causes. Trabecular meshwork inducible glucocorticoid response (TIGR) gene at fault has been identified in juvenile glaucoma, being responsible for 13% of all cases of primary open-angle glaucoma [Stone et al., 1997].

Electroretinography in glaucoma eyes can also show deterioration. ERG demonstrates an attenuation of both a-waves and b-waves after the induction of ocular hypertension using cauterization of episcleral vein experimental glaucoma model in animals [Bayer et al., 2001; Mittag et al., 2000], and also a deterioration of inner retinal function, measured by an attenuation of positive and negative scotopic threshold response (STR) [Li et al., 2006].
Primary open-glaucoma (POAG) is considered to be the most common subtype of glaucoma, and its pathogenesis remains a fascinating area for new research studies. It is almost certain that POAG develops in a multifactorial manner, and some of the risk factors may determine the appearance of the damaged glaucomatous optic nerve head [Broadway et al., 1999] (Figure 1).

1.2. Risk Factors

POAG is dependent on the presence of varying risk factors and the presence of a greater combination of these risk factors that makes it easier to develop glaucoma [Broadway et al., 1999].

Numerous potential secondary risk factors for the development of glaucoma have been studied. First of all, high ocular tension (above the normal population level, 21 mmHg) is established as the primary risk factor [Hart et al., 1979; Leibowitz et al., 1980; Armaly et al., 1980; Drance et al., 1981; Sommer, 1989; Quigley et al., 1994]. However, the existence of patients with normal-tension glaucoma (NTG) has suggested that secondary factors play an important role in the development of glaucomatous optic neuropathy in these patients whose IOPs lie within the normal statistical range [Broadway et al., 1999].

Cardiovascular disease has also been implicated as potential risk factors [Carter et al., 1990; Demailly et al., 1984; Drance et al., 1978; Freyler et al., 1988; Goldberg et al., 1981; Schulzer et al., 1990; Spaeth et al., 1975]. Other specific cardiovascular risk factors have also been proposed, such as systemic hypertension [Tielsch et al., 1995; Wilson et al., 1987], hypotension, diabetes [Graham et al., 1995; Gramer et al., 1985; Hayreh et al., 1994; Tielsch et al., 1995], increased blood viscosity or platelet aggregation [Hoyng et al., 1992; Klaver et al., 1985; Trope et al., 1987], vasospasm [Drance et al., 1988; Flammer et al., 1987; Gasser and Flammer, 1987; Gasser and Flammer, 1991; Gasser et al., 1990] and migraine [Corbett et
Increasing age, family history of glaucoma and black race are also identified risk factors for developing glaucoma [Drance et al., 1978; Hart et al., 1979; Hollows and Graham., 1996; Quigley et al., 1994; Shin et al., 1977; Sommer et al., 1991].

Myopia has also been identified as a risk factor for developing glaucoma [Daubs and Crick, 1981]. Probably, thin eye wall and large globe increases the influence of intraocular pressure over the optic disk.

### RISK FACTORS ASSOCIATED WITH INCREASED PREVALENCE OF GLAUCOMA DAMAGE

- Glaucomatous damage in the fellow eye
- High intraocular pressure
- Age
- Race
- Thin cornea
- Pseudoexfoliation
- Myopia > 4 dioptres
- Vascular risk factors:
  - Local
    - Disc haemorrhage
    - Peripapillary atrophy
  - Systemic
    - Cerebral disease
    - Cardiovascular disease
    - Vasospasm: Cold hands and feet, Raynaud’s phenomena, migraine
    - Systemic hypotension with nocturnal pressure drops
    - Low perfusion pressure
    - Hypercholesterolemia/ hyperlipidemia
    - Diabetes Mellitus
- Family history in first degree relatives

### 1.3. Intraocular Pressure

Although glaucoma is considered a multivariate and complex genetic disease, the most important risk factor, so far, is elevated intraocular pressure (IOP). Approximately 10,000 RGCs are lost per year, and by age 80, an individual with normal IOP will preserve approximately 70% of their RGCs. However, elevated IOP can accelerate RGC loss [Brubaker, 1996]. It is reasonable to think that increased IOP applies some critical stress to the ganglion cells and their axons [Nickels, 2007].

That’s why a frequent and repetitive examination of IOP has great value when following patients diagnosed with glaucoma. However, single IOP measurements do not necessarily provide adequate information about pressure, as the normal diurnal variation in IOP can be on the order of 6 mm Hg, and even larger in eyes with glaucoma, reaching 30 mm Hg [Kitazawa and Horie, 1975; Newell and Krill, 1964]. Furthermore, the fact that measurement of IOP alone is of limited value is reinforced by the existence of patients with NTG, where we can find evidence of optic nerve damage, and IOP measurements within the normal statistical range. This concept emphasizes the important role played by other pressure-independent risk factors.
factors in the development and progression of glaucomatous neuropathy. In addition, progression of glaucomatous damage continues in many patients (up to one-sixth of patients with glaucoma) despite attenuation of the initial injury (control of initially raised IOP) [Brubaker, 1996; Lisegang, 1996].

Another important clinical observation in glaucoma that would help to understand the important role of neuroprotection is the fact that patients with severe pre-existing damage are more likely to deteriorate than those previously diagnosed and treated, in spite of reaching the same or even lower IOPs [Yoles and Schwartz, 1998].

1.4. Pathogenesis

The exact mechanism of how glaucoma develops, and the way RGC degenerate is still not well-known, although there is increasing evidence for a biphasic theory.

1.4.1. Initial Injury

It is still unclear what stimulates the axons of retinal ganglion cells to begin to degenerate. Some authors believe that the initial site of damage in glaucoma is the lamina cribosa [Quigley and Addicks, 1980; Quigley et al., 1981; Quigley and Addicks, 1981]. A combined effect of vascular risk factors susceptibility and the triggering effect of increased intraocular pressure would lead to an initial damage of the axons at the level of lamina cribosa. However, another possibility will be that the direct damage of the pressure applied to the retinal ganglion cell bodies at the level of the retina will induce changes in the molecular structure of these cells and thus induce the apoptosis of the RGCs.

The classical “mechanical damage model” predicts that the compression of the axons because of increased intraocular pressure leads to their degeneration. This theory will explain that elevated IOP could induce the optic disk cupping causing optic nerve axonal compression at the lamina cribosa, and blockage of axoplasmic retrograde flow of the RGC axons. However, another hypothesis will indirectly explain the physical damage to the axons through the glial cells present in the optic nerve head and lamina cribosa that will have modified their homeostasis at this level and thus influence the RGC axons [Ransom and Behar, 2003]. These glial cells support neurons, regulate extracellular K+ levels, remove glutamate and GABA neurotransmitters (specially in synapses), renew precursor in the synthesis of glutamate, regulate extracellular pH levels and osmolarity and supply to the neuron the energy needed by the provision of lactate and glucose from glycogen stores [Ransom et al., 2003]. This primary insult may alter the gene expression of glial cells and their behaviour. In addition, both retrograde and anterograde axonal transport is blocked at the lamina cribosa in glaucomatous eyes [Minekler et al., 1977; Radius and Anderson, 1981; Dandona et al., 1991]. Some studies have identified astrocytes as the key cell type involved in the altered remodelling of the extracellular matrix (ECM) in the optic nerve head, including collagen I, IV, transforming growth factor β2 (TGF-β2) and matrix metalloproteinase (MMP)-1 in experimental animal models of glaucomatous damage [Hernandez, 2000]. In another study, Guo et al. demonstrated that RGC apoptosis in glaucoma correlates strongly with elevated
IOP, showing a link with MMP-9, and laminin degradation, suggesting that this abnormal ECM remodelling in glaucomatous retinas may increase RGC death [Guo et al., 2005].

