

Review

Apoptosis: Current therapeutic approach in retinitis pigmentosa

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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. Retinitis pigmentosa (RP) represents a group of hereditary retinal degenerations principally characterized by progressive rod-dominant photoreceptor degeneration in the initial stage and eventual cone photoreceptor degeneration in later stages. RP has been known to be initiated by photoreceptor apoptosis as a final common pathway at the cellular level, irrespective of gene mutations, and apoptosis can thus be considered as a therapeutic target. The goal of this review is to discuss the rationale behind the recent suggestions that calcium channel blockers may be useful in the treatment of retinitis pigmentosa. The National Center for Biotechnology Information (NCBI) at the US National Library of Medicine (NLM) was searched using the terms "Calcium channel blockers in retinitis pigmentosa". It is time to conduct a randomized controlled trial to provide direct evidence of the effectiveness of specific type calcium channel blockers in lowering progression of RP. New intervention as calcium channel blockers usage to prevent retinitis pigmentosa progression remains an important strategy to limit the morbidity of this significant health problem.

Key words: Apoptosis, retinitis pigmentosa, photoreceptors degeneration, calcium channel blockers, therapeutic effect.

INTRODUCTION

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. Retinitis pigmentosa represents a group of hereditary retinal degenerations 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 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 as it plays many roles in retinitis pigmentosa (Doonan and Gotter, 2004; Cottet and Schorderet, 2009).

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 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 μ M 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 down stream 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 rd1 mouse (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 Krizaj, 2009; Barabas et al., 2010).

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 retinitis pigmentosa. The National Center for Biotechnology Information (NCBI) at the US National Library of Medicine (NLM) was searched using the terms "Calcium channel blockers in retinitis pigmentosa".

CALCIUM CHANNEL BLOCKERS IN THE TREATMENT OF RETINITIS PIGMENTOSA

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. Calcium channel blockers generally dilate isolated ocular vessels and increase ocular blood flow in experimental animals, normal humans, and patients with open-angle glaucoma (Luksch et al., 2005; Koseki et al., 2008; Araie and Yamaya, 2011). 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; Pearce-Kelling et al., 2001; Pawlyk et al., 2002; Yamazaki et al., 2002; Hart et al., 2003; Sato et al., 2003; Takano et al., 2004; Vallazza-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, CNGCs 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 p23H transgenic 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 (rds) 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 neurotrophic 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 and Cotter, 2000; Yu et al., 2004; Shen et al., 2005; Komeima et al., 2006; Tuson et al., 2009; Usui 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 researchers 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 (2011a and b) 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.

CONCLUSION

In conclusion, there are potentially multiple biological bases such as decreasing the elevated intracellular concentrations of calcium ion in photoreceptor or retinal pigment epithelial cells, preventing their apoptotic degeneration; dilatation of ocular vessels and increase of ocular blood flow; photoreceptor neuroprotection through increased expression of fibroblast growth factor (FGF)2 and ciliary neurotrophic factor (CNTF) in the retina, and increased choroidal blood flow and antioxidant potency for the protective effect of calcium channel blockers on photoreceptors apoptosis in retinitis pigmentosa. Taking 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 specific crossing the blood-brain barrier calcium channel blocker and also to determine which processes are modulated by calcium channel blockers *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 of calcium channel blockers in lowering the progression of RP.

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