



# Mitochondria-Targeted Antioxidant SS-31 is a Potential Novel Ophthalmic Medication for Neuroprotection in Glaucoma

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## ABSTRACT

Glaucoma is the second leading cause of irreversible blindness and a neurodegenerative disease with a complex pathogenesis. Increasing evidence suggests that oxidative stress and mitochondrial dysfunction have crucial roles in most neurodegenerative diseases such as glaucoma. The conventional clinical treatment for glaucoma is lowering the intraocular pressure (IOP). Some patients have normal IOP, whereas other patients appear to obtain adequate control of IOP after filtration surgery or medication. However, these patients still experience progressive visual field loss. Vision field loss in glaucoma is attributed to retinal ganglion cell (RGC) apoptosis. Many recent researches demonstrated that the link between mitochondrial dysfunction and oxidative stress was a major cause of RGCs apoptosis. How oxidative stress leads to RGCs apoptosis in glaucoma is unclear but may involve the neurotoxic effects of oxidative stress-induced mitochondrial dysfunction and/or damage from reactive oxygen species (ROS). Investigations are needed concerning the mitochondria as effective targets for potential therapeutic interventions to maintain mitochondrial function and reduce oxidative stress, and thereby delay or stop RGC loss and prolong visual function. The mitochondria-targeted antioxidant Szeto-Schiller (SS) peptide is a candidate molecule. Szeto-Schiller-31 (H-D-Arg-Dmt-Lys-Phe-NH<sub>2</sub>) is an attractive mitochondria-targeted antioxidant that can protect the mitochondria and RGCs against oxidative damage. Therefore, we suggest SS-31 as a novel neuroprotective ophthalmic drug for protecting RGCs in glaucoma.

## KEY WORDS

Glaucoma, Mitochondria-targeted antioxidant SS-31, Neuroprotection, oxidative stress mitochondrial dysfunction

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## INTRODUCTION

Glaucoma is increasingly recognized as a chronic neurodegenerative disorder characterized by optic nerve head cupping and visual field defects caused by damage to and apoptosis of RGCs. Glaucoma treatment focuses

on lowering IOP via pharmacotherapy or surgery. Epidemiological investigators have recently reported that lowering IOP alone cannot prevent the progressive loss of visual field in glaucoma patients. There is improved knowledge about the mechanism of optic nerve damage. As a consequence, the concept of neuroprotection has



been increasingly proposed. Additional neuroprotective strategies may offer novel treatments to prevent RGCs loss and disease progression. Increasing IOP is a major risk factor in glaucoma; however, other concomitant factors also significantly affect the eye such as oxidative stress caused by ROS [1], increased glutamate levels [2], toxic effects and vascular alterations [3]. Recent accumulating evidence suggests the involvement of mitochondrial dysfunction in glaucoma [4,5]. Oxidative stress is a common manifestation of mitochondrial dysfunction, and it has been repeatedly implicated in the pathogenesis of glaucoma [6].

#### **Oxidative stress and mitochondrial dysfunction in glaucoma**

In brief, oxidative stress is an imbalance between processes that generate ROS and processes that remove them. More than two decades ago, oxidative stress was first proposed as a contributor of glaucoma pathogenesis [7]. Many clinical and experimental studies have assessed ROS production, antioxidant levels, and macromolecules involved in oxidative damage under glaucomatous stress. For example, in an experimental rat model of glaucoma, intracameral injection of hyaluronic acid decreased antioxidants and increased lipid peroxidation in the retina [1]. In the experimental glaucoma models, cauterization of the episcleral veins in rat leads to ocular hypertension, and the levels of ROS, nitrite as well as lipid peroxidation are markedly increased [8,9]. In another study, the researchers injected the hypertonic saline into the episcleral vein to get the increased IOP. Also, protein oxidation was detected localizing in the inner retinal layers including RGCs [10].