On the other hand, the “vascular damage model” can also be placed in the initial insult to the optic nerve head. Although the density of capillaries is similar in normal eyes and those affected by glaucoma [Quigley et al., 1984], increased IOP may lead to reduced blood flow in the capillaries of the optic nerve head, probably because of a faulty autorregulation of blood flow in the optic disk [Grunwald et al., 1984]. Astrocytes may also induce vasoconstriction of regional small capillaries by the release of vasoactive peptides during stress, associated to increased intracellular Ca2+ [Mulligan and MacVicar, 2004]. This isquemic condition and the deplection of energy stores can affect axonal Na+/K+ ATPase, that would increase intracellular Na+, leading to an overload of intracellular Ca2+ due to a greater Na+/Ca2+ exchanger activity [Osborne and Schwartz, 1994; Stys et al., 2004].

Both mechanical and vascular initial damage could stimulate axonal degeneration due to the lack of energy supply, neurotrophin withdrawal because of axoplasmic transport block, or the local involvement of the glial cells residing in the optic nerve head and lamina cribosa. This initial damage could trigger a cell self-destruction program, of which there are mainly 2 basic patterns [Beirowski et al., 2005]. The first one could be that those axons that suffered severe damage undergo what is called Wallerian degeneration, with a rapid breakdown of microfilaments and microtubules and swelling of the mitochondria [Whitmore et al., 2005; Raff et al., 2002]. The second one, which is supposed to be the most common pattern in glaucoma, would be a less severe insult to the axon resulting in a slower degeneration [Schlamp et al., 2006]. Whatever the axon degeneration pattern is, RGC’s death occurs through a final common pathway (apoptosis) [Garcia-Valenzuela et al., 1995], and it has been speculated that the primary cause of its activation is neurotrophin deprivation [Nickels, 2007]. Apoptosis of RGCs in glaucoma follows the “intrinsic” pathway, generating reactive oxygen species, a loss of ATP production and releasing cytochrome C [Green and Reed, 1998].
1.4.2. Secondary Degeneration

After an initial insult, which distorts the normal function of axons and astroglia in the optic nerve head and lamina cribosa, several changes occur, leading to the death of the injured neurons and the neighbouring intact neurons through secondary degeneration.

A). Neurotrophin Deprivation

Both retrograde and anterograde transport is blocked in glaucomatous eyes [Minckler et al., 1977; Radius and Anderson, 1981; Dandona et al., 1991], causing an alteration of axoplasmic transport of soluble growth factors called neurotrophins from target neurons in the superior colliculus and lateral geniculate nucleus in the brain [McKinnon, 1997]. RGCs, as all other neurons in CNS, require support of neurotrophins, as these small peptides regulate cellular metabolism by the activation of neuronal target-cell receptors. RGCs seem to be especially dependent upon brain-derived neurotrophic factor, neurotrophin-4, ciliary nerve trophic factor, and glial-cell derived neurotrophic factor [Mansour-Robaey et al., 1994; Sawai et al., 1996; Klocker et al., 1997; Ji et al., 2004]. Neurotrophins interact with specific receptors, including tyrosine kinase receptors (TrkA, TrkB, and TrkC), primarily activated by nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), both neurotrophin 4,5 (NT-4/5) and neurotrophin 3 (NT-3), respectively. After activation, neurotrophin/receptor complexes are retrogradely sent to the cell body [Di Stefano et al., 1992; Ehlers et al., 1995; von Bartheld et al., 1996; von Bartheld et al., 1996; Ricci et al., 1977). Immunohistochemical findings provide evidence for experimental glaucoma that causes abnormal TrkB axonal distribution, focal accumulations of TrkB and BDNF, increased label for TrkB in Ganglion Cell Layer (GCL), and increased TrkB label in glia (Pease et al., 2000). However, other results from our lab indicated that it may be other reason than the retrograde transport of
BDNF to explain the RGC survival in glaucoma. Thus, the presence of TrkB and BDNF in the complete population of the RGCs has been demonstrated [Vecino et al. 1998, 2002]. Moreover, the capacity of RGCs to survive in culture in absence of external BDNF has also been demonstrated [García et al. 2002, 2003] indicating that the deprivation of extrinsic BDNF due to the interruption of retrograde transport is not the only reason for RGCs to die in glaucoma. This is important because movement of BDNF is mediated by the TrkB receptor, and BDNF is an important trophic factor for RGCs, that inhibits apoptosis, supports survival of retinal explants and cultures and increases the rate of axonal elongation but not only when is present in the axonal terminals [Johnson et al., 1986; Thanos et al., 1989; Mansour-Robaey et al., 1994; Cohen-Cory and Fraser, 1995; Frade et al., 1997]. Neurotrophin deprivation triggers intrinsic apoptosis and activation of c-Jun N-terminal kinases, that stimulates expression of BH-3-containing proteins, that facilitate the actions of a proapoptotic protein that causes mitochondrial dysfunction (BAX) (Nickells, 2004; Putcha et al., 2003).

B) “Activation” Response of Glial Cells

After the primary injury to the optic nerve head, glia surrounding damaged axons alter their gene expression profile, characterized by the expression of two intermediate filament proteins: glial fibrillary acidic protein (GFAP) and vimentin.

This “activated” microglia can directly cause damage to axons and neighbouring cells as it has been suggested to synthesize and release nitric oxide [Neufeld et al., 1999; Liu and Neufeld, 2000]. Nitric oxide neurotoxicity occurs through a reaction with superoxide anion, forming peroxynitrite and free radical species. Peroxynitrite S-nitrosylates both proteins and nucleic acids in neighbouring cells and axons [Farkas and Grosskreutz, 2001].

C) Astrocyte Induced Vasoconstriction

Activated glia may induce vasoconstriction of regional small capillaries in the optic nerve head. During time of stress (under conditions of increased intracellular Ca2+), astrocytic end-feet, that surround small capillaries would release vasoactive peptides [Mulligan and MacVicar, 2004]. This secondary vasoconstriction associated to the initial vascular primary injury caused by mechanical compression of the optic nerve head and lamina cribosa may lead to reduced blood flow in the capillaries of the optic nerve head.

D) Activation of the Caspase Enzyme Family

RGC injury in ONT and experimental glaucoma involve activation of caspases 3 and 8, as well as the mitogen-activated protein kinase pathway (specially p38 mitogen-activated protein kinase and c-jun, facilitating mitochondrial dysfunction and destruction of RGCs [Kikuchi et al., 2000; Isenmann and Bahr., 1997; Levkovitch-Verbin et al., 2005].

E) Glutamate Induced Excitotoxicity

Glutamate is an excitatory neurotransmitter in the CNS, present in neurons in high concentrations. An excess of glutamate in the retina could damage the neurons hyperstimulating the ionotropic N-methyl-D-aspartate (NMDA) receptors, and thus generating a toxic influx of extracellular Ca2+. Abnormally high Ca2+ concentration leads to inappropriate activation of nucleases, proteases and lipases, that directly leads to the
generation of free radicals and activation of the nitric oxide pathway [Naskar and Dreyer, 2001]. The final consequence is DNA nitrosylation, fragmentation and activation of the apoptosis. Glaucoma has classically been associated to increased levels of glutamate in the vitreous [Dreyer et al., 1996; Dreyer and Lipton, 1993], and RGCs contain a high concentration of NMDA receptors, resulting very sensitive to elevated concentrations of glutamate. Moreover, Müller glial cells that normally take up glutamate fail to do so in glaucoma [Napper et al., 1999]. In addition, there is evidence that the glutamate-glutamine cycle is altered in glaucomatous eyes [Martin et al., 2002].

