Pharmacological Neuroprotection in Blinding Eye Diseases

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Abstract
Pharmacological neuroprotection aims at neutralising some of the effects of the nerve-derived toxic factors, thereby increasing the ability of the spared neurons to cope with stressful conditions. Understanding of the role of extracellular calcium transport across cell membranes in modulating various intracellular signaling processes, including the initiation of the apoptotic cascade, represents part of the rationale for interest in investigating calcium-channel blockers for neuroprotection in such blinding eye diseases as glaucoma and retinitis pigmentosa. Glaucoma is a major cause of worldwide irreversible blindness. Bilateral blindness will be present in 5.9 million people with open-angle glaucoma and 5.3 million people with angle-closure glaucoma in 2020. Currently, glaucoma is recognised as an optic neuropathy and the loss of vision in this eye disease is attributed to degeneration of the axons of the retinal ganglion cells through apoptosis. Retinitis pigmentosa (RP) represents a group of progressive hereditary diseases of the retina that lead to incurable blindness and affect two million people worldwide. RP has been known to be initiated by photoreceptor apoptosis as a final common pathway at the cellular level, irrespective of gene mutations. Apoptosis can thus be considered as a therapeutic target in glaucoma and retinitis pigmentosa. This review focuses on cellular events associated with neurodegeneration due to glaucoma and retinitis pigmentosa, and discusses pharmacological agents believed to have a neuroprotective role in these diseases. It is time to conduct a randomized controlled trial to provide direct evidence of the effectiveness of specific type calcium channel blocker in glaucoma and retinitis pigmentosa. New intervention as pharmacological neuroprotection by calcium channel blockers remains an important strategy to limit the morbidity of these eye diseases representing significant health problem.

Keywords: Glaucoma, Retinitis pigmentosa, Apoptosis, Calcium channel blockers, Neuroprotection

1. Introduction
Glaucoma is second only to cataract among visual disorders, but it is a major cause of worldwide irreversible blindness. Bilateral blindness will be present in 5.9 million people with open-angle glaucoma and 5.3 million people with angle-closure glaucoma in 2020.

By 2020, India will become second overall in number with glaucoma, surpassing Europe. There will be six million more Chinese people with glaucoma. In 2020, the Europe region will still contain the greatest number of people with open-angle glaucoma, and the proportion of all those with angle-closure glaucoma that live in Asian regions will increase further to 87.6% (Quigley and Broman, 2006). It is important to improve diagnostic and therapeutic approaches to glaucoma that can be applied worldwide.

Currently, glaucoma is recognized as an optic neuropathy and the loss of vision in this eye disease is attributed to degeneration of the axons of the retinal ganglion cells (RGC) through apoptosis (Kaushik et al., 2003). This concept emphasizes that several pressure-independent mechanisms are responsible for the development and progression of glaucomatous neuropathy and that high intra-ocular pressure (IOP) and vascular insufficiency in the optic nerve head are merely risk factors for the development of glaucoma. The central role of raised IOP is being questioned as many patients continue to demonstrate a clinically downhill course despite control of initially raised IOP (Osborne et al., 1999). In addition, up to one-sixth of patients with glaucoma develop it despite normal IOP (Brubaker, 1996). Chronic heart failure is associated with lower ocular perfusion pressure, and glaucomatous optic nerve head changes (Meira-Freitas et al., 2012).
In humans, the optic nerve consists of approximately one million axons; whose cell bodies are primarily located in the ganglion cell layer (Lisegang, 1996). RGC death, therefore, represents the final common pathway of virtually all diseases of the optic nerve including glaucomatous optic neuropathy. There is histological and electrophysiological evidence to suggest that ganglion cells are the sole neurons affected in glaucoma (Lisegang, 1996).