The mitochondria are normally protected from oxidative damage, owing to mitochondrial antioxidant systems, a multilayer network; however, if ROS production exceeds the antioxidant capacity of mitochondria, these cells experience oxidative damage [11]. With the advent of oxidative stress, excessive ROS act as signaling molecules to activate apoptotic pathways. ROS can react with neighboring molecules in the mitochondria such as nucleic acids, proteins, and lipids, and subsequently induce cell death [12]. The mitochondria perform many tasks to maintain biochemical events. Mitochondrial

function, especially the generation of adenosine triphosphate (ATP), is necessary for neuronal survival. All neuronal degeneration is associated with mitochondrial dysfunction [13,14]. During oxidative damage, the mitochondria are impaired and generate ROS more frequently than ATP. Oxidative stress and ATP depletion lead to RGCs mitochondrial dysfunction, and result in RGCs death. Oxidative damage to mitochondria leads to mitochondrial permeability transition (MPT), mitochondrial depolarization, mitochondrial swelling, further excessive ROS production and the cell death mediators such as cytochrome c (cyt c) and apoptosis-inducing factor (AIF) releasing [15].

A pathological feature of glaucoma is apoptosis of RGCs. Caspase mediation activates the proteolysis cascade, which can trigger the apoptosis of RGCs by different stimuli [16,17]. Caspases disrupt the electron transport chain of mitochondria resulting in mitochondrial dysfunction and the generation of ROS [18]. McKinnon [19] states that caspase-3 and caspase-8 were involved in RGCs apoptosis in experimental rat models of glaucoma. In experimental rat models of glaucoma, it is demonstrated that mitochondrial dysfunction has been related to RGCs apoptosis [20,21]. In vitro studies show that caspase-8 activation can occur downstream of mitochondrial dysfunction [22,23]. Caspase-8, which cleaves and activates all other caspases, has an important role in triggering apoptosis by activation of the caspase cascade. In glaucoma, the transcription factor p53 activates the proapoptotic Bax protein, which leads to RGCs apoptosis via a caspase-3–dependent pathway [24]. This process is required for cyt c release from the mitochondria [25]. Normally, Cyt c is bound to the inner mitochondrial membrane (IMM) in association with cardiolipin [26]. With MPT, calcium overload [27] or peroxidation of cardiolipin [26], cyt c is released from the IMM to the cytosol via the opening of MPT pores [28]. In the cytosol, cyt c combines with Apaf-1 (a cytosolic factor) to form an apoptosome, which then binds procaspase-9 [29]. The active caspase-9 cleaves caspase-3 and caspase-7, which then continue to cleave specific substrates within the cell [28, 30]. Caspase-3 is a common downstream caspase that mediates RGCs apoptosis. Caspase activation induces ROS production and loss of the mitochondrial transmembrane potential,



which provide feedback to the insult to mitochondrial function. Mitochondrial dysfunction is thereby aggravated. By contrast, ROS initiate the death pathway by poly (ADP-ribose)polymerase-1-mediated cleavage of AIF [31]. Apoptosis-inducing factor translocates from the mitochondria to the nucleus. It can then directly induce RGCs death via chromatin condensation, DNA fragmentation and nuclear shrinkage [32]. Tezel et al demonstrated that reducing ROS generation could temporarily protect RGCs from apoptosis [33]. Oxidative stress, increased ROS production, and mitochondrial dysfunction have emerged as an oxidative stress-mediated mitochondrial vicious cycle that promotes RGCs loss.

Based on the aforementioned evidence, we can understand that mitochondrial dysfunction in combination with oxidative stress orchestrates the apoptosis of RGCs by activating different stimuli. Mitochondrial dysfunction is responsible for cytotoxic events in RGCs apoptosis. Enhancing mitochondrial function could prolong RGCs survival. Oxidative damage to mitochondria leads to hyperpolarization of mitochondrial membrane potential, which causes mitochondrial dysfunction and releases stimulative apoptotic compounds. In addition, mitochondria are major generators of ROS and scavenge ROS to reduce ROS-evoked apoptosis signaling. We suggest that a targeted mitochondrial antioxidant could be a viable therapeutic intervention.