F) ATP Releases

There is evidence that acute glaucoma leads to an elevation in extracellular ATP levels that damages RGCs. The rise in vitreal ATP in hypertensive eyes is a physiologic response, and pannexines, stable hemichannels in the membrane, could contribute [Dahl and Locovei, 2006; Spray et al., 2006]. Pannexines gate open in response to increased pressure and release ATP from the inner retina [Bao et al., 2004]. When ATP stimulates P2X7 receptor on RGCs intracellular calcium is elevated and RCGs in vitro die [Zhang et al., 2006; Zhang et al., 2005]. In addition, ATPase apyrase and P2X7 receptor antagonists reduce RGC damage in experimental models of glaucoma [Resta et al., 2007].

G) Implication of TNF-α

Tumor necrosis factor-alpha (TNF-α) is a proinflammatory cytokine implicated in the immune response. TNF-α is produced by macrophages, lymphoid cells, endothelial cells, fibroblasts and also by glial cells, and, growing evidence supports that it may lead to neuronal cell death [Downen et al., 1999]. TNF-α is increased in the retina and optic nerve head in glaucomatous eyes. TNF-α is secreted by stressed glial cells and it can induce RGC death through TNF receptor-1-mediated caspase cascade, mitochondrial dysfunction, and oxidative damage (accumulation of reactive oxygen species (ROS)) [Tezel and Wax, 2000; Tezel and Yang, 2004]. In addition, immunohistochemical analysis of human eyes revealed an increased immunolabeling for TNF-α and TNF-R1 in the optic nerve head [Yan et al., 2000] and retina [Tezel et al., 2001] of glaucomatous eyes relative to controls, showing a predominant localization in RGCs and their axons, which are the sensitive targets for cytotoxic effects of TNF-α. Findings have also demonstrated an upregulation in gene expression for TNF-α and TNF-R1 in ocular hypertensive eyes [Ahmed et al., 2004]. Other findings provide evidence that TNF death receptor signaling is involved in the secondary degeneration of RGCs associated with JNK signaling [Tezel et al., 2004]. TNF-α in glaucoma may also be associated with another neurodestructive role by its ability to induce a highly potent secondary oxidant, nitric oxide [Goureau et al., 1997].

TNF-α also activates matrix metalloproteinases [Gottschall and Yu, 1995], associated with neurotoxicity [Guo et al., 2005]. TNF-α is also a potent stimulator of endothelin-1 (ET-1) synthesis and secretion in optic nerve head astrocytes, a potent vasoactive peptide associated with glaucomatous neurodegeneration [Desai et al., 2004].

TNF-α appears to play an important role in the immune response, ranging effects from inflammation to apoptosis, through Wallerian degeneration [Liefner et al., 2000] (Figure 4).
H) Oxidative Stress and Free Radicals

RGCs have special sensitivity to oxidative stress. Neurons lack an appropriate defences against excitotoxicity and elevated levels of reactive oxygen species (ROS), produced because of the augmented use of O2 and ATP synthesis of these tissues. ROS get accumulated in cells that undergo oxidative stress, and they react with nitric oxide producing free radicals. As a final consequence, this oxidative chain leads to mitochondrial dysfuncticon, DNA degradation, and cell failure. Free radicals cause extensive damage to the RGCs and their axons [Oku et al., 1997; Levkovitch-Verbin et al., 2000; Tezel, 2006].

I) Increased Accumulation of Self-Proteins

This phenomenon is common for many neurodegenerative diseases. As in Alzheimer’s disease, the accumulation of amyloid precursor protein has been reported in glaucoma [McKinnon et al. 2002].

J) Increased Expression of Pro-Apoptotic Genes

During glaucoma there is an unbalance between pro-apoptotic and anti-apoptotic proteins. In fact, there is an increased expression of pro-apoptotic genes, such as bax [Oltvai and Korsmeyer, 1994] and a downregulation of anti-apoptotic genes, such as bcl-xL [Levin et al., 1997].

1.5. Treatment

IOP lowering by means of antiglaucoma drugs, laser or incisional surgery is currently the only therapy approved by regulatory agencies for the treatment of glaucoma. A recent report from the Advanced Glaucoma Intervention Study (AGIS), showed that eyes with 100% of
visits and IOP less than 18 mmHg over 6 years had mean changes close to zero during follow-up [The AGIS Investigators, 2000].

However, therapeutically, we need to reduce intraocular pressure, stabilize ocular blood flow, and reduce oxidative stress.

### SIX FAMILIES OF ANTIGLAUCOMA AGENTS

<table>
<thead>
<tr>
<th>AGENTS</th>
<th>Examples (concentrations)</th>
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<tbody>
<tr>
<td><strong>ADRENERGIC AGONISTS</strong></td>
<td></td>
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<tr>
<td>Non-selective</td>
<td>Dipivefrin 0.1%</td>
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<td></td>
<td>Epinephrine 0.25-2%</td>
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<tr>
<td>Alpha-2 selective</td>
<td>Apraclonidine 0.5-1%</td>
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<td></td>
<td>Brimonidine 0.2%</td>
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<td></td>
<td>Clonidine 0.125-0.5%</td>
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<tr>
<td><strong>ADRENERGIC ANTAGONISTS</strong></td>
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<tr>
<td>Beta-1 selective</td>
<td>Betaxolol 0.5-0.25%</td>
</tr>
<tr>
<td>Non-selective</td>
<td>Befunolol 0.5%</td>
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<tr>
<td></td>
<td>Levobunolol 0.25-0.5%</td>
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<td></td>
<td>Metipranolol 0.1-0.3%</td>
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<td></td>
<td>Timolol 0.1, 0.25, 0.5%</td>
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<tr>
<td>With ISA (Intrinsic Sympathomimetic Activity)</td>
<td>Carteolol 0.5-2%</td>
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<tr>
<td></td>
<td>Pindolol 2%</td>
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<tr>
<td><strong>CARBONIC ANHYDRASE INHIBITORS</strong></td>
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</tr>
<tr>
<td>Topical</td>
<td>Brinzolamide 1%</td>
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<td></td>
<td>Dorzolamide 2%</td>
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<td>Systemic</td>
<td>Acetazolamide</td>
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<td></td>
<td>Dichlorphenamid</td>
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<td></td>
<td>Methazolamide</td>
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<tr>
<td><strong>PARASYMPATHOMIMETICS (CHOLINERGIC DRUGS)</strong></td>
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<tr>
<td>Direct</td>
<td>Pilocarpine 0.5-4%</td>
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<td></td>
<td>Aceclidine 2%</td>
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<td></td>
<td>Carbachol 0.75-3%</td>
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<td></td>
<td>Acetylcholine 1%</td>
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<tr>
<td>Indirect</td>
<td>Demecarium bromide 0.125, 0.25%</td>
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<td></td>
<td>Eclothiophate iodide 0.03-0.25%</td>
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<tr>
<td></td>
<td>Physostigmine</td>
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<tr>
<td><strong>PROSTAGLANDIN DERIVATES AND PROSTAMIDES</strong></td>
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<tr>
<td>Bimatoprost 0.03%</td>
<td>Latanoprost 0.005%</td>
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<td></td>
<td>Travoprost 0.004%</td>
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<tr>
<td></td>
<td>Unoprostone 0.12, 0.15%</td>
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<tr>
<td><strong>OSMOTICS</strong></td>
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<tr>
<td></td>
<td>Manitol 1-1.5 g/kg intravenously</td>
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</table>
2. Neuroprotection in Glaucoma

2.1. What is Glaucoma Neuroprotection?

Neuroprotection was initially investigated for disorders of the central nervous system such as amyotrophic lateral sclerosis, Alzheimer’s disease, Parkinson and head trauma, but only few therapies have been approved (nemantine for Alzheimer’s dementia).