Retinitis pigmentosa represents a group of progressive hereditary diseases of the retina that lead to incurable blindness and affect two million people worldwide (Busskamp et al., 2012) and principally characterized by progressive rod-dominant photoreceptor degeneration in the initial stage and eventual cone photoreceptor degeneration in later stages. Patients with retinitis pigmentosa (RP) mainly complain of night blindness and photophobia in the early stage, followed by gradual constriction of the visual field, decreased visual acuity, and color blindness in later stages. The prevalence of RP is roughly 1 in 4,000-5,000 people, and the condition is common in both Asian and Western countries (Liesegang et al., 2002). Significant features of RP include heterogeneity in both clinical and genetic characteristics. The severity and progression of RP vary from patient to patient even in the same family, despite affected members presumably sharing the same causative gene mutation.

Molecular genetic studies have also demonstrated that a primary lesion in RP involves photoreceptor and/or retinal pigment epithelial cells in which many causative genes are specifically expressed under physiological conditions. Photoreceptor or retinal pigment epithelial cells are known to degenerate mostly through apoptosis (Chang et al., 1993), which is now understood as a final common pathway for RP at the cellular level.

Apoptosis can thus be considered as a therapeutic target in glaucoma and retinitis pigmentosa. All animal cells are programmed for carrying out self-destruction when they are not needed, or when damaged. Apoptosis is a process rather than an event. It has been labelled a programmed cell death, or cell suicide. It is not unique to RGCs or glaucoma alone. Following an initial insult, the cells try to minimize or buffer the damage done through a variety of processes. Generation of “suicide triggers” could be one of the consequences of these processes and interactions and these molecules may start the process of apoptosis which is characterized by an orderly pattern of inter-nucleosomal DNA fragmentation, chromosome clumping, cell shrinkage and membrane blebbing (Farkas and Grosskreutz, 2001). Abnormally high Ca2+ concentration leads to inappropriate activation of complex cascades of nucleases, proteases and lipases. They directly attack cell constituents and lead to the generation of highly reactive free radicals and activation of the nitric oxide pathway (Naskar and Dreyer, 2001). The resulting interaction between intermediate compounds and free radicals leads to DNA nitrosylation, fragmentation and activation of the apoptotic programme. In summary, apoptosis is a genetically programmed mechanism of cell death in which the cell activates a specific set of instructions that lead to the deconstruction of the cell from within.

The strategy of treating a disease by preventing neuronal death is termed neuroprotection. The term is used more narrowly to describe therapies to address final common pathways of damage in many neurological diseases ranging from amyotrophic lateral sclerosis, Alzheimer’s disease and, in the context of the eye, glaucoma. The potential role of neuroprotective agents is to rescue of sick and dying cells and to maintain the integrity of healthy cells by providing resilience to a variety of hostile factors or agents (Kaushik et al., 2003).

The objective of neuroprotective therapy is to employ pharmacologic or other means to attenuate the hostility of the environment or to supply the cells with the tools to deal with these changes (Kaufman and Gabelt, 1999). According to this approach, any chronic degenerative disease may be viewed to have, at any given time, some neurons undergoing an active process of degeneration which contributes to the hostility of the environment surrounding it. The exponential loss of cells after secondary degeneration stems from the damage brought on other neurons that either escaped or were only marginally damaged by the primary injury (Schwartz et al., 1996). Neuroprotection attempts to provide protection to such neurons that continue to remain at risk (Chew and Ritch, 1997). The concept of neuroprotective therapy for glaucoma is that damage to retinal ganglion cells may be prevented by intervening in neuronal death pathways (Danesh-Meyer, 2011) and for retinitis pigmentosa may be prevented the damage of
photoreceptors and retinal pigment epithelial cells (Doonan and Gotter, 2004; Cottet and Schorderet, 2009).

Wheeler et al. (2001) proposed four criteria to assess the likely therapeutic utility of neuroprotective drugs with demonstrated utility in animal studies: the drug should have a specific receptor target in the retina/optic nerve; activation of the target must trigger pathways that enhance a neuron’s resistance to stress or must suppress toxic insults, the drug must reach the retina/vitreous in pharmacologically effective concentrations and the neuroprotective activity must be demonstrated in clinical trials.