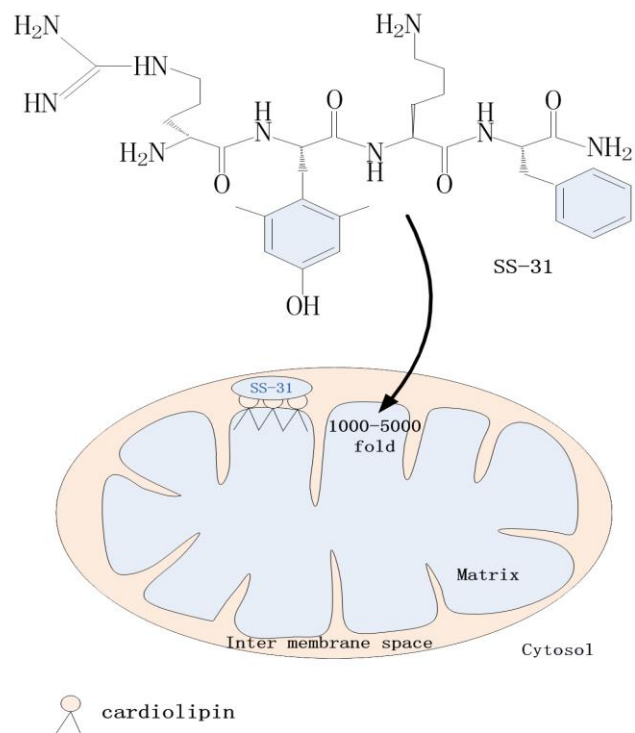
## HYPOTHESIS

Retinal ganglion cells require high energy. They rely on the mitochondria for survival and function. Pre-existing congenital or acquired mitochondrial dysfunction can increase the vulnerability of RGCs to stress from risk factors [34]. Alterations in the functional status of RGCs axon mitochondria will influence RGCs survival in glaucoma [35]. A pharmacological agent that effectively ameliorates oxidative stress and enhances RGCs' mitochondrial function should be a new treatment approach to reduce the rate of visual field loss in glaucoma. A variety of publications have suggested the use of antioxidant supplementation to help in the treatment of glaucoma [36,37]. Among the many

methods, we have concentrated on the mitochondria-targeted antioxidant SS-31 and hypothesize that SS-31 may be a logical therapeutic intervention for neuroprotection in glaucoma.

## Evaluation of the hypothesis

Hazel Szeto and Peter Schiller discovered a group of small synthetic peptides, called SS peptides, which are limited to fewer than 10 amino acid residues and contain alternating aromatic residues and basic amino acids. There are three peptide analogues in this series: SS-02, SS-31 and SS-20. Each molecule carries a 3+ net charge at physiological pH [38]. These peptides are solid-phase [39], stably water soluble, and designed to resist peptidase degradation [40]. Zhao found that these peptide antioxidants are cell-permeable and are concentrated by 1000-fold in the IMM. They are taken up into the IMM in an energy-independent, non-saturable manner [15]. This is clearly an advantage (Figure 1).



**Figure 1.** Schematic representation of a mitochondrion taking up SS-31. This molecule selectively binds to cardiolipin and accumulates 1000- to 5000-fold in mitochondria. Mitochondrial uptake of SS-31 does not depend on the mitochondrial transmembrane electric potential.