By definition, glaucoma neuroprotection must be considered independent of IOP lowering, and the target neurons should be in the central visual pathway, including RGCs.

Chronic progressive loss of retinal ganglion cells (RGC) is thought to be biphasic: initiation of damage is caused by a primary injury associated to the main risk factors of glaucoma (elevated IOP, cardiovascular risk factors, age, vasospasm...), and there’s a delayed (secondary) degeneration of neurons that either escaped the injury or were only partially damaged. The secondary degeneration may be an outcome of a hostile environment created by damaged neurons. Some factors have been identified as mediators of this secondary neuronal degeneration: high levels of potassium and calcium ions, nitric oxide, amounts of free radicals and excitatory aminoacids such as glutamate and aspartate.

Neuroprotection in glaucoma consists in preventing the death of marginally damaged neurons and the secondary denegaration of those undergoing the hostile environment created by the initial insult. In other words, neuroprotection attempts to provide protection to such retinal ganglion cells that continue to remain at risk [Chew and Ritch, 1997] (Figure 5).
2.3. Is Neuroprotective Treatment Available for Glaucoma Patients?

A neuroprotective drug is expected to prevent death of RGC in presence of chronic stress by attenuating the hostility of the environment or supplying the cells with the tools to deal with those chances [Kaufman et al., 1999].

The pharmacological profile of neuroprotective drugs should fulfil these four criteria [Wheeler et al., 2001]:

i. To have a specific target (receptors) in the retina
ii. To exhibit neuroprotective activity and have a measurable effect on RGC survival
iii. To reach the retina/vitreous in neuroprotective concentrations after clinical dosing
iv. (First three criteria must be obtained in animal models)
v. Neuroprotective activity must be demonstrated in randomized, controlled, clinical trials in humans.

Glaucoma neuroprotection offers potential as a complementary therapy to IOP-lowering for patients with previous severe damage and for those in whom pressure-lowering agents are ineffective to stop progression.
However, efficacy for neuroprotective agents has not yet been proven in glaucoma, and although there is laboratory evidence for glaucoma neuroprotection by several drugs, evidence from randomized clinical trials is still lacking [Weinreb, 2007].

2.4. Clinical Trials of Neuroprotection in Ophthalmology

To establish neuroprotective drugs for glaucoma, well-designed clinical trials are required, specially randomized clinical trials comparing neuroprotective treatments to a placebo, but there are few of them in ophthalmology.

In order to design clinical trials for neuroprotection, primary outcome measures can be:

- Functional visual acuity (The Ischemic Optic Neuropathy Decompression Trial Research Group, 2000),
- Visual field loss, although it may require several years before meaningful changes appear. Measurements of visual field have become a standard way to assess the functional progression of glaucoma.
- Some new methods for assessing visual function, like:
  - Electrophysiological testing [Berson et al., 1993]
  - Frequency Doubling Technology (FDT),
  - Short Wave Length Automated Perimetry (SWAP),
  - Contrast sensitivity,
  - The Heidelberg Retinal Tomograph (HRT) (*)
  - The GDx nerve fiber analyzer (*)
  - The optical Coherence Tomograph (OCT) (*).

2.5. Neuroprotective Drugs in Glaucoma

Some agents have been reported as having neuroprotective activity for RGCs in experimental research as well as clinical studies.

2.4.1. Antibiotics

*Minocycline*

Minocycline hydrochloride is a second-generation tetracycline, commonly used in humans because of its beneficial antimicrobial and anti-inflammatory actions. Minocycline effectively crosses the blood-brain barrier [Aronson, 1980]. This drug also has remarkable neuroprotective qualities in animal models of cerebral ischemia, traumatic brain injury, Huntington disease, and Parkinson disease [Arvin et al., 2002; Du et al., 2001; Tikka and Koistinaho 2001; Yrjanheikki et al. 1999; Yrjanheikki et al., 1998].

Levkovitch-Verbin et al. in 2006 demonstrated that systemic administration of minocycline significantly delays apoptosis of RGCs after severe injury of optic nerve transaction (ONT) [Levkovitch-Verbin et al., 2006]. Its effect is associated with inhibition of
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inducible nitric oxide synthase [Du et al. 2001], caspase 1 and caspase 3 expression [Chen et al., 2000; Sanchez et al., 2001]. It also inhibits p38 mitogen-activated protein kinase [Yrjanheikki et al., 1999; Yrjanheikki et al., 1998] and cytochrome c release [Zhu et al., 2002]. It also depresses oxygen radical release and matrix metalloproteinase activity [Brundula et al., 2002; Gabler and Creamer. 1991; Power et al. 2003; Ryan et al. 2001; Sadowski and Steinmeyer. 2001; Golub et al. 1991]. It has also been found to be neuroprotective in models of photoreceptor death [Baptiste et al., 2004; Hughes et al., 2004; Zhang et al., 2004].

Doxycycline

Doxycycline is another semisynthetic second-generation tetracycline. It also penetrates the blood-brain barrier, with well-known effects of neuroprotection [Gabler et al., 1992; Smith and Gabler, 1994; Smith and Gabler, 1995]. However, it hasn’t shown as much neuroprotective effect as minocycline in most studies.

2.4.2. Antiglaucomatous Agent

Antiglaucoma agents typically used in clinical practice exert neuroprotective effects on RGCs via distinct mechanisms.

a-2 Agonists (Brimonidine)

Brimonidine is a drug used daily in clinical practice. Brimonidine reduces the production of aqueous humor and facilitates its exit via the trabecular meshwork.

Treatment with intraperitoneal brimonidine leads to a total rescue of RGCs in glaucomatous eyes; however, the intraperitoneal application of brimonidine in animals has not been found to reduce IOP [Hernández et al., 2008]. The neuroprotective effect of brimonidine has been studied in several experimental models of optic neuropathy, including optic nerve injury [Yoles et al., 1999; Ruiz et al., 2000], transitory ischemia [Donello et al., 2001; Lafuente et al., 2001], and experimental glaucoma [Ahmed et al., 2001; Wheeler and Woldemussie, 2001; Wheeler et al., 2003; Hernández et al., 2008].

Brimonidine treatment may exert its neuroprotective effect through inhibition of the apoptotic cascade, reduction of glutamate toxicity, or enhancing the expression of BDNF. Thus, brimonidine could activate an anti-apoptotic pathway in RGCs by inducing the expression of anti-apoptotic genes such as bcl2 and bcl-xl [Lai et al., 1999]. Activation of RGC alpha-2 adrenergic receptors may inhibit pro-apoptotic mitochondrial signaling [Wheeler et al., 2001; Wheeler et al., 2001]. Also, brimonidine-mediated neuroprotection could also be due to the inhibition of glutamate-mediated excitotoxicity [Vorwerk et al., 1996]. Brimonidine could also exert its beneficial effects via neurotrophins. Thus, intravitreal injection of brimonidine has been shown to upregulate brain-derived neurotrophin factor (BDNF) expression in rat RGCs [Gao et al., 2002].

β Blockers (Betaxolol, Metipranolol and Timolol)

The ability to confer neuroprotection to retinal neurones is a common feature of three ophthalmic beta adrenoceptor antagonists (betaxolol, metipranolol and timolol).
Beta(1)-selective adrenoceptor antagonist, betaxolol, is able to protect retinal neurones in vitro and ganglion cells in vivo from the detrimental effects of either ischemia reperfusion or from excitotoxicity, after topical application. The neuroprotective effect of betaxolol is thought not to be elicited through an interaction with beta-adrenoceptors, but by its ability to reduce influx of sodium and calcium through voltage-sensitive calcium and sodium channels. Betaxolol has also been shown to increase blood flow velocity in the optic nerve head tissue [Tamaki et al., 1999]. Improvements have been made in measuring ocular blood flow velocity, but measurement difficulties still persist [Harris et al., 1999].