The general consensus is that intracellular concentrations of calcium ion are increased in apoptosis (Nicotera and Orrenius, 1998; Fox et al., 1999; Delyfer et al., 2004; Doonan et al., 2005; Sanges et al., 2006; Paquet-Durand et al., 2007). Intracellular calcium ions are provided through several types of calcium channels and transporters located on cell membranes, endoplasmic reticulum, and mitochondria. Cyclic-nucleotide-gated cation channels (CNGCs) are located in the outer segment and closed by depletion of cGMP as a result of the phototransduction reaction triggering hyperpolarization. L-type voltage-gated calcium channels (VGCCs) are located in the cell body and synaptic terminal and are closed by hyperpolarization of the cell membrane induced by phototransduction. Steele Jr. et al., (2005) suggested that the average concentration of calcium in the terminal ranges from ~350 nM in hyperpolarized light-adapted cells to more than 39 µM in cells depolarized to dark potentials in salamander rods and cones.

In addition to CNGC and VGCC, intracellular concentrations of calcium ions are regulated by many other factors, such as plasma membrane calcium ATPase, store-operated calcium entry, calcium stores in the endoplasmic reticulum, and mitochondria. Under pathological conditions, like those in rd1 mice, intracellular calcium levels significantly increase in rods, even before the detection of apoptotic cells (Doonan et al., 2005). The marked elevation of intracellular concentrations of calcium ions activates downstream reactions, including hydrolytic enzymes like calpains, and eventually leads to cell death (Nicotera and Orrenius, 1998). Excessive calcium influx is initiated in the cytosol and subsequently in mitochondria in rd1mouse (Doonan et al., 2005), suggesting that increased calcium ions may affect many biochemical cascades and reactions not only in the cytosol but also in the mitochondria (Szikra and Krizjaj, 2009; Barabas et al., 2010).

Calcium channel blockers, which alter the intracellular calcium concentration by modifying calcium flux across cell membranes and affect various intracellular signaling processes, have been long and widely used to treat essential hypertension and certain types of cardiac diseases such as angina pectoris. Among five subtypes of calcium channels, only specific agents for L-type calcium channels have been used as therapeutics.

The objective of this review is to evaluate the evidence and discuss the rationale behind the recent suggestions that calcium channel blockers may be useful in the treatment of glaucoma and retinitis pigmentosa.

2. Pharmacological Neuroprotection in Glaucoma

Calcium channel blockers generally dilate isolated ocular vessels and increase ocular blood flow in experimental animals, normal humans, and patients with open-angle glaucoma and in patients who have vascular diseases in which considerable vascular tone is present (Luksch et al., 2005; Koseki et al., 2008; Araie and Yamaya, 2011). As well, contrast sensitivity in patients with normal tension glaucoma was found ameliorated by calcium channel inhibition (Yu et al., 2004, Boehm et al., 2003). Neuroprotective effect of calcium channel blockers against retinal ganglion cell damage under hypoxia was shown by Yamada et al. (2006) and also by Garcia-Campos et al. (2007).

In a retrospective study of normal-tension and open-angle glaucoma patients who happened to be taking calcium channel blockers, Netland et al., (1993) demonstrated a decrease in glaucoma progression relative to controls. Kittazawa et al., (1989) suggested visual improvement in a significant number of patients who took nifedipine in a 6-month prospective study. Flunarizine, a potent calcium channel blocker has been demonstrated to enhance RGC survival after optic nerve transection in mice (Eschweiler and Bahr, 1993).
These findings suggest that calcium channel blockers may potentially inhibit ganglion cells and photoreceptor apoptosis in glaucoma (Araie and Yamaya, 2011).

Otori et al. (2003) evaluated the effect of diltiazem on inhibition of glutamate-induced apoptotic retinal ganglion cells death and concluded that application of diltiazem do not appear to reduce apoptosis.