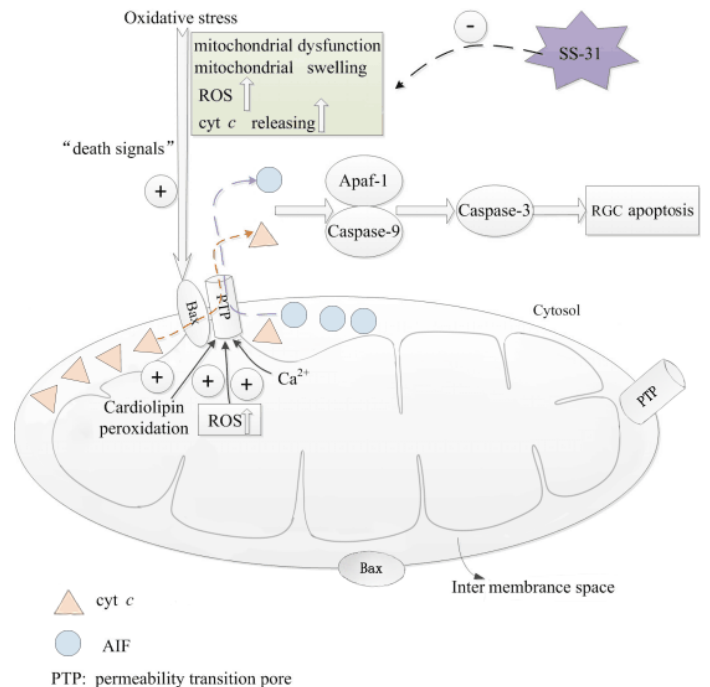


Compared to general antioxidants such as vitamin E, brimonidine, and other biological antioxidants, mitochondrial-targeted peptides show more potential therapeutic neuroprotective effects in some research. General antioxidants tend to be poorly cell-permeable and quite susceptible to the mitochondria. They have an unsatisfactory curative effect because they do not reach the relevant sites of free radical generation within the mitochondria. Vitamin E and coenzyme Q are mitochondrial-targeted antioxidants, and they are very lipophilic. They are taken up into mitochondria by exploiting the potential gradient across the IMM [41]. However, they require high concentrations to cause mitochondrial depolarization [42,43]. Therefore, we believe that mitochondrial-targeted peptides may provide greater neuroprotective effects.

Increasing evidence supports SS-31 as superior to the other SS peptide analogues such as SS-02 and SS-20. A few studies have shown that SS-31 can scavenge mitochondrial ROS in a dose-dependent manner because it possesses a tyrosine residue [15]. However, because it lacks a tyrosine residue, SS-20 cannot scavenge ROS in the same concentrations [15]. Mitochondrial ROS contribute to MPT and mitochondrial swelling; therefore, by reducing mitochondrial ROS, two scavenging SS peptides (i.e., SS-02 and SS-31) were able to inhibit MPT, prevent mitochondrial swelling, reduce cyt c release, and further reduce oxidative damage of mitochondria [44]. The peptide SS-02 was confirmed by uptake studies with [3H]SS-02 present in isolated mouse liver mitochondria at a 1000-fold concentration [15]. Using the same methods, Zhao et al showed that [3H]SS-31 was also rapidly taken up into isolated liver mitochondria, and its levels were nearly five-fold higher than those of SS-02 [45]. Based on these studies, we concluded that SS-31 is the most efficient peptide in this series.

The peptide SS-31 can inhibit MPT, reduce mitochondrial ROS production, and prevent mitochondrial swelling in isolated mitochondria [15]. In addition, SS-31 can scavenge ROS and inhibit lipid peroxidation in vitro [45]. As a mitochondrial-targeted peptide, SS-31 has been evaluated in some neurodegenerative disease models such as in islet cell transplantation [46], myocardial

infarction [47], brain ischemia reperfusion [48], amyotrophic lateral sclerosis [49] and Alzheimer's disease [50]. In all of these conditions, SS-31 exhibited remarkable neuroprotective effects (Figure 2).



**Figure 2.** Simplified scheme of oxidative stress-induced mitochondrial pathway of apoptosis. Oxidative stress leads to mitochondrial dysfunction, reactive oxygen species (ROS) production, mitochondrial swelling, and the release of cytochrome c (cyt c), and apoptosis-inducing factor (AIF) from the intermembrane compartment to the cytosol. These actions are “death signals.” The ROS production, calcium overload or peroxidation of cardiolipin promote permeability transition pore (PTP) opening. Once in the cytosol, cyt c and AIF, in cooperation with Apaf-1, activate caspase-9 and other members of the caspase family, trigger apoptosis, and eventually lead to retinal ganglion cell (RGC) apoptosis. The peptide SS-31 can reduce mitochondrial ROS production, inhibit cyt c release, and prevent mitochondrial swelling in isolated mitochondria. Activation is indicated by the symbol ⊕ and inhibition by the symbol ⊖.