It has also been shown that the non-selective beta-adrenoceptor antagonists, metipranolol and timolol when topically applied behave like betaxolol. They all attenuate the detrimental effect of ischemia-reperfusion. Protection of the retina was determined by evaluating changes in the electroretinogram and by assessing the loss of mRNA for Thy-1, which is expressed in retinal ganglion cells. In addition, studies conducted on neurones in mixed retinal cultures demonstrated that betaxolol>metipranolol>timolol were all able to partially counteract anoxia-induced cell loss and viability reduction and also to attenuate ligand-induced stimulation of calcium and sodium entry into neuronal preparations. The influence of timolol was, however, not significant [Wood et al., 2003].

Muscarinic Receptor Agonist (Pilocarpine)

Some studies demonstrated that pretreatment of pilocarpine could prevent glutamate-induced neuron death through M1 muscarinic receptor, as its effect was blocked by the non-selective antagonist atropine and the M1-selective muscarinic receptor antagonist pirenzepine. The antiapoptotic effect of pilocarpine was associated with maintaining calcium homeostasis, recovering mitochondrial membrane potential, and regulating the expression of Bcl-2 and Caspase-3 [Zhou et al. 2008].

2.4.3. Ca\(^{2+}\) Channel Blockers

In an interesting article, fifty-six patients with either open-angle or low-tension glaucoma, concurrently taking calcium channel blockers were compared to similar groups not taking such medications for a mean follow-up period of 3.4 years. In patients with low-tension glaucoma, there was a significant difference in the progression of visual field defects, with only two of 18 eyes (11%) of patients taking calcium channel blockers, compared to ten of 18 eyes (56%) of controls showing new visual field defects. Similarly, low-tension glaucoma patients taking calcium channel blocker therapy demonstrated no evidence of progressive optic nerve damage, compared to eight of 18 control eyes (44%). However, such differences didn’t appear in patients with open-angle glaucoma, suggesting that calcium channel blockers may be useful in the management of low-tension glaucoma [Netland et al., 1993]. However, excessive blood-pressure lowering properties might also decrease perfusion in the optic nerve, worsening glaucomatous damage [Caprioli, 1997].

Nifedipine

In a clinical study, it was demonstrated that in a significant number of patients with NTG who took nifedipine, visual field got improved after 6 months follow-up [Kittazawa et al., 1989].
Flunarizine

Flunarizine is a selective calcium entry blocker with calmodulin binding properties and histamine H1 blocking activity. It is effective in the prophylaxis of migraine, occlusive peripheral vascular disease, vertigo of central and peripheral origin, and as an adjuvant in the therapy of epilepsy. It may help to reduce the severity and duration of attacks of paralysis associated with the more serious form of alternating hemiplegia. Flunarizine has been demonstrated to enhance RGC survival after optic nerve transection in mice [Eschweiler and Bahr, 1993].

2.4.4. Antioxidants

Both an activation of glial cells and an oxidative stress in the axons play an important role in the secondary degeneration. Various natural compounds possess potential antioxidative value. Reduction of oxidative stress at the level of mitochondria can be achieved by gingko biloba. Polyphenolic compounds, such as tea, red wine, dark chocolate, or coffee have antioxidative properties. Coffee contains 3-methyl-1,2-cyclopentanedione (MCP), capable of scavenging peroxynitrite. Red wine-polyphenols (e.g., resveratrol), exert vasoprotective effects by inhibiting the synthesis of endothelin-1. Dark chocolate decreases blood pressure and improves endothelium-dependant vasorelaxation. Omega-3-fatty acids and magnesium can also improve blood flow regulation Anthocyanosides (bilberries) owe their antioxidative effects to their particular chemical structure. Other antioxidants include ubiquinone and melatonin, and heat shock proteins can be induced naturally by the use of sauna baths [Mozaffarieh et al., 2008; Mozaffarieh and Flammer, 2007].

<table>
<thead>
<tr>
<th>Natural compounds with antioxidative value</th>
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<tr>
<td>Polyphenolic Flavonoids</td>
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<td>Tea</td>
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<td>Coffee</td>
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<td>Vaccinium Myrtillus (Bilberry)</td>
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<td>Vitamins</td>
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<td>Alpha Lipoic Acid (ALA)</td>
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<td>Thiamin (Vitamin B1)</td>
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<td>Cobalamin (Vitamin B12)</td>
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<td>Miscellaneous</td>
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<tr>
<td>Ubiquinone (Coenzyme Q10)</td>
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<td>Melatonin</td>
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2.4.5. Cannabinoids

Since the early 1970s it was reported that smoking marijuana cigarettes could lower IOP by up to 45% [Hepler and Frank, 1971]. Later works showed that delta-9-tetrahydrocannabinol (Δ9-THC) lowered IOP intravenously [Purnell and Gregg, 1975], orally [Merritt et al., 1980] or by inhalation [Merritt et al., 1980]. Other results suggested that
topical application of a synthetic cannabinoid receptor agonist (WIN55212-2), lowers IOP in human glaucoma, and that the IOP-lowering effects of WIN55212-2 are, probably, mediated through CB1 cannabinoid receptors [Porcella et al., 2001].

Nowadays, there is important progress in the role of cannabinoids in affording neuroprotection in traumatic, ischemic, inflammatory and neurotoxic damage in neurons [Van der Stelt and Di Marzo, 2005; de Lago and Fernández-Ruíz, 2007; Mechoulam and Shohami, 2007].

Rat retina has a functional endocannabinoid system and high IOP-induced ischemia-reperfusion reduces the endogenous anandamide (AEA) via enhanced expression of fatty acid amide hydrolase (FAAH), implicating this phenomenon in retinal cell loss caused by high IOP. It has been shown that FAAH inhibition or the administration of a stable analogue of AEA reduces cell loss in the RGC layer caused by ischemia-reperfusion. This effect seems to occur via activation of CB1 and TRPV1 receptors, suggesting a neuroprotective effect through engagement of these receptors [Nucci et al., 2007].

Other studies have shown that CB1 agonists (THC and cannabidiol) protect RGCs from glutamate-induced excitotoxicity [El Remessy et al., 2003; Opere et al., 2006], and ischemia secondary to an experimental model of glaucoma in rat [Crandall et al., 2007].

HU-211 is a nonpsychotropic cannabinoid that behaves as a noncompetitive NMDA antagonist [Feigenbaum et al., 1989], and systemic administration provides neuroprotection for RGCs after nerve axotomy [Yoles et al., 1996].

2.4.6. NMDA Antagonists, P38 MAPK, and Caspase Inhibitors

Glutamate mediates synaptic transmission essential for normal function of the nervous system. Hence, complete blockade of NMDA receptor activity causes unacceptable side effects. Open-channel blocker of the NMDA receptors, memantine, blocks only excessive NMDA receptor activity while leaving normal function relatively intact.

This NMDA antagonist is neuroprotectant in several ways: preventing excessive calcium influx by inhibition of over-stimulation of the NMDA receptor, and inhibiting OPA1 release from mitochondria by blockage of NMDA receptor. OPA1 effect is accompanied by increased Bcl-2 expression, decreased Bax expression and apoptosis blockade [Ju et al., 2008].