Nimodipine is an isopropyl calcium channel blocker which readily crosses the blood-brain barrier due to its high lipid solubility. Its primary action is to reduce the number of open calcium channels in cell membranes, thus restricting influx of calcium ions into cells.

Several clinical trials have unequivocally shown that nimodipine is capable of preventing neurological deficits secondary to aneurysmal subarachnoid haemorrhage. The results of the VENUS (Very Early Nimodipine Use in Stroke) study do not support the concept that early nimodipine exerts a beneficial effect in stroke patients (Horn et al., 2001). On the other hand oral nimodipine showed an enhanced acute reperfusion if applied within 12 hours of onset of acute stroke (Infeld et al., 1999; Horn et al., 2001; Yamada et al., 2006). Yamada et al., (2006) in experimental in vitro model revealed that nimodipine have a direct neuroprotective effect against retinal ganglion cells damage related to hypoxia.

Michelson et al., (2006) have evaluated the impact of nimodipine on retinal blood flow in double-blind, two-way, crossover study of healthy subjects and found that orally administered at a dosage of 30 mg three times a day nimodipine significantly increases retinal perfusion in healthy subjects. Based on experimental findings Shahsuvaryan (2008) investigated the efficacy of nimodipine in the prospective comparative clinical interventional study of patients with nonarteritic anterior and posterior optic neuropathy. The author stated that increase in visual acuity was higher in the posterior ischemic neuropathy subgroup than in the anterior ischemic subgroup. Visual field testing during the follow-up also revealed positive transformation of visual field defects size and location, which correlated to visual acuity changes. These encouraging findings need to be confirmed by double-blind study.

The impact of nimodipine on ocular circulation in normal tension glaucoma has been evaluated in many clinical studies.

Piltz et al., (1998) have described a performance-corrected improvement in visual field deviation and contrast sensitivity in patients with normal tension glaucoma (NTG) and in control subjects in a prospective, placebo-controlled double-masked study after oral administration of nimodipine (30 mg twice a day). Other authors (Michalk et al., 2004) also stated that a single dose of 30mg nimodipine normalizes the significantly reduced retinal blood flow in NTG patients with clinical signs of vasospasmic hyperactivity. Luksch et al., (2005) have examined the impact of 60 mg nimodipine in NTG patients 2 hours after oral administration. Results disclosed that nimodipine increased the blood flow of the optic nerve head by 18% and improved color-contrast sensitivity.

Thus, nimodipine is potentially useful calcium channel blocker for eye disorders treatment due to its high lipid solubility and ability to cross the blood-brain barrier.

Recent experimental evidences suggest that Nilvadipine appear to have beneficial effects on different ocular structures. Ogata et al., (2000) have evaluated the effects of nilvadipine on retinal blood flow and concluded that this agent may directly and selectively increase retinal tissue blood flow, while having only minimal effect on systemic circulation including arterial blood pressure. Another experimental study conducted by Uemura and Mizota (2008) have also advocated the use of nilvadipine for the treatment of glaucoma or other retinal diseases that have some relation to apoptosis, based on claims that nilvadipine has high permeability to retina and neuroprotective effect to retinal cells. Otori et al., (2003) in the experimental study of different calcium channel blockers protective effect against glutamate neurotoxicity in purified retinal ganglion cells has found that nilvadipine significantly reduce glutamate-induced apoptosis.

In addition to direct effects of calcium channel blockers on intracellular concentrations of calcium ion in ganglion cells, other indirect effect is expected such as increased choroidal blood flow (Koseki et al., 2008).

Several clinical trials have shown the effectiveness of nilvadipine in glaucoma. Yamamoto et al., (1998), Tomita et al., (1999), Niwa et al., (2000) have found that nilvadipine reduces...
vascular resistance in distal retrobulbar arteries and significantly increases velocity in the central retinal artery in patients with normal tension glaucoma.

Tomita et al., (1999) also stated that reduced orbital vascular resistance after a 4-week treatment with 2 mg oral nilvadipine consequently increases the optic disc blood flow.