In the field of ophthalmology, Huang and colleagues [51] assessed the protective action of SS-31 in the retinas of diabetic rats. They reported that the most prominent neuronal abnormality in diabetic rats is apoptosis of RGCs [52] and that the RGCs of diabetic rats express several proapoptosis molecules that are very vulnerable to oxidative stress [53]. The authors observed that SS-31 could protect retinal structures and inhibit the breakdown of the inner blood retinal barrier (iBRB) by



reducing oxidative damage and thus preventing apoptosis of RGCs [51]. The mechanism of the results indicated that iBRB dysfunction was detected by the presence of extracellular fluid in the retina and sequential RGCs exposure to serum constituents, and consequently result in RGC apoptosis, which could result in immunoreactions with immunoglobulins and neurotoxicity with increasing glutamate levels [54]. In their study, they also found that SS-31 treatment promoted antiapoptotic protein expression and inhibited apoptosis-related protein overexpression in the retinas of diabetic rats [51]. We suggest that SS-31 may reduce cell apoptosis and prevent mitochondrial dysfunction, thus restoring retinal function so that RGCs are protected from apoptosis. In addition, a previous study demonstrated that SS-31 could decrease ROS production and prevent the release of cyt c from the mitochondria, which attenuates high glucose-induced injuries in human retinal endothelial cells [55]. Moreover, neuroprotection in glaucoma requires preventing RGCs apoptosis induced by oxidative damage. The opinions of Huang et al sustained our hypothesis that SS-31 could be a potent ophthalmic drug to prevent the apoptosis of RGCs and protect the optic nerve in glaucoma.

## CONCLUSION

Glaucoma is a multifactorial optic neuropathy characterized by progressive degeneration of RGCs. Oxidative stress occurs in glaucoma and contributes to RGCs degeneration. Mitochondria have a central role as generators and as targets of oxidative stress, and there has been growing evidence of mitochondrial dysfunction in the pathogenesis of glaucoma [56]. We suggest that the targeted mitochondrial antioxidant SS-31 has therapeutic potential to ameliorate the mitochondrial vicious cycle and thus protect RGCs from glaucomatous degeneration. To assess whether SS-31 could be a potent neuroprotective drug in glaucoma, further studies on oxidative damage in experimental glaucoma and clinical trials evaluating SS-31 treatment should be performed.

## DISCLOSURE

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## REFERENCES

1. Moreno MC, Campanelli J, Sande P, et al. Retinal oxidative stress induced by high intraocular pressure. *Free Radic Biol Med*. 2004 Sep 15;37(6):803-12. PMID:15384194.
2. Shen F, Chen B, Danias J, et al. Glutamate-induced glutamine synthetase expression in retinal Muller cells after short-term ocular hypertension in the rat. *Invest Ophthalmol Vis Sci*. 2004 Sep;45(9):3107-12. PMID:15326127.
3. Flammer J, Haefliger IO, Orgul S, Resink T. Vascular dysregulation: a principal risk factor for glaucoma damage? *J Glaucoma* 1999 Jun;8(3):212-9. PMID:10376264
4. Osborne NN, Lascaratos G, Bron AJ, Chidlow G, Wood JP. A hypothesis to suggest that light is a risk factor in glaucoma and the mitochondrial optic neuropathies. *Br J Ophthalmol*. 2006 Feb; 90(2):237-41. PMID:16424541.
5. Osborne NN. Mitochondria: their role in ganglion cell death and survival in primary open angle glaucoma. *Exp Eye Res*. 2010 Jun; 90(6):750-7. PMID: 20359479.
6. Tezel G. Oxidative stress in glaucomatous neurodegeneration: mechanisms and consequences. *Prog Retin Eye Res*. 2006 Sep;25(5):490-513. PMID:16962364.
7. Alvarado J, Murphy C, Polansky J, Juster R. Age-related changes in trabecular meshwork cellularity. *Invest Ophthalmol Vis Sci*. 1981 Nov;21(5):714-27. PMID:7298275.
8. Ferreira SM, Lerner SF, Brunzini R, Reides CG, Evelson PA, Llesuy SF. Time course changes of oxidative stress markers in a rat experimental glaucoma model. *Invest Ophthalmol Vis Sci*. 2010 Sep;51(9):4635-40. PMID:20357192.
9. Ko ML, Peng PH, Ma MC, Ritch R, Chen CF. Dynamic changes in reactive oxygen species and antioxidant levels in retinas in experimental glaucoma. *Free Radic Biol Med*. 2005 Aug;39(3):365-73. PMID:15993335.
10. Tezel G, Yang X, Cai J. Proteomic identification of oxidatively modified retinal proteins in a chronic pressure-induced rat model of glaucoma. *Invest Ophthalmol Vis Sci*. 2005 Sep;46(9):3177-87. PMID:16123417.
11. Andreyev AY, Kushnareva YE, Starkov AA. Mitochondrial metabolism of reactive oxygen species. *Biochemistry(Mosc)* 2005 Feb;70(2):200-14. PMID:15807660.
12. Moller P, Folkmann JK, Danielsen PH, et al. Oxidative stress generated damage to DNA by gastrointestinal exposure to insoluble particles. *Curr Mol Med* 2012 Jul;12(6):732-45. PMID:22292440.
13. Boveris A, Navarro A. Brain mitochondrial dysfunction in aging. *IUBMB Life* 2008 May;60(5):308-14. PMID:18421773.