Memantine effectively blocks the excitotoxic response of retinal ganglion cells both in culture and in vivo [Vorwerk et al., 1996]. Memantine reduced ganglion cell loss when is systematically applied in a rat model of retinal ischemia [Lagreze et al., 1998]. Although memantine has demonstrated exciting results for neuroprotection in laboratories, the phase III clinical trial of Memantine failed to prove such activity [Ge, 2008].

NMDA receptors and downstream signaling pathways, triggered by p38 mitogen activated protein kinase (MAPK) and caspases, are also potential targets of intervention for glaucoma, and improved memantine derivatives, p38 MAPK, and caspase inhibitors seem plausible therapeutics to prevent RGC death [Seki and Lipton, 2008].

2.4.7. Nitric Oxide Synthase (NOS-2) Inhibitors

Nitric-oxide synthase (NOS-2) is elevated in the optic nerve heads from human glaucomatous eyes and from rat eyes with chronic, moderately elevated IOP.
Aminoguanidine

Aminoguanidine is a relatively specific inhibitor of NOS-2. It has been shown that aminoguanidine protects RGCs in a rat model of glaucoma; after unilateral induction of monolateral ocular hipertension, some animals were treated for 6 months. At 6 months, untreated animals had pallor and cupping of the optic disks in the eyes with elevated IOP, but aminoguanidine-treated animals with similar elevations of IOP appeared normal. After quantification, eyes with elevated IOP in the untreated group lost 36% of their retinal ganglion cells, and eyes with similarly elevated IOP in the aminoguanidine-treated group lost less than 10% of their retinal ganglion cells. Pharmacological neuroprotection by inhibition of NOS-2 may prove useful for the treatment of patients with glaucoma [Neufeld et al., 1999].

2.4.8. Gingko Biloba Extract (GBE)

This freely available nutritional supplement exerts protective effects against free radical damage and lipid peroxidation, preserves mitochondrial metabolism and ATP production and also behaves as a scavenger of superoxide radicals and nitric oxide [Janssens et al., 1995].

Ginkgo biloba extract (GBE) on glaucoma patients improves ocular blood flow. A Phase I cross-over trial of GBE with placebo control in 11 healthy volunteers was performed. Color Doppler imaging was used to measure ocular blood flow before and after treatment. Ginkgo biloba extract did not alter arterial blood pressure, heart rate, or IOP, but significantly increased diastolic velocity in the ophthalmic artery in the ophthalmic artery [Chung et al., 1999].

2.4.9. 17beta-Estradiol

17beta-estradiol (E2) is a steroid hormone, which has been shown to increase the viability, survival, and differentiation of primary neuronal cultures from different brain areas including amygdala, hypothalamus, and neocortex. 17 beta-estradiol (E2), has shown cytoprotective activities in animal models of stroke, myocardial infarct and neurodegenerative diseases.

Systemic administration of E2 significantly reduces RGC loss induced by acute increase of IOP in rat. In addition, pretreatment with E2, 30 min before ischemia, minimizes the elevation of glutamate observed during the reperfusion period. These effects seem to be in part mediated by the activation of the estrogen receptor, since a pre-treatment with ICI 182-780, a specific estrogen receptor antagonist, partially counteracts the neuroprotection afforded by the estrogen [Russo et al., 2008].

Later studies suggested the possible involvement of estrogen receptor/Akt/CREB/thioredoxin-1, and estrogen receptor/MAPK/NF-kappaB, in estrogen-mediated retinal ganglion cell protection [Zhou et al., 2007].

However, three synthetic estrogen analogues (ZYC-1, ZYC-3, ZYC-10) showed efficacy of neuroprotection against glutamate-induced RGC cell death. These compounds seem to affect neuroprotection via pathways independent of the classical estrogen receptors, as inclusion of an estrogen receptor antagonist (ICI 182,780) did not reverse their neuroprotective properties against glutamate insult. These results support the hypothesis that estrogen analogues may be useful in neuroprotection of retinal ganglion cells in ocular...
pathologies such as glaucoma [Kumar et al., 2005]. This receptor–independent effect may be explained by the fact that estrogens and novel analogs prevent cell death in large measure by maintaining functionally intact mitochondria [Simpkins et al., 2005].

2.4.10. Erythropoietin

Erythropoietin is a glycoprotein hormone that controls erythropoiesis. It is produced by the liver and kidney. It is the precursor or erythrocytes in the bone marrow, and it also has other known biological functions. For example, erythropoietin plays an important role in the brain's response to neuronal injury [Siren et al., 2001]. EPO is also involved in the wound healing process [Haroon et al., 2003].

In the DBA/2J mouse model of glaucoma EPO promoted RGC survival without affecting IOP. These results suggest that EPO may be a potential therapeutic neuroprotectant in glaucoma [Zhong et al., 2007].

3. What’s next? New Neuroprotective Treatment Targets in Glaucoma

Elucidation of specific signalling pathways is crucial to finding new treatment targets. Targeted proteomic approaches are expected to indentify signalling molecules in glaucomatous neurodegeneration.

3.1. Neurotrophins

Neurotrophin administration in glaucoma is being evaluated; however the main problem may be in delivering these substances so that they could reach the retina.

BDNF, ciliary neurotrophin factor and basic fibroblastic growth factor have been shown to protect human RGC from death in culture and in vivo [Rabacchi et al., 1994]. Glial cell line–derived neurotrophic factor also showed neuroprotection of RGCs in mice and it’s still under study [Ward et al., 2007].

3.2. Immunomodulatory Compounds

T lymphocytes also provide a certain neuroprotection by freeing neurotrophins or growth factors for microglia and monocytes, according to a specific and active process involving antigen-presenting cells. Activated. Such activated microglia and macrophages can take up glutamate, debris, and produce growth factors. But those T cells are controlled by regulatory T cells, part of the physiological immune network. Glaucoma appears to be characterized by an increase in both TNF-alpha of the glia in the optic-nerve head and its type 1 receptor in the ganglion cells of the retina, which makes them particularly easy to stimulate by TNF-alpha.
Glial cells, both activated during glaucoma and by TNF-alpha, and secreting TNF-alpha, could serve as antigen-presenting cells and thus constitute a new way to neuroprotection [Baudouin and Liang, 2006].

3.3. Glatiramer Acetate(Ga) (Copolymer A® Copraxone®)

It is a synthetic 4-amino-acid copolymer, currently used for multiple sclerosis. In a rat model of experimental chronic glaucoma, vaccination slowed down the progression of glaucoma, by making milieu of the nerve and retina less hostile to RGC survival [Schwartz et al., 2007]. Prevention of ganglion cells loss was also observed by prior immunization of animals using a synthetic polymer close to myelin (COP1), capable of stimulating a specific lymphocyte reaction of neuronal impairment without inducing uveitis [Baudouin and Liang, 2006].

3.4. Anti-TNF-a STRATEGIES

There is growing evidence that TNF-alpha-mediated neurotoxicity plays a key role in the neurodegenerative injury in glaucoma. Targeting TNF-alpha signaling for RGC rescue should block the caspase cascade and improve the ability of these neurons to survive. However, ability of anti-TNF-alpha strategies against axonal injury also needs to be clarified.

3.5. Gene Therapy

Several gene families have been identified to play roles in RGCs apoptosis or survival. Caspases stimulate apoptosis and carry out disassembly of the cells [Hetts, 1998]. Tumor suppression protein, p53 can upregulate the expression of the pro-apoptotic gene bax and down-regulate the expression of the anti-apoptotic gene bcl-2 [Nickells, 1999]. On the other hand, Bcl-2 and related proteins are important inhibitors of apoptosis. They inhibit intermediate proteins that activate caspases [Adams and Cory, 1998].