Koseki et al., (2008) conducted a randomized, placebo-controlled, double-masked, single-center 3-year study of nilvadipine on visual field and ocular circulation in glaucoma with low-normal pressure. No topical ocular hypotensive drugs were prescribed.

The authors concluded that nilvadipine (2 mg twice daily) slightly slowed the visual field progression and maintained the optic disc rim, and the posterior choroidal circulation increased over 3 years in patients with open-angle glaucoma with low –normal intraocular pressure. The results of this study add to the growing body of evidence that nilvadipine may be useful for neuroprotection in glaucoma.

Thus, nilvadipine is potentially useful calcium channel blocker for eye disorders treatment due to its hydrophobic nature with high permeability to the central nervous system, including the retina and the highest antioxidant potency among calcium channel blockers.

The latest experimental study (Karim et al., 2006) evaluated a neuroprotective effect of another new calcium channel blocker – lomerizine. The authors stated that lomerizine alleviates secondary degeneration of retinal ganglion cells induced by an optic nerve crush injury in the rat, presumably by improving the impaired axoplasmic flow. Tamaki et al., (2003) also investigated the effects of lomerizine on the ocular tissue circulation in rabbits and on the circulation in the optic nerve head and choroid in healthy volunteers and have found that lomerizine increases blood velocity, and probably blood flow, in the optic nerve head and retina in rabbits, and it also increases blood velocity in the optic nerve head in healthy humans, without significantly altering blood pressure or heart rate.

3. Pharmacological Neuroprotection in Retinitis Pigmentosa

Frasson et al., (1999) first reported the effects of d-cis-diltiazem, a benzothiazepin calcium channel antagonist which blocks both cnGC and vGcC, on photoreceptor protection in rd1 mice, several investigators have reported positive and negative effects of calcium channel blockers on animal models of rp (Bush et al., 2000; Kelling et al., 2001; Pawlyk et al., 2002; Yamazaki et al., 2002; Hart et al., 2003; Sato et al., 2003; Takano et al., 2004; Yallazza-Deschamps et al., 2005; Sanges et al., 2006; Takeuchi et al., 2008).

Since rd1 is caused by a mutation in the gene encoding the β-subunit of rod cGMP-phosphodiesterase, one of the key enzymes in the phototransduction pathway, cnGC located in the outer segment cannot be closed despite light stimulation in the rod receptor cells. Inhibition of light-induced hyperpolarization, caused by a mutation in the rod cGMP-phosphodiesterase gene, also does not close vGcC. These phenomena increase calcium influx in both outer and inner segment in rd1 mice. The intracellular concentration of calcium ions is subsequently elevated, leading to photoreceptor apoptosis (Frasson et al., 1999), possibly by upregulation of calpains and other proteins (Paquet-Durand et al., 2007). Sanges et al. (2006) demonstrated that systemic administration of d-cis-diltiazem reduced intracellular concentrations of calcium, downregulating calpains and photoreceptor apoptosis in rd1 mice. Direct inhibitory effects of d-cis-diltiazem on l-type vGcC have been reported by Hart et al. (2003), and d-cis-diltiazem effectively blocks photoreceptor light damage in mouse models by inhibiting photoreceptor apoptosis (Vallazza-Deschamps et al., 2005). In contrast, l-cis isomer inhibits l-type vGcC similarly to d-cis isomer (Cia et al., 2005). The difference in action between d-cis- and l-cis-diltiazems on photoreceptor neuroprotection suggests that cnGC might also be important for photoreceptor neuroprotection (Frasson et al., 1999). Read et al.(2002) also reported that the β-subunit of vGcC knock-out rd1 mice showed retardation of photoreceptor degeneration, suggesting that blockage of calcium influx may partially contribute to photoreceptor rescue in these animal models although it did not prevent photoreceptor degeneration. Despite these studies, however, Pawlyk et al. (2002) and Takano et al. (2004) found no rescue effects of d-cis-diltiazem on retinal degeneration in rd1 mice, and Bush et al. (2000) also reported that d-cis-diltiazem was ineffective for photoreceptor rescue in rhodopsin p23hrtransgenic rats. While the effects of diltiazem on animal models of retinal degeneration remain controversial, another type
of calcium channel blockers, nilvadipine a member of the dihydropyridine derivatives, is another candidate therapeutic agent for rp. nilvadipine has low-voltage-activated calcium blocking actions in addition to l-type high-voltage calcium blocking actions. The hydrophobic nature induced by the chemical structure of nilvadipine allows high permeability to the central nervous system, including the retina (Tokuma et al., 1987). Systemic administration of nilvadipine has been shown to be effective for protecting photoreceptors in rcs rats (Yamazaki et al., 2002; Sato et al., 2003), rd1 mice (Takano et al., 2004), and heterozygous rd2 (rd2) mice (Takeuchi et al., 2008)