14. Browne SE. Mitochondria and Huntington's disease pathogenesis: insight from genetic and chemical models. *Ann N Y Acad Sci.* 2008 Dec;1147:358–82. PMID:19076457.
15. Zhao K, Zhao GM, Wu D, et al. Cell-permeable peptide antioxidants targeted to inner mitochondrial membrane inhibit mitochondrial swelling, oxidative cell death, and reperfusion injury. *J Biol Chem.* 2004 Aug;279(33):34682–90. PMID:15178689.
16. Tezel G, Wax MB. The mechanisms of hsp27 antibody-mediated apoptosis in retinal neuronal cells. *J Neurosci.* 2000 May;20(10):3552–62. PMID:10804196.
17. Tezel G, Wax MB. Increased production of tumor necrosis factoralpha by glial cells exposed to simulated ischemia or elevated hydrostatic pressure induces apoptosis in cocultured retinal ganglion cells. *J Neurosci.* 2000 Dec;20(23):8693–700. PMID:11102475.
18. Ricci JE, Gottlieb RA, Green DR. Caspase-mediated loss of mitochondrial function and generation of reactive oxygen species during apoptosis. *J Cell Biol.* 2003 Jan;160(1):65–75. PMID:12515825.
19. McKinnon SJ, Lehman DM, Kerrigan-Baumrind LA, et al. Caspase activation and amyloid precursor protein cleavage in rat ocular hypertension. *Invest Ophthalmol Vis Sci.* 2002 Apr;43(4):1077–87. PMID:11923249.
20. Mittag TW, Danias J, Pohorenc G, et al. Retinal damage after 3 to 4 months of elevated intraocular pressure in a rat glaucoma model. *Invest Ophthalmol Vis Sci.* 2000 Oct;41(11):3451–9. PMID:11006238.
21. Tatton WG, Chalmers-Redman RM, Sud A, Podos SM, Mittag TW. Maintaining mitochondrial membrane impermeability. an opportunity for new therapy in glaucoma? *Surv Ophthalmol.* 2001 May;45Suppl 3:S277-83. PMID:11377449
22. Slee EA, Harte MT, Kluck RM, et al. Ordering the cytochrome c-initiated caspase cascade: hierarchical activation of caspases-2, -3, -6, -7, -8, and -10 in a caspase-9-dependent manner. *J Cell Biol.* 1999 Jan;144(2):281-92. PMID:9922454.
23. Viswanath V, Wu Y, Boonplueang R, et al. Caspase-9 activation results in downstream caspase-8 activation and bid cleavage in 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine-induced Parkinson's disease. *J Neurosci.* 2001 Dec;15;21(24):9519-28. PMID:11739563.
24. Mo J, Marshall B, Covar J, Zhanq NY, Smith SS, zhanq M. Role of Bax in Death of Uninfected Retinal Cells During Murine Cytomegalovirus Retinitis. *Invest Ophthalmol Vis Sci.* 2014 Oct;55(11):7137-46. PMID:25298417.
25. Nickells RW. Apoptosis of retinal ganglion cells in glaucoma: an update of the molecular pathways involved in cell death. *Surv Ophthalmol.* 1999 Jun;43 Suppl 1:S151-61. PMID:10416758.
26. Petrosillo G, Ruggiero FM, Paradies G. Role of reactive oxygen species and cardiolipin in the release of cytochrome c from mitochondria. *FASEB J.* 2003 Dec;17(15):2202-8. PMID:14656982.
27. Szeto HH. Cell-permeable, Mitochondrial-targeted, Peptide Antioxidants. *AAPS J.* 2006 Apr;8(2):E277-83. PMID:16796378.
28. Shidoji Y, Hayashi K, Komura S, et al. Loss of molecular interaction between cytochrome c and cardiolipin due to lipid peroxidation. *Biochem. Biophys Res Commun.* 1999 Oct;264(2):343-7. PMID:10529366.