Transgenic mice allowed expression of the apoptosis-inhibiting gene Bcl-2 in rat neurons, with a 50% increase in retinal ganglion cell number. A gene can be delivered to the damaged tissue via viruses, artificial liposomes, and direct transfer, and even induce the cell to express some genes by its own regulatory pathways.

Recently, rat RGCs were able to be transfected by recombinant adeno-associated virus – BDNF (rAAV-BDNF) in vitro. The transfected cells could express BDNF gene at the level of both mRNA and protein. Apoptosis rate was lower in the transfected cells. This study indicated that rAAV-BDNF transfection can be used for the potential gene therapy in glaucomaneuroprotection [Li et al., 2008].
3.6. siRNA

Since its discovery in plants in the early 1990s, RNA interferente (RNAi) has begun to emerge as a promising technology to apply to therapeutics. This phenomenon consists of a specific and selective inhibition of gene expression in an efficient manner. RNAi is mediated by small interfering RNA (siRNA), consisting of 19-23 nucleotide double-stranded RNA, that promote specific nucleolytic cleavage of mRNA targets through an RNA-induced silencing complex. In this way, siRNAs offers a powerful tool that can be used to determine functional significance of newly identified molecules as treatment targets. This technology can also serve along with other genomic or pharmacologic treatments to provide neuroprotection in glaucoma.

Besides, siRNA can be locally and topically administrated for glaucoma in controlled doses, avoiding treating the whole body.

3.7. Stem Cells

Numerous stem cell sources exist, with embryonic and fetal stem cells liable to be superseded by adult-derived cells. Stem cell transplantation is currently being explored as a therapy for many neurodegenerative diseases including glaucoma. Cellular therapies have the potential to provide chronic neuroprotection after a single treatment, and possible neuroprotective mechanisms offered by stem cell transplantation include the supply of neurotrophic factors and the modulation of matrix metalloproteinases and other components of the CNS environment to facilitate endogenous repair [Bull et al., 2008].

REFERENCES


[19] Chen, M; Ona, VO; Li, M; Ferrante, RJ; Fink, KB; Zhu, S; Bian, J; Guo, L; Farrell, LA; Hersch, SM; Hobbs, W; Vonsattel, JP; Cha, JH; Friedlander, RM. Minocycline inhibits caspase-1 and caspase-3 expression and delays mortality in a transgenic mouse model of Huntington disease. *Nat Med*, 2000; 6: 797-801.


[26] Dahl, G; Locovei, S. Pannexin: To gap or not to gap, is that a question? *IUBMB Life*, 2006; 58: 409–419.


[31] Di Stefano, PS; Friedman, B; Radziejewski, C; Alexander, C; Boland, P; Shick, CM; Lindsay, RM; Wiegang, SJ; Schulzer, M; Britton, RJ. The neurotrophins BDNF, NT-3 and NGF display distinct patterns of retrograde axonal transport in peripheral and central neurons. *Neuron*, 1992; 8: 983-993.

[32] Donello, JE; Padillo, EU; Webster, ML; Wheeler, LA; Gil, DW. Alpha (2)-adrenoceptor agonists inhibit vitreal glutamate and aspartate accumulation and preserve retinal function after transient ischemia. *J Pharmacol Exp Ther*, 2001; 1: 216-223.


[34] Dreyer, EB; Lipton, SA. A proposed role of excitatory amino acids in glaucoma visual loss. *IOVS*, 1993; 34: 1504.
Current Trends in Glaucoma: What about Neuroprotection?  

[39] Dreyer, EB; Zurakowski, D; Schumer, RA; Podos, SM; Lipton, SA. Elevated glutamate levels in the vitreous body of humans and monkeys with glaucoma. Arch Ophthalmol, 1996; 114: 299-305.

[40] Du, Y; Ma, Z; Lin, S; Dodel, RC; Gao, F; Bales, KR; Triarhou, LC; Chernet, E; Perry, KW; Nelson, DL; Luecke, S; Phiebus, LA; Bymaster, FP; Paul, SM. Minocycline prevents nigrostriatal dopaminergic neurodegeneration in the MPTP model of Parkinson's disease. Proc Natl Acad Sci U S A, 2001; 98:14669-14674.


[42] El Remessy, AB; Khalil, IE; Matragoon, S; Abou-Mohamed, G; Tsai, NJ; Roon, P; Caldwell, RB; Caldwell, RW; Green, K; Liou, GI. Neuroprotective effect of delta(9)-tetrahydrocannabinol and cannabidiol in N-methyl-D-aspartate-induced retinal neurotoxicity: involvement of peroxynitrite. Am J Pathol, 2003; 163: 1997-2008.


[45] Feigenbaum, JJ; Bergmann, F; Richmond, SA; Mechoulam, R; Nadler, V; Kloog, Y; Sokolovsky, M. Nonpsychotropic cannabinoid acts as a functional N-methyl-D-aspartate receptor blocker. Proc Natl Acad Sci USA, 1989; 86: 9584-9587.


[47] Frade, JM; Bovolenta, P; Martinez-Morales, JR; Arribas, A; Barbas, JA; Rodriguez-Tebar, A. Control of early cell death by BDNF in the chick retina. Development, 1997; 124: 3313-3320.

[48] Freyler, H; Menapace, R. Ist die Erblindung an Glauckom vermeidbar? Spektrum Augenheilkd 2; 121, 1988;


Gasser, P; Flammer, J; Guthauser, U; Mahler, F. Do vasospasms provoke ocular diseases? *Angiology*, 1990; 41: 213-220.


Goureau, O; Amiot, F; Dautry, F and Courtois, Y. Control of nitric oxide production by endogenous TNF-α in mouse retinal pigmented epithelial and Muller glial cells. *Biochem Biophys Res Commun*, 1997; 240: 132-135.


Grunwald, JE; Riva, CE; Stone, RA; Keates, EU; Petrig, BL. Retinal autoregulation in open-angle glaucoma. *Ophthalmology*, 1984; 91: 1690-1694.

Guo, L; Moss, SE; Alexander, RA; Ali, RR; Fitzke, FW; Cordeiro, MF. Retinal ganglion cell apoptosis in glaucoma is related to intraocular pressure and IOP-induced effects on extracellular matrix. *Invest Ophthalmol Vis Sci*, 2005; 46: 175-182.


Harris, A; Chung, HS; Ciulla, TA; Kagemann, L. Progress in measurement of ocular blood flow and relevance to our understanding of glaucoma and age-related macular degeneration. *Prog Retin Eye Res*, 1999; 18: 669-687.


Hughes, EH; Schlichtenbrede, FC; Murphy, CC; Broderick, C; van Rooijen, N; Ali RR; Dick, AD. Minocycline delays photoreceptor death in the rods mouse through a microglia-independent mechanism. *Exp Eye Res*, 2004; 78: 1077-1084.


Janssens, D; Michiels, C; Delaive, E; Eliaers, F; Drieu, K; Remacle, J. Protection of hypoxia induced ATP decrease in endothelial cells by Ginkgo biloba extract and bilobalide. *Biochem Pharmacol*, 1995; 50: 991-999.


Johnson, JE; Barde, YA; Schwab, M; Thoenen, H. Brain-derived neurotrophic factor (BDNF) supports the survival of cultured rat retinal ganglion cells. *J Neurosci*, 1986; 6: 3031-3038.