in addition to direct effects of calcium channel blockers on intracellular concentrations of calcium ion in photoreceptor cells, other indirect effects are expected such as increased expression of fibroblast growth factor (fgf)2 (Sato et al., 2003; Takano et al., 2004) and ciliary neurotrophic factor (cntf) (Takeuchi et al., 2008) in the retina, and increased choroidal blood flow (Koseki et al., 2008). Since fgf2 and cntf are known to exert photoreceptor-protective effects (Bok et al., 2002; Tao et al., 2002; Schlichtenbrede et al., 2003; Wen et al., 2006), upregulating such intrinsic neurotropic factors by nilvadipine may demonstrate beneficial effects against rp. cntf has also been applied as a clinical trial for rp by Sieving et al. (2006).

oxidative stress as have been shown in multiple studies (Carmody et et, 2000; Yu et al., 2004; Shen et al., 2005; Komeima et al., 2006; Tuson et al., 2009; Usi et al., 2009) may be involved in photoreceptor death in rp, and nilvadipine has the highest antioxidant potency among calcium channel blockers (Sugawara et al., 1996).

The beneficial effect of calcium channel blockers on photoreceptor degeneration achieved in animal models of rp, have encouraged researches to conduct the human trials.

Pasantes-Morales et al. (2002) reported that a combination of d-cis-diltiazem, taurin, and vitamin e has beneficial effects on the visual field progression, although the study did not clarify whether diltiazem alone demonstrated beneficial effects. Ohguro (2008) reported the photoreceptor rescue effects of nilvadipine in a small patient group. Nakazawa et al., (2010; 2011) expanded his nilvadipine study for rp patients to confirm the results. Although both treated and control groups are still small, authors results have shown significant retardation of the mean deviation (md) slope as calculated by the central visual field (humphry visual field analyzer, 10-2 program) after a mean of 48 months of observation.

5. Conclusion

In conclusion, there are potentially multiple biological bases for the protective effect of calcium channel blockers on eye structures, as was shown above. Understanding of the role of extracellular calcium transport across cell membranes in modulating various intracellular signaling processes, including the initiation of the apoptotic cascade, represents part of the rationale for interest in investigating calcium-channel blockers for pharmacological neuroprotection in glaucoma and retinitis pigmentosa.

Taken into account that not all calcium channel blockers are equally effective, the challenge for future laboratory research will be to determine the best type and dosage of calcium channel blockers and also to determine which processes are modulated by these drugs in vivo and therefore are primarily responsible for the apparent beneficial effects observed in the previous studies.

Clearly, further observational studies cannot adequately address many unanswered questions. It is time to conduct a randomized controlled trial to provide direct evidence of the effectiveness of specific type calcium channel blocker in glaucoma and retinitis pigmentosa. New intervention as pharmacological neuroprotection by calcium channel blockers in such blinding eye diseases as glaucoma and retinitis pigmentosa remains an important strategy to limit the morbidity of this significant health problem.
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