29. Li P, Nijhawan D, Budihardjo I, et al. Cytochrome c and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade. *Cell.* 1997 Nov;91(4):479-89. PMID:9390557.
30. Green DR, Reed JC. Mitochondria and apoptosis. *Science.* 1998 Aug;281(5381):1309-12. PMID:9721092.
31. Li GY, Osborne NN. Oxidative-induced apoptosis to an immortalized ganglion cell line is caspase independent but involves the activation of poly(ADP-ribose)polymerase and apoptosis-inducing factor. *Brain Res.* 2008 Jan;1188:35-43. PMID:18053973.
32. Dawson VL, Dawson TM. Deadly conversations: nuclear-mitochondrial cross-talk. *J Bioenerg Biomembranes.* 2004 Aug;36(4):287-94. PMID:15377859.
33. Tezel G, Yang X. Caspase-independent component of retinal ganglion cell death, in vitro. *Invest Ophthalmol Vis Sci.* 2004 Nov;45(11):4049-59. PMID:15505055.
34. Kong GY, Van Bergen NJ, Trounce IA, Crowston JG. Mitochondrial dysfunction and glaucoma. *J Glaucoma.* 2009 Feb;18(2):93-100. PMID:19225343.
35. Osborne NN. Pathogenesis of ganglion "cell death" in glaucoma and neuroprotection: focus on ganglion cell axonal mitochondria. *Prog Brain Res.* 2008;173:339-52. PMID:18929120.
36. Baltmr A, Duggan J, Nizari S, Salt TE, Cordeiro MF. Neuroprotection in glaucoma — is there a future role? *Exp Eye Res.* 2010 Nov;91(5):554-66. PMID:20800593.
37. Chidlow G, Wood JP, Casson RJ. Pharmacological neuroprotection for glaucoma. *Drugs.* 2007;67(5):725-59. PMID:17385943.
38. Zhao K, Luo G, Zhao GM, Schiller PW, Szeto HH. Transcellular transport of a highly polar 3+ net charge opioid tetrapeptide. *J Pharmacol Exp Ther.* 2003 Jan;304(1):425-32. PMID:12490619.
39. Schiller PW, Nguyen TM, Berezowska I, et al. Synthesis and in vitro opioid activity profiles of DALDA analogues. *Eur J Med Chem.* 2000 Oct;35(10):895-901. PMID:11121615.
40. Szeto HH, Lovelace JL, Fridland G, et al. In vivo pharmacokinetics of selectivemu-opioid peptideagonists. *J Pharmacol Exp Ther.* 2001 Jul;298(1):57-61. PMID:11408525.
41. Murphy MP, Smith RA. Drug delivery to mitochondria: the key to mitochondrial medicine. *Adv Drug Deliv Rev.* 2001 Jul;298(1):57-61. PMID:11408525.
42. Smith RA, Porteous CM, Coulter CV, Murphy MP. Selective targeting of an antioxidant to mitochondria. *Eur J Biochem.* 1999 Aug;263(3):709-16. PMID:10469134.
43. Kelso GF, Porteous CM, Coulter CV, et al. Selective targeting of a redox-active ubiquinone to mitochondria within cells: antioxidant and antiapoptotic properties. *J Biol Chem.* 2001 Feb;276(7):4588-96. PMID:11092892.
44. Batandier C, Leverve X, Fontaine E. Opening of the mitochondrial permeability transition pore induces reactive oxygen species production at the level of the respiratory chain complex I. *J Biol Chem.* 2004 Apr;279(17):17197-204. PMID:14963044.
45. Zhao K, Luo G, Giannelli S, Szeto HH. Mitochondria-targeted peptide prevents mitochondrial depolarization and apoptosis induced by tertbutyl hydroperoxide in neuronal cell lines. *Biochem Pharmacol.* 2005 Dec;70(12):1796-806. PMID:16216225.