Ju, WK; Kim, KY; Angert, M; Duong-Polk, KX; Lindsey, JD; Ellisman, MH; Weinreb, RN. Memantine blocks mitochondrial OPA1 and cytochrome c release, and subsequent apoptotic cell death in glaucomatous retina. *Invest Ophthalmol Vis Sci*, 2008. 20. [Epub ahead of print]


Kumar, DM; Perez, E; Cai, ZY; Aoun, P; Brun-Zinkernagel, AM; Covey, DF; Simpkins, JW; Agarwal, N. Role of nonfeminizing estrogen analogues in


[100] Li, RS; Tay, DK; Chan, HH; So, KF. Changes of retinal functions following the induction of ocular hypertension in rats using argon laser photocoagulation. *Clin Exp Ophthalmol*, 2006; 34: 575-583.


[102] Liebfner, M; Siebert, H; Sachse, T; Michel, U; Kollias, G; Bruck, W. The role of TNF-alpha during Wallerian degeneration. *J Neuroimmunol*, 2000; 108: 147-152.


Current Trends in Glaucoma: What about Neuroprotection?


[112] Mittag, TW; Danias, J; Pohorenc, G; Yuan, HM; Burakgazi, E; Chalmers-Redman, R; Podos, SM; Tatton, WG. Retinal damage after 3 to 4 months of elevated intraocular pressure in a rat glaucoma model. *Invest Ophthalmol Vis Sci*, 2000; 41: 3451–3459.


[116] Napper, GA; Pianta, MJ; Kalloniatis, M. Reduced glutamate uptake by retinal glial cells under ischemic/hypoxic conditions. *Vis Neurosci*, 1999; 16: 149-158.


Sergio Pinar and Elena Vecino


[124] Nucci, C; Gasperi, V; Tartaglione, R; Cerulli, A; Terrinoni, A; Bari, M; De Simone, C; Finazzi Agró, A; Morrone, LA; Corasaniti, MT; Bagetta, G. and Maccarrone, M. Involvement of the endocannabinoid system in retinal damage after high intraocular pressure-induced ischemia in rats. *Invest Ophthalmol Vis Sci*, 2007; 48: 2997-3004.


[134] Putcha, GV; Le, S; Frank, S; Besirli, CG; Clark, K; Chu, B; Alix, S; Youle, RJ; LaMarche, A; Maroney, AC; Johnson, EMJr. JNK mediated BIM phosphorylation potentiates BAX-dependent apoptosis. *Neuron*, 2003; 38: 899–914.


[146] Resta, V; Novelli, E; Vozzi, G; Scarpa, C; Caleo, M; Ahluwalia, A; Solini, A; Santini, E; Parisi, V; Di Virgilio, F; Galli-Resta, L. Acute retinal ganglion cell injury caused by intraocular pressure spikes is mediated by endogenous extracellular ATP. *Eur J Neurosci*, 2007; 27: 2741–2754.


[149] Russo, R; Cavaliere, F; Watanabe, C; Nucci, C; Bagetta, G; Corasaniti, MT; Sakurada, S; Morrone, LA. 17Beta-estradiol prevents retinal ganglion cell loss induced by acute rise of intraocular pressure in rat. *Prog Brain Res*, 2008; 173: 583-590.


[152] Sanchez Mejia, RO; Ona, VO; Li, M; Friedlander, RM. Minocycline reduces traumatic brain injury-mediated caspase-1 activation, tissue damage, and neurological dysfunction. *Neurosurgery*, 2001; 48: 1393-1401.

[153] Sawai, H; Clarke, DB; Kitterlova, P; Bray, GM; Aguayo, AJ. Brain-derived neurotrophic factor and neurotrophin-4/5 stimulate growth of axonal branches from regenerating retinal ganglion cells. *J Neurosci*, 1996; 16: 3887–3894.


[160] Sirén, AL; Fratelli, M; Brines, M; Goemans, C; Casagrande, S; Lewczuk, P; Keenan, S; Gleiter, C; Pasquali, C; Capobianco, A; Mennini, T; Heumann, R; Cerami, A; Ehrenreich, H; Ghezzi, P. "Erythropoietin prevents neuronal apoptosis after cerebral ischemia and metabolic stress". *Proc Natl Acad Sci USA*, 2001; 98: 4044-4049.


[168] Stone, EM; Fingert, JH; Alward, WLM; Nguyen, TD; Polansky, JR; Sunden, SLF; Nishimura, D; Clark, AF; Nystuen, A; Nichols, BE; Mackey, DA; Ritch, R; Kalenak, JW; Craven, ER; Sheffield, VC. Identification of a gene that causes primary open angle glaucoma. *Science*, 1997; 275: 668-670.


Current Trends in Glaucoma: What about Neuroprotection?


[189] von Bartheld, CS; Williams, R; Lefcort, F; Clary, DO; Reichardt, LF; Bothwell, M. Retrograde transport of neurotrophins from the eye to the brain in chick embryos: roles of the p75NTR and TrkB receptors. J Neurosci, 1996; 16: 2995-3008.
[190] Vorwerk, CK; Lipton, SA; Zurakowski, D; Hyman, BT; Sabel, BA; Dreyer, EB. Chronic low-dose glutamate is toxic to retinal ganglion cells. Toxicity blocked by memantine. Invest Ophthalmol Vis Sci, 1996; 37: 1618-1624.

[205] Yrjanheikki, J; Tikka, T; Keinanen, R; Goldsteins, G; Chan, PH; Koistinaho, JA. A tetracycline derivative, micocycline, reduces inflammation and protects against focal cerebral ischemia with a wide therapeutic window. *Proc Natl Acad Sci USA*, 1999; 96: 13496-13500.

[206] Zangwill, LM; Bowd, C; Berry CC; Williams, J; Blumenthal, EZ; Sánchez-Galeana, CA; Vasile, C; Weinre, RN. Discriminating between normal and glaucomatous eyes using the Heidelberg retina tomography, GDx Nerve Fiber Analyzer, and Optical Coherence Tomograph. *Arch Ophthalmol*, 2001; 119(7): 985-993.

[207] Zhang, C; Lei, B; Lam, TT; Yang, F; Sinha, D; Tso, MO. Neuroprotection of photoreceptors by minocycline in light-induced retinal degeneration. *Invest Ophthalmol Vis Sci*, 2004; 45: 2753-2759.

[208] Zhang, X; Reigada, D; Zhang, M; Laties, AM; Mitchell, CH. Increased ocular pressure increases vitreal levels of ATP. *Association for Research in Vision and Ophthalmology* (ARVO), 2006; Abstract 426.


[210] Zhang, X; Zhang, M; Laties, AM; Mitchell, CH. Balance of purines may determine life or death as A3 adenosine receptors prevent loss of retinal ganglion cells following P2X7 receptor stimulation. *J Neurochem*, 2006; 98: 566-575.

[211] Zhong, L; Bradley, J; Schubert, W; Ahmed, E; Adamis, AP; Shima, DT; Robinson, GS; Ng, YS. Erythropoietin promotes survival of retinal ganglion cells in DBA/2J glaucoma mice. *Invest Ophthalmol Vis Sci*, 2007; 48(3): 1212-1218.


[213] Zhou, X; Li, F; Ge, J; Sarkisian, SRJr, Tomita H; Zaharia, A; Chodosh, J; Cao, W. Retinal ganglion cell protection by 17-beta-estradiol in a mouse model of inherited glaucoma. *Dev Neurobiol*, 2007; 67(5): 603-616.

[214] Zhu, S; Stavrovskaya, IG; Drozda, M; Kim, BY; Ona, V; Li, M; Sarang, S; Liu, AS; Hartley, DM; Wu, DC; Gullans, S; Ferrante, RJ; Przedborski, S; Kristal, BS; Friedlander, RM. Minocycline inhibits cytochrome c release and delays progression of amyotrophic lateral sclerosis in mice. *Nature*, 2002; 417: 74-78.