46. Thomas DA, Stauffer C, Zhao K, et al. Mitochondrial targeting with antioxidant peptide SS-31 prevents mitochondrial depolarization, reduces islet cell apoptosis, increases islet cell yield, and improves posttransplantation function. *J Am Soc Nephrol*. 2005 Dec;70(12):1796-806. PMID:16216225.
47. Cho J, Won K, Wu D, et al. Potent mitochondriatargeted peptides reduce myocardial infarction in rats. *Coron Artery Dis*. 2007 May;18(3):215-20. PMID:17429296.
48. Cho S, Szeto HH, Kim E, Kim H, Tolhurst AT, Pinto JT. A novel cell-permeable antioxidant peptide, SS31, attenuates ischemic brain injury by down-regulating CD36. *J Biol Chem*. 2007 May;18(3):215-20. PMID:17429296.
49. Petri S, Kiaei M, Damiano M, et al. Cell-permeable peptide antioxidants as a novel therapeutic approach in a mouse model of amyotrophic lateral sclerosis. *J Neurochem*. 2006 Aug;98(4):1141-8. PMID:16895581.
50. Manczak M, Mao P, Calkins MJ, et al. Mitochondria-targeted antioxidants protect against amyloid-beta toxicity in Alzheimer's disease neurons. *J Alzheimers Dis*. 2010;20 Suppl 2:S609-31. PMID:20463406.
51. Huang J, Li X, Li M, et al. Mitochondria-Targeted Antioxidant Peptide SS31 Protects the Retinas of Diabetic Rats. *Current Molecular Medicine*. 2013 Jul;13(6):935-45. PMID:23745582.
52. Barber AJ, Lieth E, Khin SA, et al. Neural apoptosis in the retina during experimental and human diabetes, Early onset and effect of insulin. *J Clin Invest*. 1998 Aug;102(4):783-91. PMID:9710447.
53. AbuElAsrar AM, Dralands L, Missotten L, et al. Expression of apoptosis markers in the retinas of human subjects with diabetes. *Invest Ophthalmol Vis Sci*. 2004 Aug;45(8):2760-6. PMID:15277502.
54. Barber AJ. A new view of diabetic retinopathy: a neurodegenerative disease of the eye. *Prog Neuropsychopharmacol Biol Psychiatry*. 2003 Apr;27(2):283-90. PMID:12657367.
55. Li J, Chen X, Xiao W, et al. Mitochondria-targeted antioxidant peptide SS31 attenuates high glucose-induced injury on human retinal endothelial cells. *Biochem Biophys Res Commun*. 2011 Jan;404(1):349-56. PMID:21134355.
56. Lee S, Van Bergen NJ, Kong GY, et al. Mitochondrial dysfunction in glaucoma and emerging bioenergetic therapies. *Exp Eye Res*. 2011 Aug;93(2):204-12. PMID:20691180.